Vol. 84, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1978 Pages 611-617

KINETICS OF OXYGEN CONSUMPTION BY PHAGOCYTOSING HUMAN NEUTROPHILS

Anthony W. Segal and Stephen B. Coade

Division of Cell Pathology, Clinical Research Centre,
Watford Road, Harrow, Middlesex HA1 3UJ

Received August 14, 1978

Summary: Oxygen consumption by phagocytosing human neutrophils commences after a lag of ~ 25 secs after particle uptake, reaches a maximal rate of ~35 nmols/10^7 cells/min and remains linear for ~ 60 secs. A strict temporal and stoichiometric relationship exists between particle uptake and oxygen consumption. For each particle taken up, 0.2 fmols of oxygen is consumed in a very brief and self limiting process.

INTRODUCTION

Neutrophilic polymorphonuclear leukocytes (neutrophils) provide the primary cellular defence against bacterial infection. There is a marked enhancement of oxygen consumption by neutrophils in association with phagocytosis (1). This oxygen is not utilized for mitochondrial respiration by these cells, which contain relatively few mitochondria and can phagocytose normally under anaerobic conditions (2), but is important for the actual killing of some bacteria (3, 4).

The characteristics of the pattern of this oxygen consumption have not been extensively studied in human cells. We have investigated the characteristics of oxygen consumption by human neutrophils after stimulation with latex particles. It was found that an initial latent period is followed by a burst of oxygen consumption which ceases spontaneously after a surprisingly short time. Comparison of the kinetics of particle uptake and oxygen consumption indicate that, contrary to contemporary thought, oxygen consumption within each individual phagocytic vacuole is a very brief event.

MATERIALS AND METHODS

Separation of neutrophils. Buffy coats from citrated venous blood of healthy controls were used. Erythrocytes were sedimented with 1% Dextran 500 (Pharmacia) and neutrophils were separated from the supernatant leukocyte rich plasma by

0006-291X/78/0843-0611$01.00/0

density centrifugation (5), freed of residual erythrocytes by hypotonic lysis and suspended in RPMI 1640 medium (Flow Labs, Irvine) containing heparin (5 iu/ml Paines and Pyrne, Greenford).

Preparation of particles. Latex particles (Difco 0.81 μ diam) were opsonised with human IgG (Lister Institute, Elstree) or pooled human serum by incubating 1.0 ml of the particles with 0.2 ml of IgG (30 mg), or serum, and 1.0 ml of 100 mmol/l Tricine buffer pH 8.5, at 37°C for 30 mins. The particles were then centrifuged at 8000 g for 2 mins in an Eppendorf 3200 centrifuge, washed and then finally resuspended in Hanks balanced salt solution at a concentration of 1×10^{10} particles/ml. The concentration of IgG on the surface of the latex particles coated with IgG was measured by incubating them for 10 hours at 4°C with iodinated rabbit anti-human γ-chain antibodies (6), and then washing them 3 times in (0.15 mol/l) NaCl containing (10 mmol/l) sodium phosphate and 1 mg/ml bovine serum albumin (Sigma). These methods measured an IgG concentration of $\sim 3 \times 10^7$ molecules/particles.

Oxygen consumption. Oxygen consumption was measured in a closed thermostatically controlled (37°C unless stated otherwise) plastic chamber attached to a Clark type oxygen electrode (Rank) calibrated by the addition of dithionite taking the normal oxygen tension in water as 230 nmols/ml at room temperature (7). An aliquot of the cell suspension (generally 2×10^7 cells in 1.0 ml) was added to the chamber and incubated until a steady resting respiratory rate was achieved. An aliquot of the latex suspension (generally 1×10^9 particles in 0.1 ml) was then added through a vent in the chamber stopper.

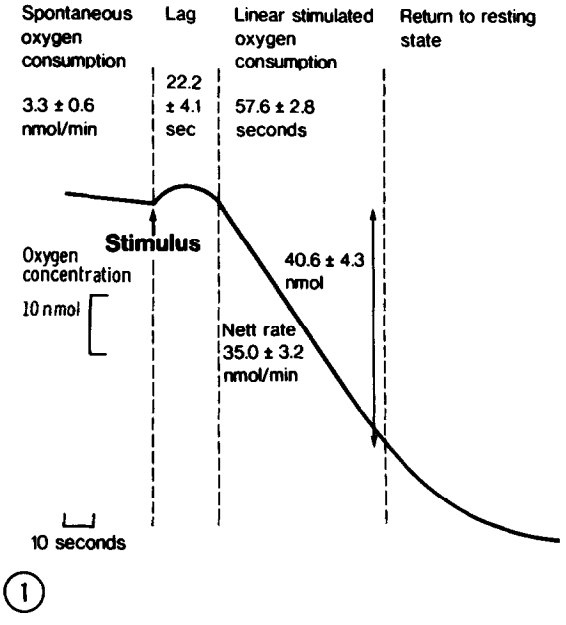
Measurement of phagocytosis. Phagocytosis was arrested by the addition to the chamber of 4 ml of ice-cold Hanks balanced salt solution containing 1.0 mmol/l tetrasodium-EDTA adjusted to pH 7.4 with NaOH. The cell suspensions were layered onto 2.0 ml of ficoll/sodium metrizoate (density 1.077, Ficoll-Paque, Pharmacia) diluted to 80% of its original concentration with 0.15 mol/l NaCl and centrifuged at 400 g x 20 mins at 4°C in an MSE 6 L rotor. The latex particles that passed through the gradient attached to cells were counted microscopically and the mean number in 100 cells was determined. Latex uptake was also quantitated spectrophotometrically after extraction into dioxane (8).

RESULTS

Characteristics of latex induced oxygen consumption

The characteristics of the burst of oxygen consumption by neutrophils after the addition of latex particles opsonised with IgG are summarized in Figure 1. Under resting conditions oxygen is consumed at a rate of approximately 3 nmols/10^7 cells/min. Upon the addition of latex there is an initial lag of approximately 25 secs, followed by a phase of linear oxygen consumption at a rate of approximately 35 nmols/10^7 cells/min which lasts approximately 60 secs, before a final phase decline.

The effect of varying the temperature on the phase of linear consumption was studied. It was found that as the temperature is elevated from 20 to 50°C the rate of O_2 uptake increases, but that the duration for which it remains linear



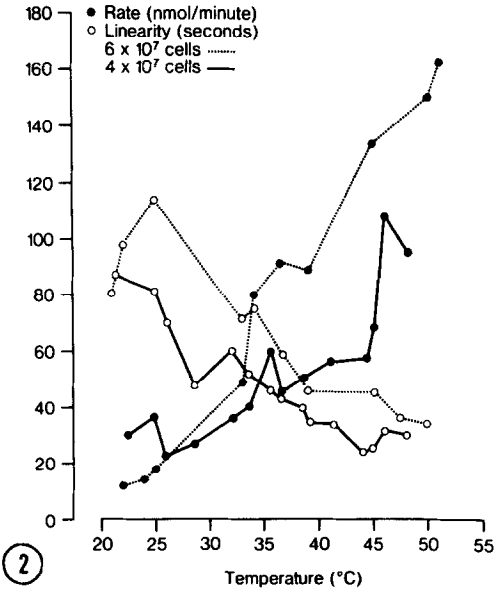


Figure 1 Polarographic measurement of oxygen consumption by human neutrophils (1×10^7) after addition of IgG coated latex particles (5×10^8). Schematic representation of averaged data (mean ± SE) from 30 studies.

Figure 2 Effect of temperature on linear oxygen consumption by phagocytosing neutrophils (10 particles/cell).

falls, resulting in a fairly constant product (Figure 2). The cessation of oxygen consumption does not appear to be due to exhaustion of the substrate of the oxidase system. If a submaximal stimulus is administered to the same cell suspension prior to a maximal stimulus, oxygen consumption follows a similar time course, but with a lower amplitude, and recommences after the addition of the second stimulus, the sum of the rates roughly equalling that of a single maximal stimulus (Figure 3).

Relationship between oxygen consumption and latex uptake

There is a linear relationship between the rate of oxygen consumption and the logarithm of the number of particles added to the cell suspension (Figure 4). The variation in oxygen consumption is due to a change in the rate of linear consumption rather than its duration, and there is little variation in the initial lag phase (data not shown). This somewhat surprising logarithmic

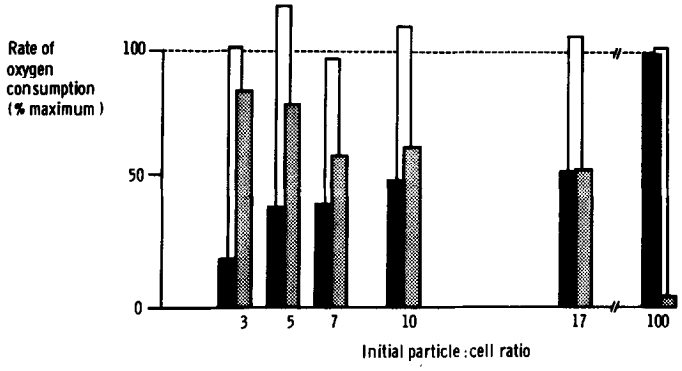


Figure 3 Rate of oxygen consumption after addition of a maximal stimulus of latex ▨ to human neutrophils previously stimulated with a submaximal number of latex particles ■ . The sum of these rates is also shown □ .

relationship is due to a similar relationship between the number of particles administered and the number taken up by the cells (Figure 4). The kinetics of particle uptake are similar to those of oxygen consumption once this has commenced after the initial lag phase (Figure 5). This relationship was observed both with particles opsonised with IgG and with serum. The initial rate of particle uptake and oxygen consumption is slower and more prolonged with the serum coated particles, and with both opsonins the stoichiometry of the oxygen consumption is approximately 0.2 fmols/particle taken up. Similar results were obtained when particle uptake was quantitated spectrophotometrically (8, not shown).

DISCUSSION

These data indicate that latex particles opsonised with human IgG are rapidly phagocytosed by human neutrophils, that oxygen consumption does not commence for a further 25 seconds, and that when the oxygen consumption does occur, the time course and extent of the response closely parallels that of particle uptake (Figure 1).

The reason for the latent period between particle uptake and oxygen consumption has not been established. It seems likely that it is either

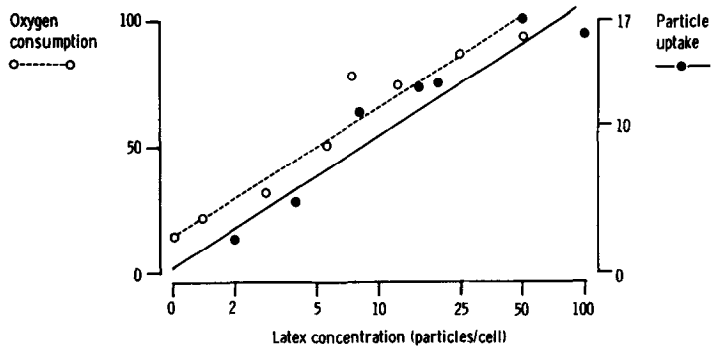


Figure 4 Effect of varying the number of latex particles on the rate of particle uptake (particles/cell 1 min after addition of particles) and rate of oxygen consumption (% of maximum rate at 1 min after addition of an excess of particles.

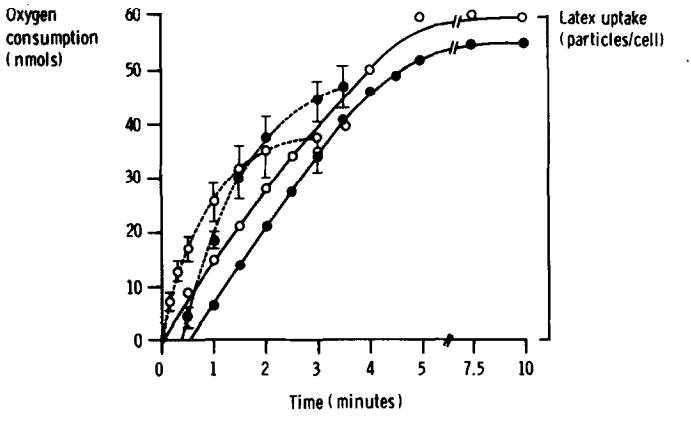


Figure 5 Relationship between particle uptake ● and oxygen consumption O after addition of latex particles opsonised with serum (———) or with IgG (— — —) mean ± SE, n = 3).

related to closure of, or degranulation into, the vacuole. Initial processes in the activation of the oxidase system, which seems to include a newly described cytochrome b (9), can be measured by superoxide dependent reduction of cytochrome c (10). This cytochrome c reduction occurs within the first few seconds of the addition of particles and is almost complete by the time that oxygen consumption commences (Segal and Meshulam, unpublished data).

The most interesting observation is that oxygen consumption starts off in a linear fashion and then begins to decrease after less than 1 minute. The

cessation of oxygen consumption is not due to exhaustion of the oxygen within the incubation chamber, or of substrate of the oxidase system within the cell. A similar time course is observed if oxygen consumption is varied by adding different numbers of particles to the cells, and oxygen uptake recommences if further particles are added to the cells after an initial submaximal stimulus (Figure 3). Once it commences the kinetics of oxygen consumption closely parallel those of particle uptake, and there is a similar relationship between the number of particles to which the cells are exposed and both particle uptake and oxygen consumption (Figure 5). This indicates that the duration of oxygen consumption within each individual phagocytic vacuole must be very brief, in the order of seconds or fractions thereof, rather than minutes (2) otherwise the kinetics of oxygen consumption would be different, showing an additive effect as each new phagocytic vacuole contributes to oxygen consumption by the system as a whole.

Few studies (2, 11, 12) have directly measured the consumption of oxygen by neutrophils, most conventional methods of studying neutrophil metabolism use indirect techniques such as superoxide dismutase inhibitable reduction of cytochrome c (10), H_2O_2 production in the presence of cyanide or azide (13) and activity of the hexose monophosphate shunt (14) - all of which are unsuitable for studying the kinetics of the burst of oxygen metabolism to which they cannot be directly related. Phagocytosis induced oxygen consumption has been shown to continue in a linear fashion for between 15 (11, 12) and 45 (2) mins. However oxygen uptake was not correlated with particle uptake in those studies in which prolongation of the process was probably due to a low rate of phagocytosis (11).

The identification that oxygen consumption by each vacuole is very brief is important because it indicates the type of system that might be involved and the methodology necessary to study it. Attempts to recreate the oxidase system from cell components *in vitro* are unlikely to be successful unless the transitory nature of the oxidase process is recognised. The system requires activation and

after a brief burst of oxygen consumption it becomes refractory despite conditions within the cell which permit oxygen consumption by other phagocytic vacuoles. This suggests activation followed by inactivation of an enzyme system, or a change in the local physico-chemical environment such as the creation or abolition of a membrane potential across the wall of the phagosome. These processes could well be related to electron transport by the cytochrome b system which has recently been described in neutrophil phagosomes (9).

Acknowledgments: Dr. A.C. Allison for assistance and Dr. T. Platts-Mills for the gift of ^{125}I labelled rabbit anti-human γ-chain antibodies.

REFERENCES

1. Baldrigde, C.W., and Gerard, R.W. (1933) Am. J. Physiol. 103, 235-236.
2. Sbarra, A.J., and Karnovsky, M.L. (1959) J. Biol. Chem. 234, 1355-1362.
3. McRipley, R.J., and Sbarra, A.J. (1967) J. Bacteriol. 94, 1417-1424.
4. Mandell, G.L. (1974) Infect. Immun. 9, 337-341.
5. Böyum, A. (1968) Scand. J. Clin. Lab. Invest. 21, 31-50.
6. Platts-Mills, T.A.E., and Ishizaka, K. (1975) J. Immunol. 114, 1605-1610.
7. Umbreit, W.W., Burris, R.H., and Stauffer, J.M. (1964) Manometric Techniques 4th Ed. pp. 46-59, Burgess Publishing Co., Minneapolis.
8. Baehner, R.L., Murrmann, S.K., Davis, J., and Johnston, R.B. (1975) J. Clin. Invest. 56, 571-576.
9. Segal, A.W., Jones, O.T.G., Webster, D., and Allison, A.C. (1978) Lancet in press.
10. Babior, B.M., Kipnes, R.S., and Curnutte, J.T. (1973) J. Clin. Invest. 52, 741-744.
11. Rossi, F., and Zatti, M. (1964) Brit. J. Exp. Path. 45, 548-559.
12. Reiss, M., and Roos, D. (1978) J. Clin. Invest. 61, 480-488.
13. Homan-Müller, J.W.T., Weening, R.S., and Roos, D. (1975) J. Lab. Clin. Med. 85, 198-207.
14. Zatti, M., and Rossi, F. (1965) Biochim. Biophys. Acta 99, 557-561.