Reactive forms of oxygen and chemiluminescence in phagocytizing rabbit alveolar macrophages

P. R. MILES, V. CASTRANOVA, AND PING LEE
Appalachian Laboratory for Occupational Respiratory Diseases, National Institute for Occupational Safety and Health, U. S. Public Health Service, Department of Health, Education, and Welfare, Morgantown, West Virginia 26505; and Department of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, West Virginia 26505

MILES, P. R., V. CASTRANOVA, AND PING LEE. *Reactive forms of oxygen and chemiluminescence in phagocytizing rabbit alveolar macrophages*. Am. J. Physiol. 235(3): C103–C108, 1978 or Am. J. Physiol.: Cell Physiol. 4(2): C103–C108, 1978.—Chemiluminescence (CL), superoxide anion (O_2^-) production, and particle uptake were measured to determine the role of antibacterial substances in the chemiluminescent response associated with phagocytosis in rabbit alveolar macrophages (AM). Exposure of AM to zymosan particles induced both CL and the production of extracellular O_2^-. CL is inhibited by superoxide dismutase, an enzyme which catalyzes the conversion of O_2^- to hydrogen peroxide (H_2O_2), by catalase, an enzyme which destroys H_2O_2, and by the hydroxyl radical (·OH) scavengers, benzoate and ethanol. Superoxide dismutase and catalase probably exert their effects in the extracellular fluid. CL can also be produced by the addition of NaO_2 or H_2O_2 to zymosan in a noncellular system. The chemiluminescent response occurs before particle uptake is complete, which also indicates that CL occurs in the extracellular fluid. These results suggest that CL induced by zymosan in AM is due to the extracellular reaction between various reactive forms of oxygen and zymosan.

antibacterial substances; superoxide anion

WHEN ALVEOLAR MACROPHAGES from rabbits or dogs are exposed to foreign particles such as zymosan or polystyrene beads, chemiluminescence (CL) occurs (17, 19). This phenomenon was first reported to occur during phagocytosis of both foreign particles and bacteria in polymorphonuclear leukocytes from human blood (2, 3). During the past few years much work has been done in an attempt to determine the origin of the chemiluminescent response in white blood cells (3, 6, 24). Most of the results indicate that CL is related to the production of reactive forms of oxygen by these cells, e.g., superoxide anion, hydrogen peroxide, singlet oxygen, and hydroxyl radical (3, 6, 24). However, the mechanism of the chemiluminescent response associated with phagocytosis in alveolar macrophages is unknown.

The exposure of alveolar macrophages to foreign particles leads to the initiation of many events which may be related to CL. For example, during phagocytosis in these cells, there is an increase in respiratory rate and glucose metabolism, primarily in hexose monophosphate shunt activity and oxidative phosphorylation (9, 12, 16). Also, substances which are known to kill bacteria are produced by the cells. Phagocytosis in alveolar macrophages from most animals leads to the production of both superoxide anion (9) and hydrogen peroxide (13, 16). Therefore, it is possible that the production of antibacterial substances is related to the chemiluminescent response in lung macrophages.

This study was undertaken to investigate the origin of the chemiluminescent response that occurs when rabbit alveolar macrophages are exposed to foreign particles. The specific objective is to determine the role of the various reactive forms of oxygen, i.e., superoxide anion, hydrogen peroxide, and hydroxyl radical, in chemiluminescence. The experiments were performed by exposing rabbit alveolar macrophages to zymosan particles and measuring the subsequent chemiluminescence, production of superoxide anion, and uptake of the zymosan particles by the cells.

METHODS

Alveolar macrophages were harvested from male New Zealand white rabbits (1–3 kg body wt) by tracheal lavage according to the method of Myrvik et al. (20). The solution used in the lavage procedure was Hank's Balanced Salt Solution (HBSS; Grand Island Biological Co., Grand Island, N.Y.), which contained 5 mM glucose and 5 U/ml heparin and was maintained at pH 6.5. The cells were separated from the lavage fluid by centrifugation at 120 × *g* for 5 min. Then the cells were resuspended in 3–4 ml HBSS, and the number of cells per milliliter of this suspension was determined by using a hemocytometer. The average yield of macrophages per rabbit (± SE) was 8.7 (± 1.6) × 10^7. The HBSS used to resuspend the cells and in all remaining experimental procedures contained 5 mM glucose and was maintained at pH 6.5, because this is the optimal pH for phagocytosis in these cells (21). The desired number of cells per milliliter in the cell suspension was then obtained by addition of the appropriate amount of HBSS. Histological preparations were made in order to determine the types of cells obtained from the lung lavage, i.e., in particular, the number of polymorphonuclear leukocytes (PMN) present. In all cases there was less than 0.1% PMN in the lavage fluid. So in these experiments we were dealing with a very pure popula-

tion of alveolar macrophages. In addition, the viability of the cells was measured for each experimental condition by trypan blue exclusion. Cell viability was not affected by any treatment used in this study.

The foreign particle used in all experiments reported here is zymosan, an extract from the cell walls of *Saccharomyces cerevisiae* yeast (Sigma Chemical Co., St. Louis, Mo.). The zymosan was prepared by boiling 20–50 mg in 2–3 ml HBSS for 15 min. Then the zymosan was separated from the solution by centrifugation and resuspended in fresh HBSS so that the final concentration was 4–20 mg/ml. This procedure seemed to prevent the zymosan particles from clumping during the course of the experiment. In all of the experiments reported here we used 2 mg zymosan per 1×10^7 alveolar macrophages.

For measurements of chemiluminescence 1×10^7 alveolar macrophages were incubated at 37°C in 4.5 ml HBSS in dark-adapted plastic vials for 15 min. Then at zero time, 2 mg zymosan (in 0.5 ml HBSS) was added to each vial, and CL was measured immediately. The CL was measured as counts per minute in the tritium channel of a liquid scintillation counter (model LS-345; Beckman Instrument Co., Fullerton, Calif.) operated in the out-of-coincidence mode. The zero time measurement, which was always between 15,000 and 19,000 cpm, represents the background level. After the zero time measurement, the vials were returned to the incubator where they were maintained at 37°C. Measurements of CL were made at various later times and expressed as counts per minute minus the background level. The relationship between CL and time was presented in an earlier publication (19). Chemiluminescence begins after the addition of zymosan to the cells, reaches a peak in 15–20 min, and gradually disappears within 3 h. In all experiments presented in this paper, measurements were made only for 1 h, because this is the time during which maximal CL occurs.

Superoxide anion production was measured by very slight modification of the method used by Babior et al. (4). This method is based on the fact that superoxide anion causes reduction of cytochrome *c* which can be measured spectrophotometrically at 550 nm. In these experiments 4×10^7 alveolar macrophages were incubated at 37°C for 15 min in HBSS with 0.12 mM cytochrome *c* (type VI; Sigma). Then at zero time, zymosan (8 mg) was added to the cells (final volume, 15.5 ml). At various later times 2-ml samples were taken, and the cells were immediately separated from the supernatant by centrifugation at $500 \times g$ for 30 s in a refrigerated centrifuge (model RC2-B; Sorvall Instrument Co., Norwalk, Conn.). Optical densities of the supernatants were determined at 550 nm with a Gilford spectrophotometer (model 300-N; Gilford Instrument Laboratories, Oberlin, Ohio). To be certain that reduction of the cytochrome *c* was due to superoxide, experiments were done both in the presence and in the absence of superoxide dismutase (SOD; Sigma), an enzyme which catalyzes the conversion of superoxide to hydrogen peroxide and oxygen (10, 18). There were never any changes in the optical densities of the supernatants when the cells were exposed to SOD (0.4 mg/ml). In addition, resting superoxide anion production was determined by using cells that were not exposed to zymosan, and this was subtracted from the zymosan-induced levels. Therefore, superoxide anion production was expressed as zymosan-induced superoxide-dependent cytochrome *c* reduction in OD (optical density) units per 1×10^7 alveolar macrophages.

The time course of the uptake of zymosan particles by alveolar macrophages was determined by using histological preparations. The cells (4×10^7) were incubated in HBSS (15 ml) at 37°C for 15 min before the addition of zymosan (8 mg). Aliquots of this suspension were removed at various times after the addition of zymosan and spun in a centrifuge in order to obtain a cell pellet. Smears of the cells were made from the pellet on microscope slides. The smears were dried, stained with hematoxylin and eosin, and examined under a microscope. At least 100 cells from each slide were examined, and the number of zymosan particles inside the cells was counted. The results were expressed as number of zymosan particles per alveolar macrophage.

RESULTS

It has been shown previously that phagocytosis in alveolar macrophages leads to the production of superoxide anion by the cells (12). Once superoxide is produced, a number of reactions can occur to cause the formation of hydrogen peroxide, singlet oxygen, and hydroxyl radical (10, 14, 15). The reaction scheme for all of these antibacterial substances, as well as the enzymes and other modifiers which affect these reactions, is shown in Fig. 1. This scheme is the framework upon which the experiments of this study are based.

Role of superoxide anion in chemiluminescence. To determine the role of superoxide anion in CL, superoxide dismutase (SOD), an enzyme which catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen (10, 18), was used. With SOD in the incubation medium there should be no superoxide anion present in the system. The effect of SOD on the chemiluminescent response is shown in Fig. 2. In the presence of 0.4 mg/ml SOD, a concentration which causes a maximal effect, CL is virtually abolished. Lower concentrations of SOD cause lesser amounts of inhibition. Since 0.4 mg/ml SOD is a large amount of enzyme, the effects of heat-inactivated SOD or 0.4 mg/ml bovine serum albumin (BSA) on CL were measured in order to check for nonspecific protein effects. However, neither the inactivated SOD nor the BSA had an effect on CL. This result indicates that superoxide plays an extremely

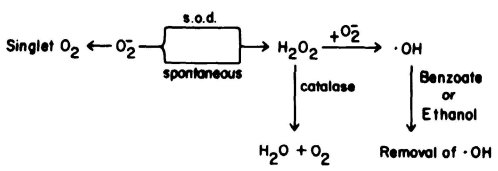

FIG. 1. Reaction scheme for production of other antibacterial substances from superoxide anion (8, 11, 12). Actions of superoxide dismutase (SOD), catalase, benzoate, and ethanol are shown at appropriate locations in the scheme.

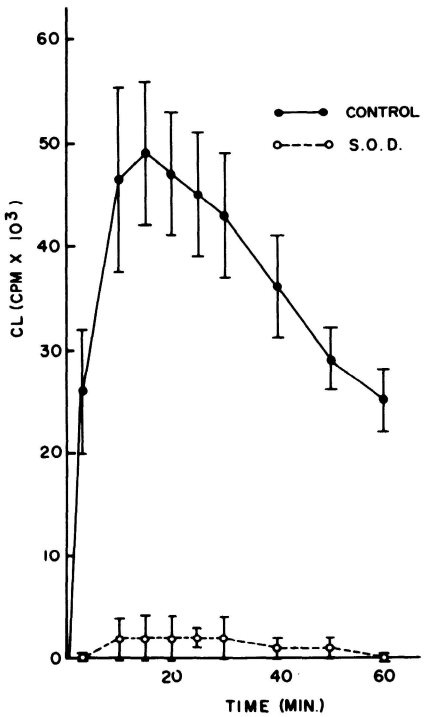

FIG. 2. Effect of superoxide dismutase (SOD) on CL. These measurements were made as paired experiments; i.e., one control and one experimental vial were used for cells from each animal. In the SOD experiments, enzyme (0.4 mg/ml) was added to incubation medium 10 min prior to addition of zymosan. Points are mean values from 6 experiments and bars are SE.

important role in CL; i.e., when no superoxide anion is present, there is very little CL. Furthermore, superoxide dismutase is a large molecule and may not be able to penetrate the membrane. Therefore, it is possible that SOD has its effect outside the cells and that CL is dependent upon extracellular superoxide anion.

Since it appeared that extracellular superoxide could be important in the chemiluminescent response, the superoxide anion in the extracellular fluid produced in response to zymosan was measured. The alveolar macrophages were exposed to zymosan at zero time and superoxide production was measured at various later times. In addition, resting levels of superoxide production were determined and subtracted from the zymosan-induced levels. Zymosan-induced superoxide levels were plotted against time, and the results are shown in Fig. 3. The amount of superoxide anion in the medium increased for 15–20 min and then reached a maximum. This time course is very similar to that for chemiluminescence; i.e., CL also reaches a peak 15–20 min after the addition of zymosan. These results then also suggest that CL is related to the release of superoxide anion into the extracellular fluid.

Because CL seems to be related to the appearance of superoxide anion in the extracellular fluid, the question arises as to how light is produced extracellularly. One possibility is that CL is produced by the reaction of superoxide anion with zymosan. If this is the case, CL should be obtained by the addition of solid NaO_2 (Alfa Products; Beverly, Mass.) to zymosan. The results from such experiments are shown in Table 1. The addition of solid NaO_2 to zymosan produces a high level of light emission, indicating that one possible mechanism for CL is the reaction between superoxide anion and zymosan. The results of all experiments thus far indicate that CL is caused by the reaction between superoxide anion—or some product of the dismutation of superoxide—and zymosan and that this reaction occurs extracellularly. The following experiments deal with the possible role of other antibacterial substances that may be produced from superoxide anion.

Role of other reactive forms of oxygen in chemiluminescence. Once superoxide anion is formed, a number of reactions can occur to cause the formation of hydrogen peroxide, singlet oxygen, and hydroxyl radical (see Fig. 1). To study the role of hydrogen peroxide in the

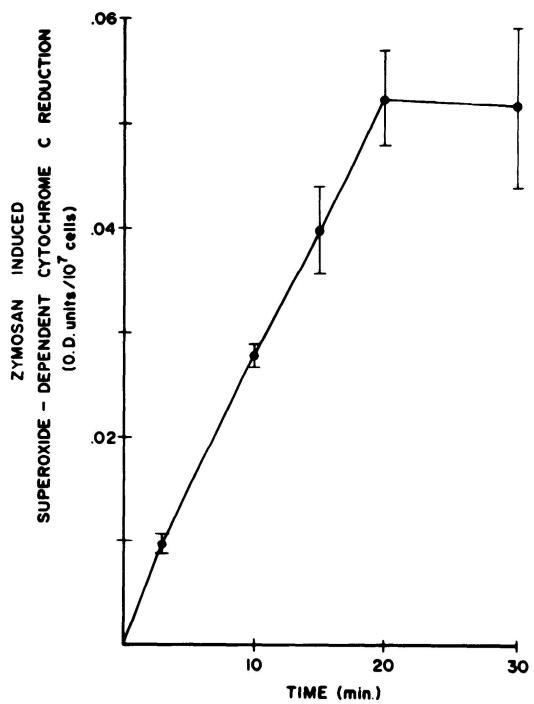

FIG. 3. Relationship between superoxide anion production in response to zymosan and time. Superoxide anion was measured as difference in reduction of cytochrome *c* in the presence of cells with zymosan and in the presence of cells alone. To be certain that reduction of cytochrome *c* was due to superoxide, experiments were done both in the presence and absence of SOD (0.4 mg/ml). Reduction of cytochrome *c* (final concentration, 0.12 mM) was measured as a change in optical density at 550 nm. Points represent mean values for 6 experiments and bars are SE.

TABLE 1. *Chemiluminescence induced by NaO_2 and H_2O_2*

Treatment	CL, cpm × 10^3	*n*
NaO_2 alone	92 ± 13	6
NaO_2 + zymosan	748 ± 70	6
H_2O_2 alone	13 ± 4	6
H_2O_2 + zymosan	179 ± 17	6

NaO_2 (final concentration, 1 mM) was added as a solid and H_2O_2 (1 ml of a 30% solution) as a liquid to the medium containing zymosan at zero time. CL values shown are maximal values which occur immediately after the addition of NaO_2 or H_2O_2. Final volume of the medium was 5 ml and amount of zymosan used was 2 mg. Numbers shown are means ± SE from *n* experiments. In each case the appropriate background level of CL (i.e., no H_2O_2 or NaO_2 present) was determined and subtracted from the other responses.

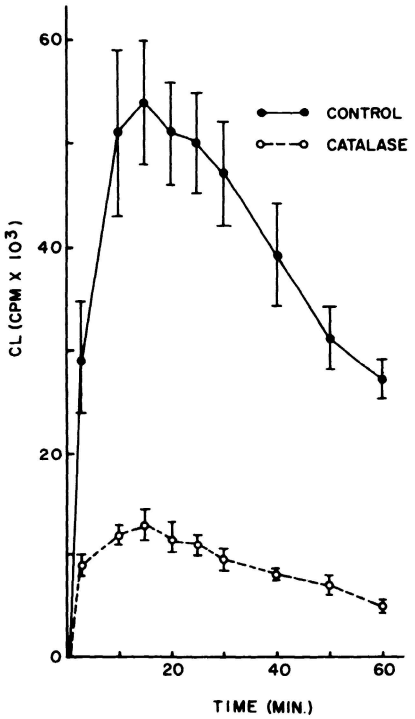

FIG. 4. Effect of catalase on CL. Catalase (4 mg/ml) was added to incubation medium 10 min prior to addition of zymosan. These measurements were made as paired experiments. Points represent mean values from 6 experiments and bars are SE.

chemiluminescent response, catalase, an enzyme which destroys H_2O_2, was added to the incubation medium. The results from these experiments are shown in Fig. 4. A maximal effect of catalase occurs at a concentration of 4 mg/ml, and this causes an inhibition of CL by approximately 70%. Nonspecific protein effects can be ruled out because 4 mg/ml BSA has no effect on CL. This concentration of catalase results in a highly colored solution. Thus, it is possible that the inhibition produced is due to color quenching of the light. However, we performed experiments in which the solution containing catalase surrounded another vial containing the cells and zymosan. We found that at this concentration the effect of catalase is not due to color quenching. Furthermore, the effect of catalase is probably extracellular since it is presumably too large to penetrate the membrane. Therefore, we tested the effect of direct addition of H_2O_2 to zymosan, and the results are shown in Table 1. Although H_2O_2 by itself produces some CL, the effect is more than 10-fold higher when zymosan is present. Thus, CL in rabbit alveolar macrophages may result from the reaction between H_2O_2 and zymosan as well as from the reaction between O_2^- and zymosan.

Haber and Weiss (14) proposed that hydroxyl radical is produced by a reaction between H_2O_2 and superoxide anion (see Fig. 1). Therefore, it is possible that the CL produced when alveolar macrophages are exposed to zymosan is due to the reaction between $\cdot OH$ and zymosan. To test this possibility, the effects of ethanol and benzoate, two scavengers of $\cdot OH$ (5), on CL were determined. Both of these substances cause inhibition of the chemiluminescent response. When 0.2 ml ethanol is added to the incubation medium 5 min prior to the addition of zymosan, there is absolutely no CL produced. Lesser amounts of ethanol cause lesser amounts of inhibition. Sodium benzoate also produces inhibition of the CL. The results are shown in Fig. 5. Although only the effects of 20 mM benzoate are shown here, lesser amounts cause lesser inhibition, and greater amounts produce greater inhibition. The results of these experiments then suggest that CL may also involve hydroxyl radicals.

The possibility that ethanol and benzoate cause inhibition of CL via an effect on superoxide anion generation must be ruled out. Therefore, we determined the effect of these two substances on the superoxide produced in response to zymosan. Neither ethanol nor benzoate had any effect on superoxide generation. Furthermore, Gee et al. (11) have shown that ethanol does not affect H_2O_2 production in rabbit alveolar macrophages. Thus, they probably act by scavenging $\cdot OH$ in this system just as they do in other systems. The results of these experiments then indicate that the CL produced when alveolar macrophages are exposed to zymosan is due to the reaction between any one, or all, of the reactive forms of oxygen and zymosan.

Relationship of particle uptake to chemiluminescence. In this paper we have suggested that CL results from the extracellular reaction of zymosan with various reactive forms of oxygen. This suggestion is based on the following points: *1*) superoxide anion is released extracellularly in response to zymosan; *2*) the time courses for CL and the appearance of superoxide anion in the medium are similar; *3*) reactive forms of oxygen produce CL when exposed to zymosan in noncellular

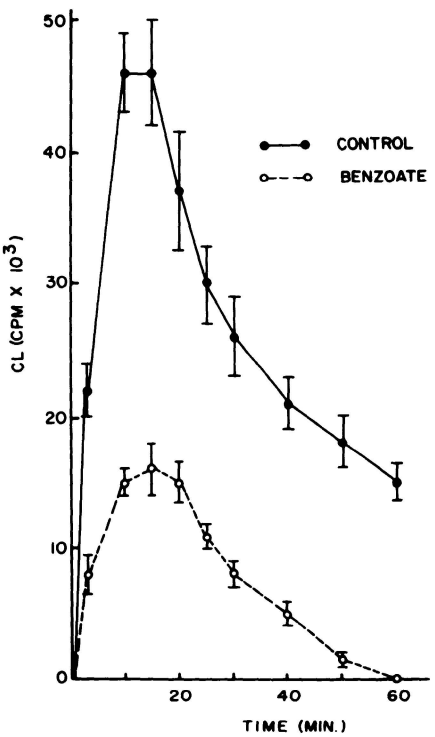

FIG. 5. Effect of benzoate on CL. Sodium benzoate (20 mM) was added to the incubation medium 10 min prior to addition of zymosan. These measurements were made as paired experiments. Points represent mean values from 6 experiments and bars are SE.

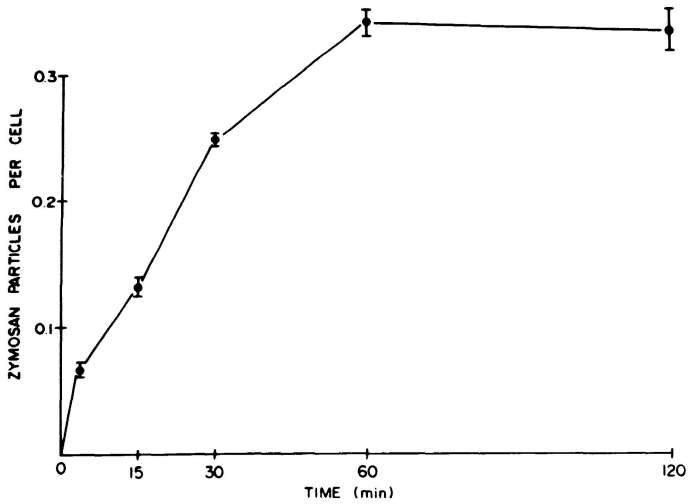

FIG. 6. Uptake of zymosan by rabbit alveolar macrophages as a function of time. Zymosan was added to the cells at zero time. Samples were taken at various later times. Slides were made for microscopic examination, and number of zymosan particles inside the alveolar macrophages was determined and expressed as particles per cell. Each point represents the mean value for 3–5 experiments and bars are SE. See text for details.

systems; and *4*) catalase and SOD, which greatly affect CL, are probably not able to penetrate cell membranes. If CL is due to an extracellular reaction, then it should occur before the uptake of zymosan. The time course for zymosan uptake in normal rabbit alveolar macrophages is shown in Fig. 6. In contrast with superoxide production and CL, both of which peak in 15–20 min, particle uptake is slower and does not reach a maximum for at least 1 h. Therefore, it seems likely that the CL is due to an extracellular reaction between the various reactive forms of oxygen and zymosan.

DISCUSSION

The results of these experiments indicate that the CL in this system is caused by the extracellular reaction between reactive forms of oxygen and zymosan. For example, the reactions between superoxide anion and zymosan and between H_2O_2 and zymosan cause CL, as shown in Table 1. In addition, some chemiluminescence may be associated with singlet oxygen, a substance which Allen et al. (3) showed could be produced in polymorphonuclear leukocytes. Some CL could be produced by the reaction between singlet oxygen and zymosan. Also light may result from the relaxation of singlet oxygen molecules to the ground state (3). In fact, this may be the source of the weak chemiluminescence which occurs when NaO_2 is placed in HBSS (see Table 1). The latter mechanism may be important in the CL induced by polystyrene particles in alveolar macrophages (19), because in this case the foreign particles are inert, i.e., they do not contain lipid as does zymosan.

It is possible that a certain amount of the reactive forms of oxygen is always present in the extracellular fluid and that CL merely arises when the zymosan particles are there to initiate the reaction. However, we have some experimental evidence to show that this is not the case. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) is a substance which, in the presence of reactive forms of oxygen, emits light (1). In our system in the presence of luminol, the level of background counts is the same as when no luminol is present. Furthermore, the level of CL increases upon exposure of the cells to zymosan in exactly the same manner both in the presence or absence of luminol. Determinations of extracellular superoxide anion indicate that very little superoxide is produced until the alveolar macrophages are exposed to zymosan (see Fig. 3). Thus, exposure of the cells to zymosan does lead to an actual increase in the production of antibacterial substances by the cells.

The amount of superoxide anion produced by macrophages at rest and after exposure to foreign particles shows a great deal of species variability. For example, we find no O_2^- production in resting rabbit alveolar macrophages, yet cells from rats, mice, and guinea pigs produce a high resting level of O_2^- (8, 9, 22). Although we do find a marked increase in extracellular O_2^- when rabbit alveolar macrophages are exposed to zymosan, this response is not as large as found in the rat or mouse (8, 9, 22). It is interesting that even though guinea pig alveolar macrophages exhibit a high resting level of O_2^- production, there is no increase in O_2^- production in response to foreign particles (9). The reason for the lack of response in guinea pig cells is unknown. There is a report which, in contrast to our data, states that no O_2^- is produced by rabbit alveolar macrophages even after exposure to zymosan (7). This discrepancy may result from differences in the age of rabbits used in each study, i.e., rabbits in our study were rather young (1–3 kg). Our data indicate that as rabbits grow older there is a tendency for CL to decrease (unpublished data).

The results of these experiments indicate that exposure of alveolar macrophages to zymosan leads to the production of superoxide anion and subsequently to CL. The events which initiate superoxide formation and the site of formation are unknown. In polymorphonuclear leukocytes there is evidence to suggest that hexose monophosphate shunt activity is important in producing NADPH for superoxide formation (4, 23). In alveolar macrophages both oxidative phosphorylation and hexose monophosphate shunt activity appear to play a role (12, 21). We have found that two metabolic inhibitors which inhibit CL, 2,4-dinitrophenol and fluoride (19), also inhibit the production of superoxide anion (preliminary results not reported here). Thus, it appears that the production of superoxide is dependent upon cellular metabolism, but the events which initiate this production and the subcellular site of formation remain unknown.

The results from this study, together with those from a previous publication (19), may be used to construct a model for the mechanism of the chemiluminescent response. The exposure of alveolar macrophages to zymosan causes an increase in the production of superoxide anion by the cells. The mechanism of this sequence of events is unknown. This eventually leads to an increase in the concentration of superoxide in the extracellular fluid. Hydrogen peroxide, singlet oxygen,

and hydroxyl radical are formed spontaneously from superoxide anion. It is the extracellular reaction between these antibacterial substances and zymosan which is actually responsible for the light that is produced. At this time the mechanism of the reaction(s) which produces CL is unknown. This scheme is not unlike the one proposed by Cheson et al. (6) for the origin of the chemiluminescent response in phagocytizing granulocytes.

In summary, chemiluminescence is produced when alveolar macrophages are exposed to zymosan. The light is due to the extracellular reaction between the antibacterial substances that are produced by the cells and zymosan. The significance of the chemiluminescent response is unknown at this time. It is possible that the extracellular level of antibacterial substances is a reflection of the intracellular level, i.e., it is possible that a "spilling over" of the antibacterial substances occurs. On the other hand, it is also possible that external release of these substances normally occurs in these cells. Perhaps CL is a good indicator of bactericidal potential. Experiments which should yield some information about the significance of CL are now being performed in our laboratory.

We are most grateful for the excellent technical assistance provided by Linda Bleigh and for helpful discussions and suggestions from Michael Trush, Knox Van Dyke, and Theresa Sweeney.

Received 10 August 1977; accepted in final form 24 April 1978.

REFERENCES

1. ALLEN, R. C., AND L. D. LOOSE. Phagocytic activation of a luminol-dependent chemiluminescence in rabbit and peritoneal macrophages. *Biochem. Biophys. Res. Commun.* 69: 245–252, 1976.
2. ALLEN, R. C., R. L. STJERNHOLM, AND R. H. STEELE. Evidence for the generation of an electronic excitation state(s) in human polymorphonuclear leukocytes and its participation in bactericidal activity. *Biochem. Biophys. Res. Commun.* 47: 679–684, 1972.
3. ALLEN, R. C., S. J. YEVICH, R. W. ORTH, AND R. H. STEELE. The superoxide anion and singlet molecular oxygen: their role in the microbicidal activity of the polymorphonuclear leukocyte. *Biochem. Biophys. Res. Commun.* 60: 909–917, 1974.
4. BABIOR, B. M., R. S. KIPNES, AND J. T. CURNETTE. Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. *J. Clin. Invest.* 52: 741–744, 1973.
5. BEAUCHAMP, C., AND I. FRIDOVICH. A mechanism for the production of ethylene from methional. The generation of hydroxyl radical by xanthine oxidase. *J. Biol. Chem.* 245: 4641–4646, 1970.
6. CHESON, B. D., R. L. CHRISTENSEN, R. SPERLING, B. E. KOHLER, AND B. M. BABIOR. The origin of the chemiluminescence of phagocytosing granulocytes. *J. Clin. Invest.* 58: 789–796, 1976.
7. DECHATELET, L. R., D. MULLIKIN, AND C. E. MCCALL. The generation of superoxide anion by various types of phagocyte. *J. Infectious Diseases* 131: 443–446, 1975.
8. DRATH, D. B., A. A. HARPER, AND M. L. KARNOVSKY. Biochemical characteristics of alveolar macrophages. In: *Lung Cells in Disease*, edited by A. Bouhuys. Amsterdam: Elsevier, 1976.
9. DRATH, D. B. AND M. L. KARNOVSKY. Superoxide production in phagocytic leukocytes. *J. Exptl. Med.* 141: 257–262, 1975.
10. FRIDOVICH, I. Superoxide radical and superoxide dismutase. *Accounts Chem. Res.* 5: 321–326, 1972.
11. GEE, J. B. L., J. KASKIN, M. P. DUNCOMBE, AND C. L. VASSALLO. The effects of ethanol on some metabolic features of phagocytosis in the alveolar macrophage. *J. Reticuloendothelial Soc.* 15: 61–68, 1974.
12. GEE, J. B. L., AND A. S. KHANDWALA. Oxygen metabolism in the alveolar macrophage: friend and foe? *J. Reticuloendothelial Soc.* 19: 229–236, 1976.
13. GEE, J. B. L., C. L. VASSALLO, P. BELL, J. KASKIN, R. E. BASFORD, AND J. B. FIELD. Catalase-dependent peroxidative metabolism in the alveolar macrophage during phagocytosis. *J. Clin. Invest.* 49: 1280–1287, 1970.
14. HABER, F., AND J. WEISS. The catalytic decomposition of hydrogen peroxide by iron salts. *Proc. Roy. Soc. London Ser. A* 147: 332–351, 1934.
15. KHAN, A. U. Singlet molecular oxygen from superoxide anion and sensitized fluorescence of organic molecules. *Science* 168: 476–477, 1970.
16. KLEBANOFF, S. J., AND C. B. HAMON. Antimicrobial systems of mononuclear phagocytes. In: *Mononuclear Phagocytes in Immunity, Infection, and Pathology*, edited by R. van Furth. Oxford: Blackwell, 1976.
17. LEE, P., P. R. MILES, G. TSEKOURAS, AND M. SMITH. Chemiluminescence associated with phagocytosis in dog alveolar macrophages (Abstract). *Biophys. Soc. Abstr.* 17: 166a, 1977.
18. MCCORD, J. M., AND I. FRIDOVICH. Superoxide dismutase. An enzyme function for erythrocuprein (hemocuprein). *J. Biol. Chem.* 244: 6049–6055, 1969.
19. MILES, P. R., P. LEE, M. A. TRUSH, AND K. VAN DYKE. Chemiluminescence associated with phagocytosis of foreign particles in rabbit alveolar macrophages. *Life Sci.* 20: 165–170, 1977.
20. MYRVIK, Q. N., E. S. LEAKE, AND B. FARISS. Lysozyme content of alveolar and peritoneal macrophages from the rabbit. *J. Immunol.* 86: 133–136, 1961.
21. OUCHI, E., R. J. SELVARAJ, AND A. J. SBARRA. The biochemical activities of rabbit alveolar macrophages during phagocytosis. *Exptl. Cell Res.* 40: 456–468, 1965.
22. SWEENEY, T. D., V. CASTRANOVA, L. BLEIGH, P. LEE, AND P. R. MILES. Factors which influence superoxide anion production in rat alveolar macrophages. *Federation Proc.* 37: 3106, 1978.
23. TSAN, M., B. NEWMAN, AND P. A. MCINTYRE. Surface sulfhydryl groups and phagocytosis-associated oxidative metabolic changes in human polymorphonuclear leucocytes. *Brit. J. Haematol.* 33: 189–204, 1976.
24. WEBB, L. S., B. H. KEELE, AND R. B. JOHNSTON. Inhibition of phagocytosis-associated chemiluminescence by superoxide dismutase. *Infection Immunity.* 9: 1051–1056, 1974.