MJBU, Vol 13, No. 1&2, 1995

CHEMILUMINESCENCE STUDY OF PHAGOCYTIC FUNCTION OF WHOLE BLOOD ON STIMULATION BY BARIUM SULPHATE CRYSTALS.

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ABSTRACT

Lumional-dependent chemiluminescence (CL) of whole blood on stimulation by barium sulphate crystals was developd to estimate the phagocytic function of leukocytes. CL was effectively detected by a multipurpose photon counting system. It is rapid and simple method to use and allows temperature within the reaction vessel to controlled accuratly.
The peak CL of whole blood is determined by the number of leukocytes. Red blood cells contamination apparently reduces the count of peak CL but had no influence on time showing peak CL.

INTRODUCTION

Phagositic cells emit light while ingesting microorganisms and other particles. This phenomenon of chemiluminescence (CL) and it's meas urement has become a well used and effective tool in studies and examination of phagocyte functions. CL of phagocytes was first described by Allen et al[1], who noted the emission of light quanta by granulocytes following phagocytosis. The exact nature of this CL, measurable in a liquid scintillation counter, is thought to be due to the result of the interaction of biologically active oxygen products and excitable substrates within the cell[2]. In further studies, CL has been found to be closely linked

to bactericidal activities in various types of phagocytic cells[3,4]. This phenomenon has been used subsequently as a functional assay of granulocyte activity in patients with chronic granulomatous disease[5] which has been diagnosed by using the nitrosoblue dye exclusion test. CL can be amplified by luminol (5-amino-2,3-dihydro-1,4-phtlalazinedione) which is converted to an excited aminophthalate ion in the presence of oxidizing species like superoxide anion (O_{-2}),hydrogen peroxide (H_2O_2),hydroxyl radical (-OH),and single oxygen ($1\ O_2$) (6). Using luminol as an amplifier, trace amounts of activated oxygen species in a sample can be measured[4,7].

The estimation of CL as a phagocytic function in patients is usually carried out using purified granulocytes. It is well known that the course of preparation of purified granulocytes has many influences on granulocyte functions.

Furthermore, this method requires a high volume of blood,and thus sometimes cannot be used for patients with neutropenia. In the present study, using luminol-dependent CL, we have tried to assess phagocytic function of whole blood on stimulation with $BaSO_4$4 crystals.

MATERIALS AND METHODS

Preparation of Blood Samples:

Venous blood samples (0.08 ml) were obtained from healthy adults. Each sample was mixed with 0.02 ml of 5% sodium citrate as anticoagulant in a measuring vial, and then kept at 4 C until the start of the assay (usually CL was measured within 1 hr.). In some experiments pure granulocytes were separated for changing artificially the number of granulocytes in a tested sample as follow: 10 ml of citrated blood mixed with 1 ml of 6% (W/V) Dextran 500 in saline (0.9% W/V NaCl in H20) were incubated for 30 min at room tem-

perature then the leukocyte-rich plasma layer was removed after erythrocytes were settled, and centrifuged at 1800 rpm for 15 min. To the bottom leukocytes-rich portion were added 10 ml of PBS, which was then centrifuged at 1700 rpm for 10 min. We added 10 ml of diluted PBS (1:1 with H_2O) to the pellet to lyse the erythrocytes. The cells were then centrifuged as above, resuspended in 1 ml of PBS, and the leukocytes were counted.

Chemicals and Reagents:

Luminol solution was prepared by dissolving 20 mg of luminol (Sigma Chemical Co.) in 2 ml of 0.2 M NaoH . This stock solution was diluted up to 100 ml with deionized water prior to use.

CL inducer: In order to activate granulocytes to burst luminol-dependent CL, a medium of the following composition (mM) was used: 165 NaCl, 15 Tris HCl, 2.25 $CaCl_2$ and 25 $BaSO_4$ (pH 7.4). $BaSO_4$ was in this medium in suspension form.

Chemiluminescence measurement

This was carried out using the principle of oxidation of luminol by reactive oxygen species produced during phagocytosis in phagocytic cells to increase the amount of measurable light[1,6,9]. Luminol-dependent CL in stimulated granulocytes was measured in an ultra sensitive, multipurpose photon counting system built up in our department (Fig 1) (A.H.Mohammed and F.H.Mohammed unpuplished work). The reaction mixture consisted of 2 ml CL inducer, 0.2 ml NaoH and 0.2 ml luminol in a 5 ml beaker. To this mixture 0.02 ml whole blood was added and agitated to mix well before it was poured into the measuring cuvette of the photon counting system, where the temperature is kept at 37Co.

CL was continuously recorded until the CL peaked and demonstrated a definite decline. All results

were taken in relative to standard-arbitrary units.

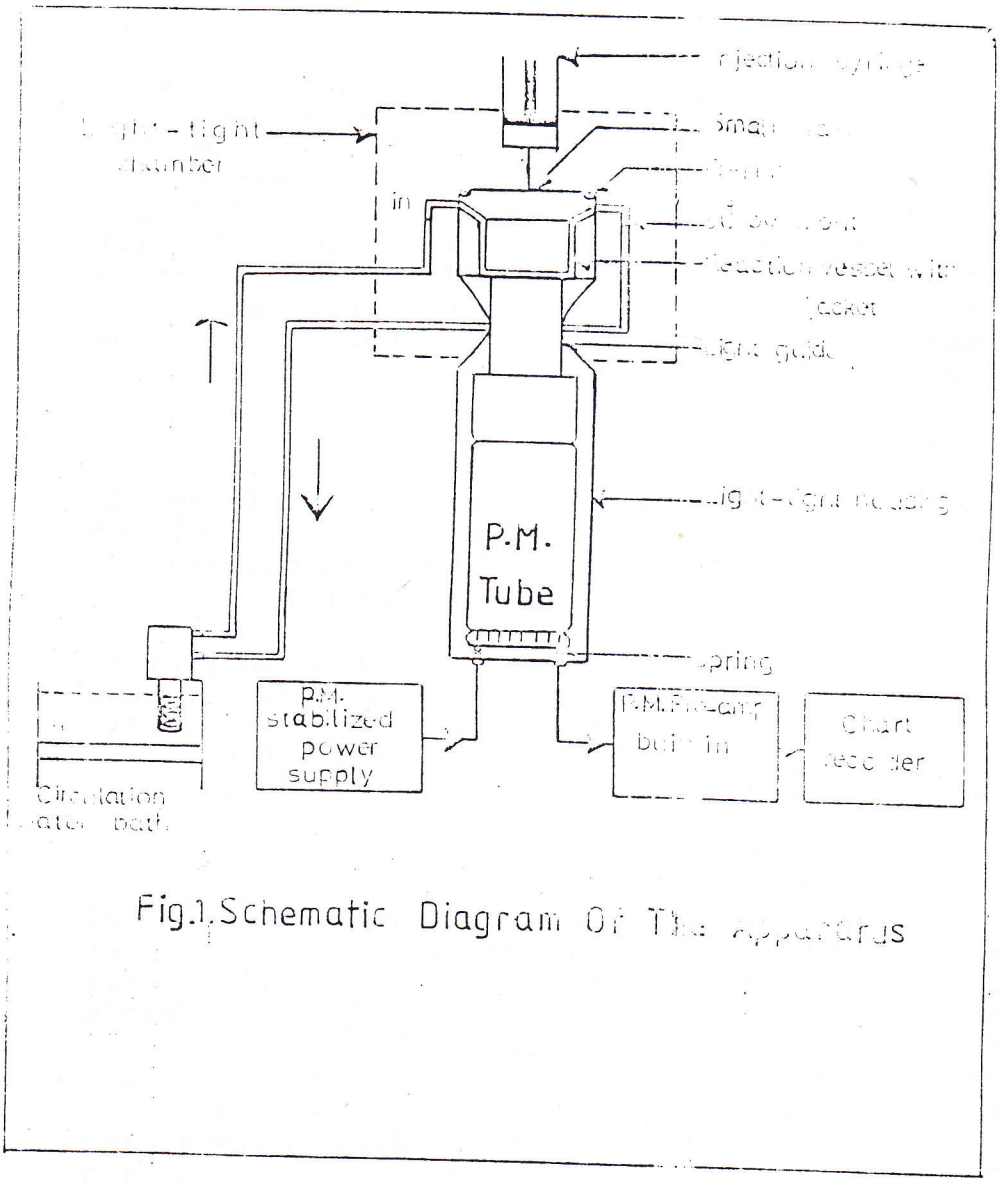


Fig.1 Schematic Diagram Of The apparatus

RESULTS

Kinetics of Whole Blood CL Induced by BaSO4
Figure 2 present a typical curves of luminol-dependent CL of whole blood stimulated by BaSO4 crystals in (A) and the absence of CL burst when only the reaction mixture without blood was poured into the measuring chamber of the machine in (B).
CL kinetics of whole blood during stimulation by $BaSO_4$ from 11 apparently normal adults were presented in Fig 3 sharp increase in CL count was observed generally within 3 to 6 min. from the addition of whole blood to the reaction mixture and a peak count within 4 to 8 min. The variation in CL count observed may be due to the number of phagocytes among the specimens.

Relationship Between Whole Blood CL and the number of Granulocytes: First we tried to determined the relationship between the peak CL of whole blood induced by $BaSO_4$ and the number of granulocytes present in a specimen. The peak CL was significantly related to the number of granulocytes ($P < 0.01$) (Fig 4A). Next Peak CL and the amount of time peak CL is shown were estimated for whole blood in which the number of granulocytes was artificially changed. As shown in Fig 4B there was a linear relationship between peak CL and the number of granulocytes. On other hand the number of granulocytes had no influence on the time showing peak CL.

These results showed that the peak CL of whole blood is determined by the number of granulocytes.

Influence of Red Blood Cells on Whole Blood CL: It is well known that red blood cells and hemoglobin reduce photon counts which are measurable in a liquid scintillation counter (8). In the present study the number of red blood cells in the tested samples was changed to study the influence of red blood cells contamination on

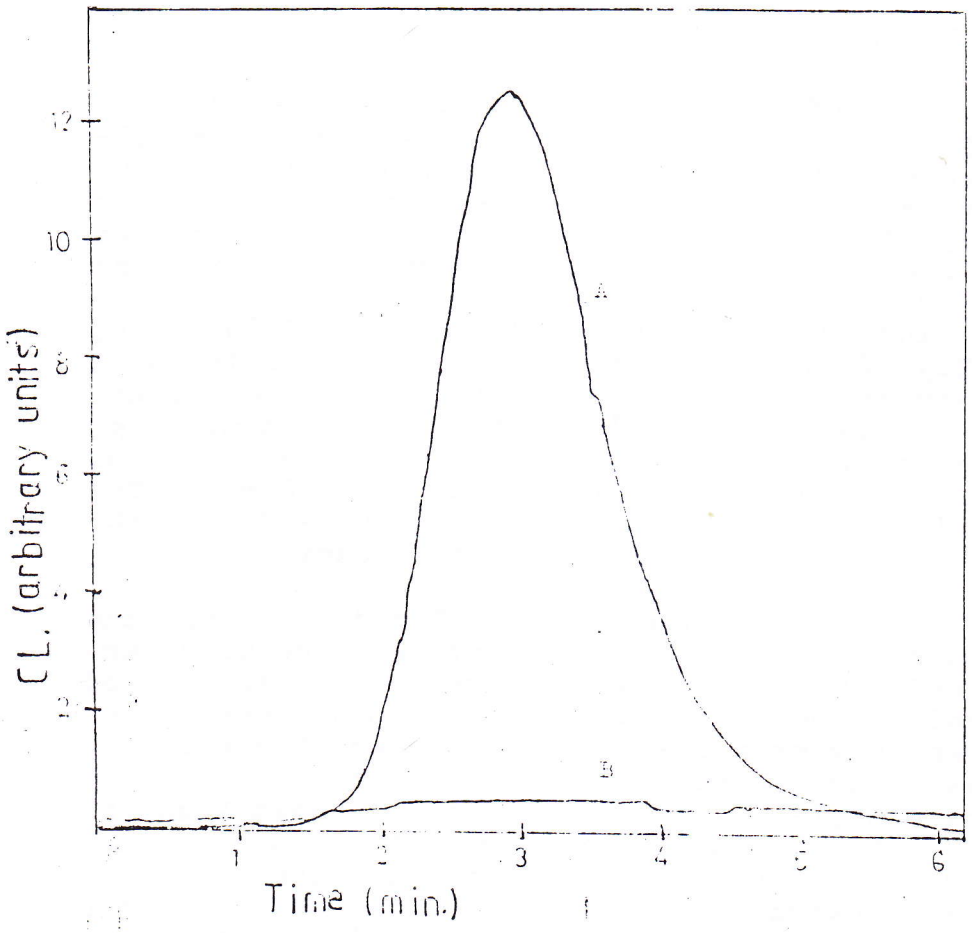


Fig 2: A typical leukocyte chemiluminescence kinetics of whole blood stimulated by $BaSO_4$ crystals.

A: Reaction mixture with 0.02 ml blood.

B: Reaction mixture without blood.

Temperature 37C°; pH 7.4. Abscissa-time in min.; ordinate intensity of CL in relative arbitrary units.

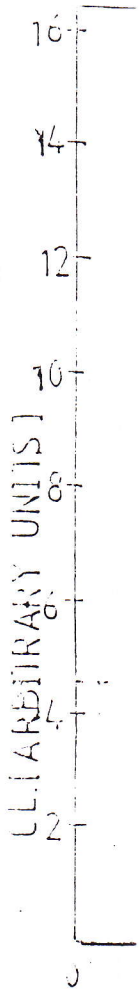


Figure 3
Page 11

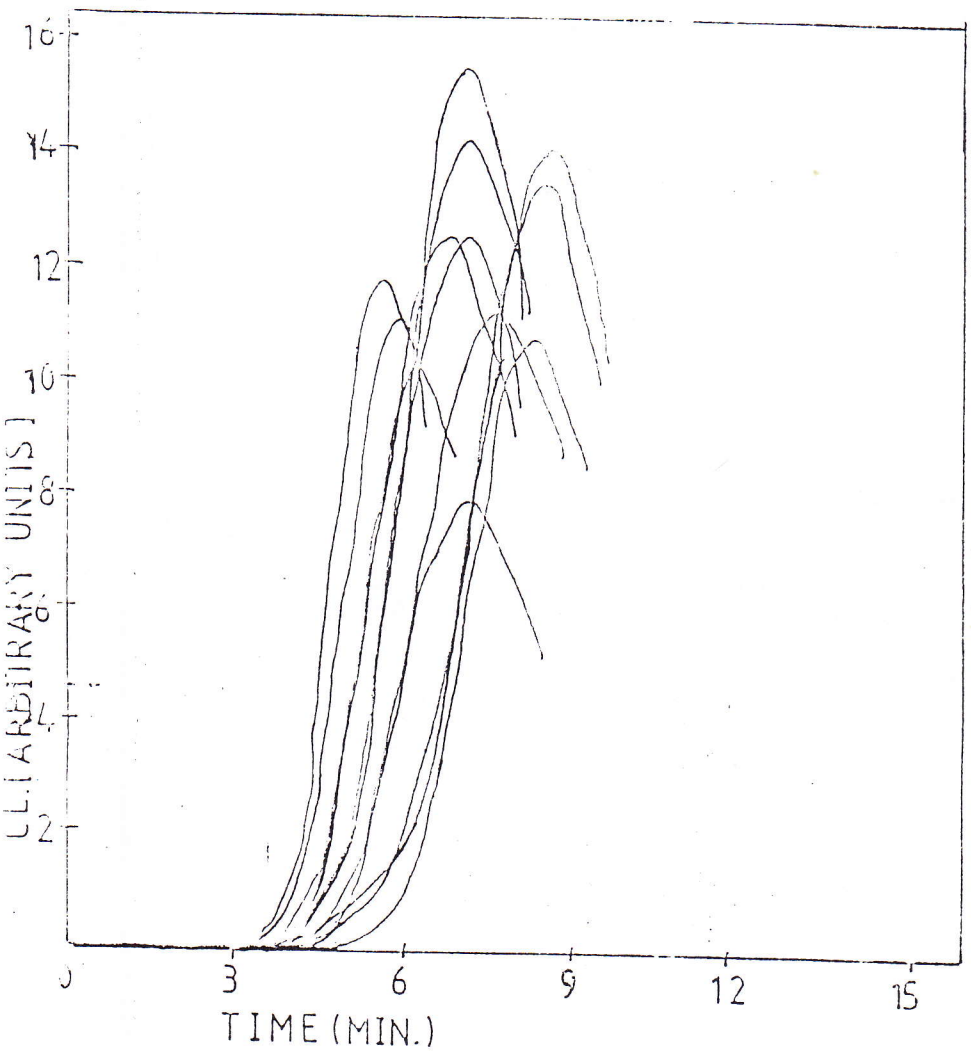


Figure 3: Actual kinetics of luminol-dependent CL of [illegible] from 11 normal adults induced by $BaSO_4$ crystals.

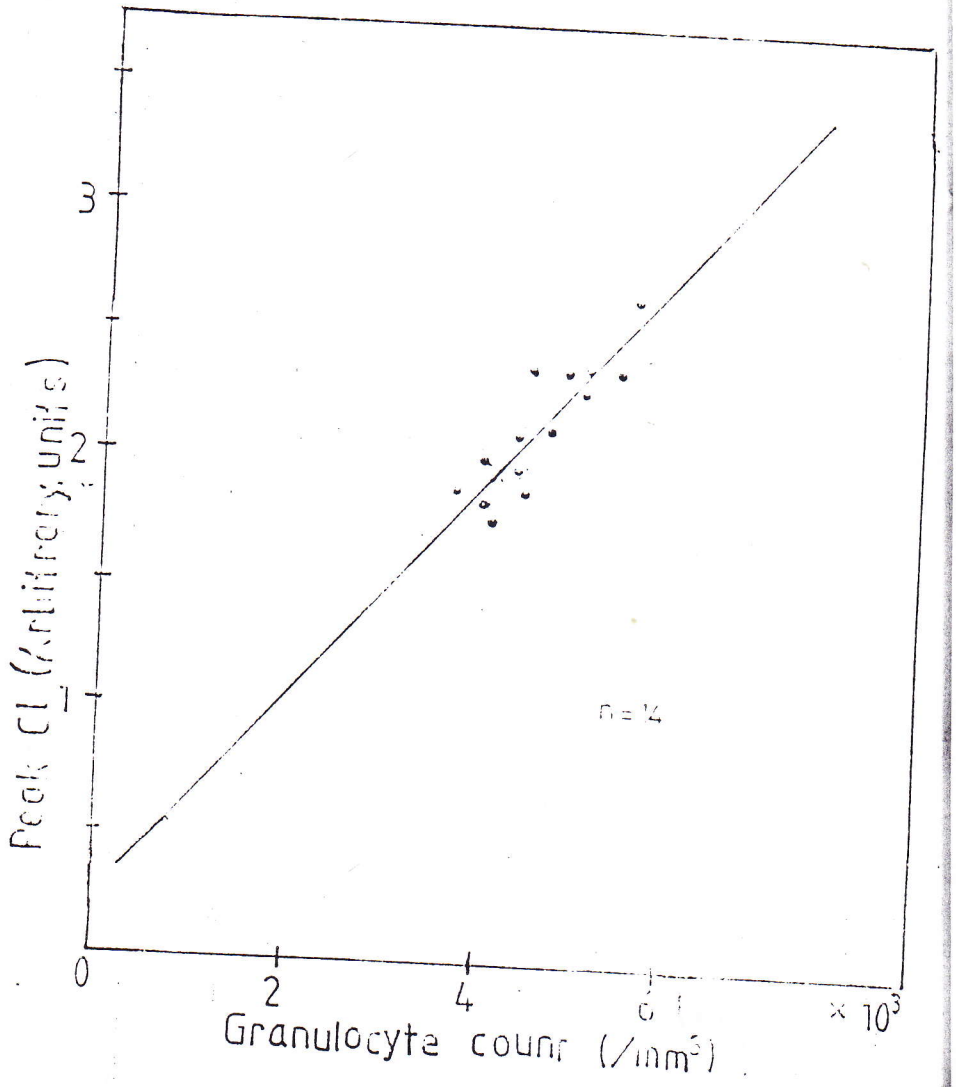
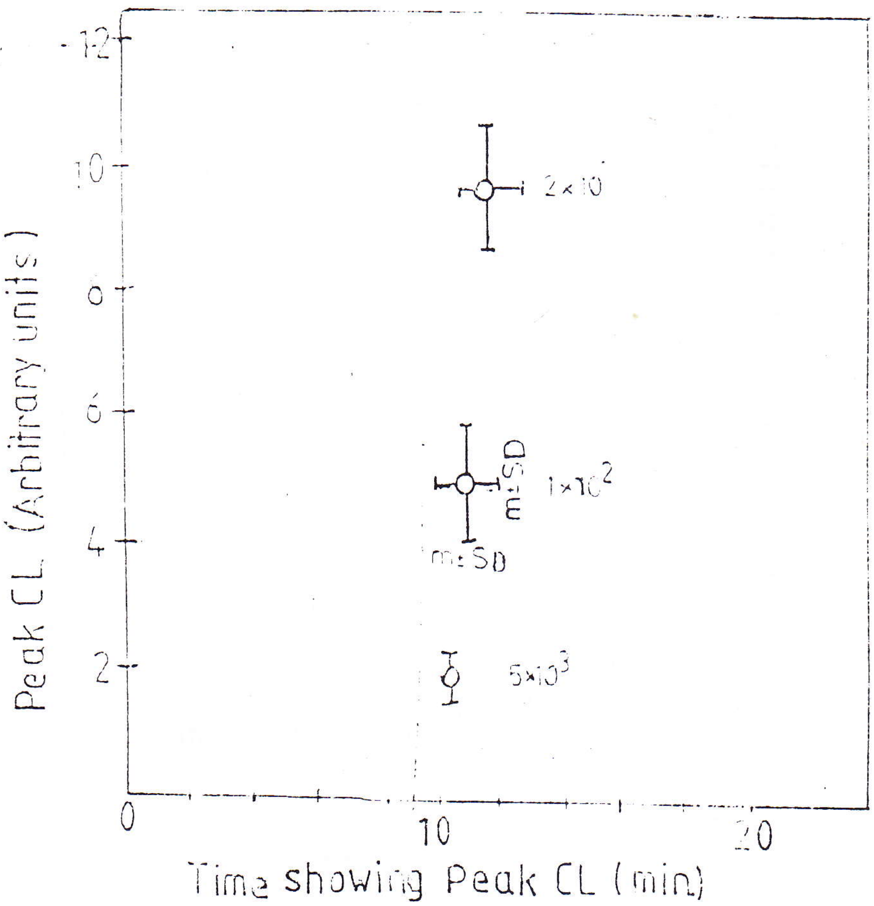


Figure (47): Relationship between whole blood CL and number of granulocytes.

(n): Peak CL was significantly related to the number of granulocytes present in a sample of whole blood (correlation coefficient: 0.95; significant at $p < 0.05$).



[illegible]igure [illegible]-(b) peak CL and the time showing p[illegible] CL o[illegible] who[illegible] [illegible]lood [illegible] [illegible] number [illegible] leukocytes was [illegible]rtificially change[illegible]. P[illegible] CL d[illegible]p[illegible]nds on the [illegible] [illegible] [illegible]eukocytes of the s[illegible]ple[illegible] [illegible] the other hand[illegible] the time showing p[illegible] CL [illegible] [illegible]esults [illegible] expressed as [illegible] [illegible] ± SD for five readings.

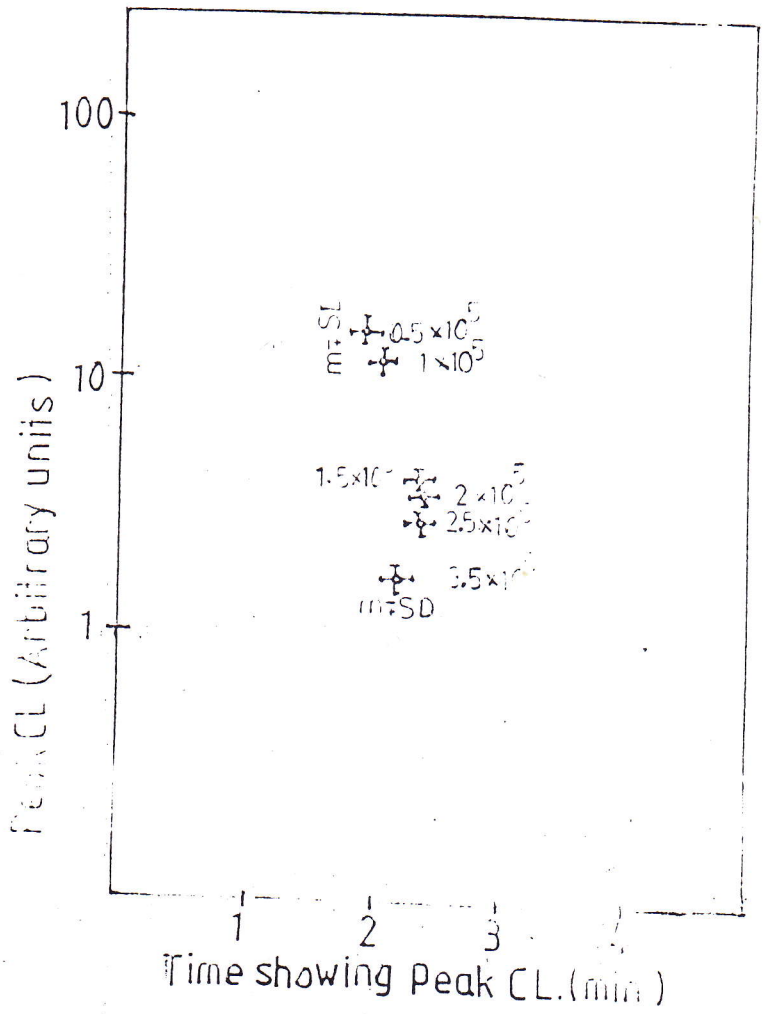


Figure (6): Influence of red blood cells on leucocytes CL induced by [illegible] tats.

(A): Varying number of red blood cells were added to samples of 5×10^5 leucocytes suspended in plasma. Peak CL and the time showing peak CL were measured in five samples of each group.

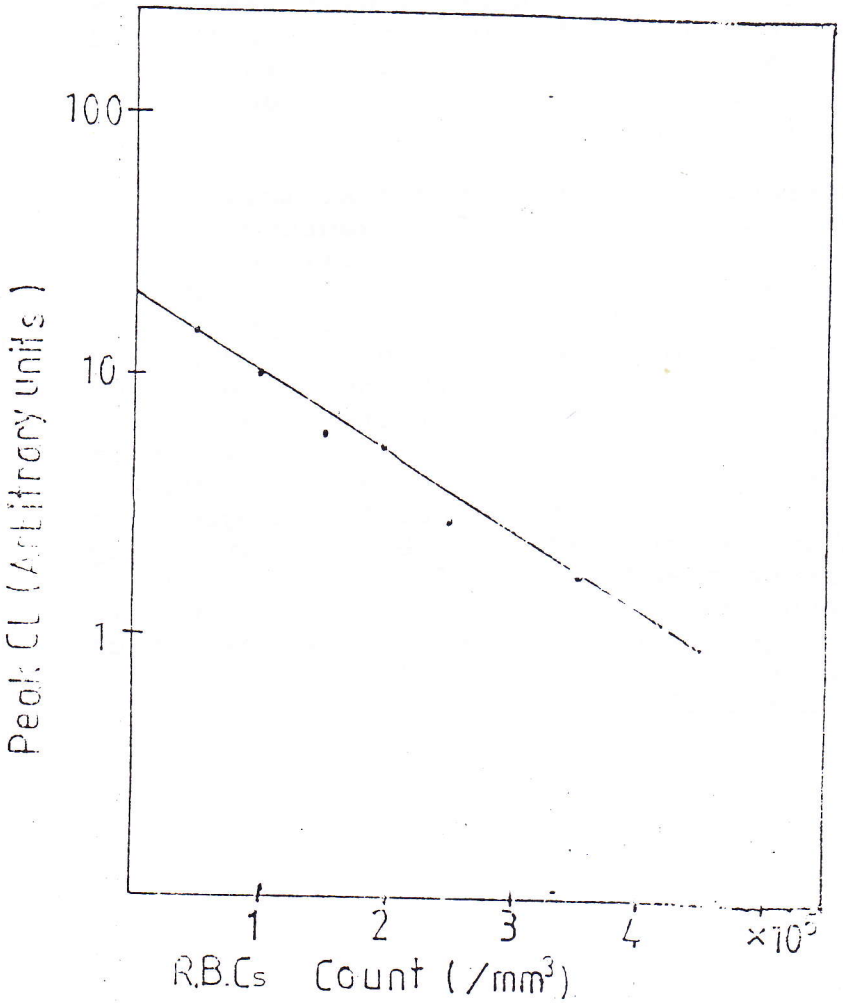


Figure (3a): Red blood cells inhibited CL of leukocytes in a dose dependent manner. The relationship linear on the logarithmic graph.

whole blood CL. Red blood cells apparently reduced the counts of peak CL but had no influence on the time showing peak CL (Fig 5A). The relationship between peak CL and the number of red blood cells was linear on a logarithmic graph (Fig 5B).

DISCUSSION

Chemiluminescence has been widely used to estimate functions of phagocytes (4,7,8,9). However CL does reflect not only the phagocytic function of cells but also the intracellular oxidative metabolic response through which activated oxygen species are generated. Thus CL is thought to be closely linked to bactericidal activities in phagocytic cells (4).

In the present study whole blood stimulated by BaSO4 crystals was used for CL measurement. This method possesses many merits, it utilizes only a small volume of blood 0.08 ml, the speed and ease with which results obtained and phagocytes were activated by the available, simple to prepare BaSO4 suspension. Although whole blood CL may be influenced by many factors we demonstrated that the number of granulocytes in a specimen is important for determining CL multiple linear regression formulae. The regression relationship determined in the present study Table 5 for boys and Table 6 for girls could be reliably applied to Iraqi children belonging to different age, height and weight groups which provide a base line data for lung indices in healthy Iraqi children.

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