KINETIC STUDY ON PHAGOCYTOSIS OF BOVINE LEUKOCYTES MEASURED BY OXYGEN UPTAKE

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1. Introduction

Phagocytosis by leukocytes has been a prime interest from the standpoint of biochemistry. In order to clarify the mechanism of phagocytosis, the method for determining the rate of phagocytosis by leukocytes had to be explored. One of the methods, measurement of oxygen uptake induced by phagocytosis of leukocytes from guinea pig was done using polystylene latex beads or starch granules by Sbarra and Karnovsky [1]. Roberts and Quastel [2] measured the absorbance at 253 nm of polystylene polymer extracted by dioxane after ingestion of polystylene particles into human leukocytes. Stossel et al. [3] measured the rate of phagocytosis by human leukocytes by determining spectrophotometrically the amount of oil red 0 ingested into leukocytes.

In the present paper, the authors isolated leukocytes from bovine blood in a large scale and the initial rate of oxygen uptake of bovine leukocytes in the presence of different kinds of bacterial cells was measured. Affinity of leukocyte for bacterial cell, K_m, and the maximum rate of oxygen uptake for each kind of bacteria, V_{max}, were determined by applying the kinetics of an enzyme-catalized reaction to phagocytosis phenomenon.

2. Material and methods

Isolation of bovine leukocytes was performed as follows. Bovine blood with acid–citrate–dextrose, ACD, (6:1) [4] was centrifuged for 10 min at 3000 *g* and the mixture of leukocytes and platelets (whitish layer), present between plasma and the erythrocyte fraction, was collected and then recentrifuged for 5 min at 1500 *g* in order to remove erythrocytes as a sediment. The whitish layer in each centrifugation tube was collected and centrifuged again at 200–300 *g* for 5 min. By this method, a large number of leukocytes together with erythrocytes was deposited in the bottom of tubes and platelets were suspended in the upper layer (plasma). The upper layer was discarded. By repeating the above centrifugation (200–300 *g*) several times after addition of plasma to the sediment, a large number of platelets was removed. The sediment obtained by last centrifugation consists of two layers with an indistinct boundary; the upper layer (whitish purple) contains mainly leukocytes with erythrocytes and the lower layer (blackish purple) erythrocytes. Whitish purple layer was taken out carefully*, to which three-fold vol of chilled buffered saline [5]** was added and mixed gently. On letting the mixture in a polycarbonate tube in an ice bath stand, leukocytes

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* In order to isolate leukocytes in a high yield, a ratio of number of leukocytes to erythrocytes should be higher than 1 : 100.

** The mixture of 0.9% NaCl and 310 milliosmolar sodium phosphate buffer at pH 7.4 (19 : 1).

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were deposited in 30–60 min as a white paste at the bottom of the tube. The upper part containing erythrocytes was discarded and leukocytes remaining in the tube were washed several times by the same method as that described above. Then the highly purified leukocytes were obtained. The number of leukocytes isolated from one liter of bovine blood was approximately $1.0–2.0 \times 10^9$ cells (recovery 30%). The leukocyte preparation contains hardly erythrocytes and platelets (less than 0.01% in number). The leukocytes obtained were found to be viable by testing with trypan blue and 60% of the cells was polymorphonuclear by Giemsas staining. The rate of oxygen uptake by phagocytizing leukocytes from bovine blood was 5.8×10^{-15} mol O_2/cell·min, which is comparable with 4.9×10^{-15} mol O_2/cell · min for guinea pig polymorphonuclear leukocytes [6]. The initial rate of oxygen uptake by leukocytes was determined in the presence of bacterial cells such as *Micrococcus lysodeikticus, Clostridium histricum* or *Escherichia coli,* which are heat-killed and washed with buffered saline. To the mixture (3.5 ml) of serum and buffered saline (1:2) was added 50 μl of the leukocyte suspension followed by the addition of 50 μl bacterial suspension. The initial rate of oxygen uptake by leukocytes was measured polarographically with a Clark electrode at 37°C.

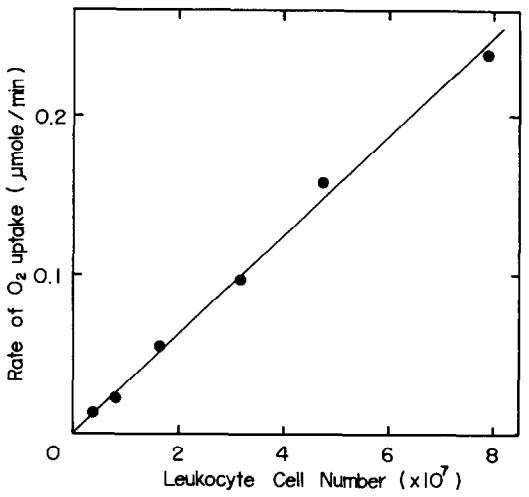

Fig.1. Plotting of the initial rate of oxygen uptake by bovine leukocytes against number of leukocyte cells in the presence of a constant number of *M. lysodeikticus* (1.1×10^9 cells). 37°C.

3. Results and discussion

Fig.1 shows the rate of oxygen uptake by bovine leukocytes measured at various numbers of leukocytes (1.1×10^9 cells). The rate of oxygen uptake is enhanced linearly by increasing the number of leukocyte cells.

The initial rate of oxygen uptake by leukocytes was measured in the presence of *E.coli, M.lysodeikticus* or *C.histricum* cells. The plotting of the rate of oxygen uptake against the number of bacterial cells shows that at lower numbers of bacterial cells the rate of oxygen uptake is proportional to the number of cells and is thus first order with respect to the number of cells. However, as the number of bacterial cells is increased, the rate of oxygen uptake becomes constant and independent of the number of bacterial cells, indicating that the rate of oxygen uptake is zero-order with respect to the number of cells. This phenomenon is the same as the effect of the substrate concentration on the rate of an enzyme-catalyzed reaction, and may be analyzed by Michaelis–Menten Equation. Double reciprocal plot of the initial rate of oxygen uptake, $1/v$, and the cell number, $1/S$, obtained for each kind of bacteria is shown in fig.2. Curves A, B and C are the results obtained for *E.coli, M.lysodeikt-*

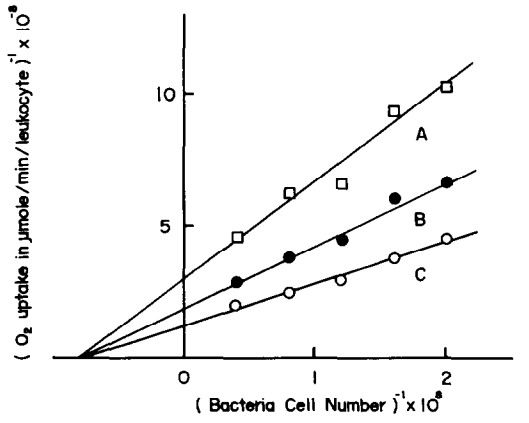

Fig.2. Double reciprocal plot of the initial rate of oxygen uptake against the number of bacterial cells. Curve A; *E. coli,* Curve B; *M. lysodeikticus,* Curve C; *C. histricum.*

Table 1
The values of V_{max} and K_m obtained for phagocytosis by bovine leukocytes in the presence of various bacterial cells or yeast.

	$V_{max} \times 10^9$			$K_m \times 10^{-8}$		
E. coli	2.4	3.5	3.6	3.2	4.2	2.6
M. lysodeikticus	3.0	3.0	5.6	3.2	4.2	2.6
C. histricum	4.8	4.0	8.3	3.2	4.2	2.6
S. cerevisiae			0.8			0.02

icus and *C.histricum* cells, respectively. Each curve shows a straight line with slope of K_m/V_{max} (affinity of leukocyte for bacterial cell/the maximum rate of oxygen uptake) and with intercept of $1/V_{max}$ on the $1/v$ axis. The values of V_{max} and K_m were determined from curves A, B and C, which are summarized in table 1. These V_{max} and K_m values change with a deviation of approximately 40%, which is due to the difference of the individual leukocytes obtained from different origin. So far as leukocytes from the same origin were used, the K_m value does not change in kinds of bacteria, while the V_{max} value does. As seen in table 1, the K_m value is constant ($2.6–4.2 \times 10^{-8}$ cell/ml) for each kind of bacteria. The V_{max} value for *C.histricum* is greater than that of *E.coli* or *M.lysodeikticus,* which may reflect the difference in the surface area of each kind of bacteria (*E.coli* and *M.lysodeikticus*; 2–5 μ^2, *C.histricum*; 5–10 μ^2).

A similar study was carried out using yeast (*Saccharomyces cerevisiae*) and latex beads in place of bacterial cells. The values of K_m and V_{max} obtained for yeast is much smaller than those for bacterial cells. The oxygen uptake by leukocytes in the presence of latex beads does not take place, although leukocyte internalizes latex beads.

Comparison of these K_m and V_{max} values obtained by kinetic studies on phagocytosis may give an important clue to clarify the mechanism of phagocytosis by leukocytes.

References

[1] Sbarra, A. J. and Karnovsky, M. L. (1959) J. Biol. Chem. 234, 1355–1362.
[2] Roberts, J. and Quastel, J. H. (1963) Biochem. J. 85, 150–156.
[3] Stossel, T. P., Mason, R. J., Hartwig, J. and Vaughan, M. (1972) J. Clin. Invest. 51, 615–624.
[4] Aster, R. H. and Jandl, J. H. (1964) J. Clin. Invest. 43, 843–856.
[5] Dodge, J. T., Mitchell, C. and Hanohan, D. J. (1963) Arch. Biochem. Biophys. 100, 119–130.
[6] Stahelin, H., Suter, E. and Karnovsky, M. L. (1956) J. Exp. Med. 104, 121–136.