

METABOLIC PATTERNS IN THREE TYPES OF PHAGOCYTIZING CELLS

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ABSTRACT

Some chemical and metabolic characteristics of polymorphonuclear leukocytes and monocytes from peritoneal exudates of the guinea pig, and of alveolar macrophages from the same animal, have been compared. Changes in the metabolic patterns of these three types of cell have been followed during the act of phagocytosis. The effect of conventional inhibitors of metabolism, and of anaerobiosis on the phagocytic ability of each of the three cell types mentioned has also been determined. From these studies it was found that alveolar macrophages depend to a considerable degree upon oxidative phosphorylation to provide energy for phagocytosis. The other two types of cell depend only on glycolysis as the source of metabolic energy for that function. In some experiments aimed at obtaining information on the possible role of complex lipids in the function of the cell membrane, it was noted that phagocytosis stimulated the incorporation of inorganic phosphate- P^{32} into the phosphatides of both types of cell from peritoneal exudates—whether these were free-swimming or adherent to a surface. This phenomenon has not yet been detected in the case of alveolar macrophages.

During the past several years, a number of studies have been carried out on the metabolic concomitants of the phagocytic act in leukocytes. Almost all of these observations were made on polymorphonuclear leukocytes. Only a few data have been reported for monocytes (1-4). The object of the present experiments was to examine the metabolic basis of the phagocytic event in mononuclear cells and in alveolar macrophages and to compare these cells with polymorphonuclear leukocytes. This seemed to be of importance, because, although polymorphonuclear leukocytes are the most immediate phagocytizing cells during infection, they are very short-lived. The macrophages, of which the peritoneal mononuclear leukocytes and the alveolar macrophages may be representa-

tive, are of major importance as defenses against invading organisms. Alveolar macrophages are particularly intriguing cells and have recently been the subject of considerable morphological study (5). There has been a good deal of discussion of their origin (5-8) and function (6, 8).

This paper presents information on the chemical characteristics of these three types of phagocyte, all obtained from the guinea pig, as well as comparisons of the metabolic changes which accompany phagocytosis. Definite differences with respect to the source of metabolic energy for phagocytosis, between the alveolar macrophages on the one hand and the peritoneal exudate monocytes and polymorphonuclear leukocytes on the other, were found.

MATERIAL AND METHODS

1. *Materials*

The casein used for eliciting polymorphonuclear or mononuclear cells, sodium caseinate (nutrose), was obtained from Difco Laboratories, Inc., Detroit. Glucose-U-C¹⁴ (uniformly labeled glucose), glucose-1-C¹⁴ and glucose-6-C¹⁴ were obtained from the New England Nuclear Corporation Inc., Boston. Starch granules from *Amaranthus cruentus* (9) were obtained through the courtesy of the Northern Regional Research Laboratory, Peoria, Illinois. Samples of insoluble starch were prepared in this laboratory from *Amaranthus caudatus* seeds by the method of MacMasters *et al.* (9). This starch was quite suitable for phagocytosis experiments, although the granules were somewhat larger than those of the *Amaranthus cruentus* species. Polystyrene spherules 1.171 μ in diameter were obtained through the courtesy of Dr. J. Vanderhoff of The Dow Chemical Co., Midland, Michigan. It was necessary to dialyze these particles against many changes of distilled water (providing a possible dilution of 1×10^{11}) over 5 days at 4°C to remove a preservative which had been added to some batches. All other chemicals were of analytical grade where procurable.

2. *Preparation of Polymorphonuclear*

*Leukocytes*¹

Guinea pigs weighing between 330 and 350 gm were fed a diet of chow pellets and cabbage *ad libitum*. Each animal received an injection (15 to 20 ml) of sterile neutral casein (12 per cent weight/volume) in physiological saline, into the peritoneal cavity. It was found advantageous to shave the abdomen prior to administering the injection. Eighteen hours later serum was collected for various purposes from a cardiac puncture and the animals were killed by injection of air into the heart while immobilized on an animal board. The abdomen was then opened and the peritoneal fluid withdrawn. Non-specific cellular debris in the harvested exudates was greatly decreased by using a small J-shaped pipette with holes in the upper surface of the curve of the J. This permitted a minimum of contact with, or disturbance of, the peritoneal wall.

¹ The polymorphonuclear leukocytes have also been referred to as "neutrophilic granulocytes." The former term will be used here, and abbreviated as "PMN." The monocytes obtained from the peritoneum have also been called "peritoneal macrophages." We shall use the term "monocytes" or "mononuclear cells" and the abbreviation "MN." The alveolar macrophages will be abbreviated as "AM."

The peritoneal cavity was washed until no further cells were obtained, using approximately 15 cc of physiological saline for each wash. The cells were collected in graduated 40 ml thick-walled glass centrifuge tubes or in plastic centrifuge tubes. They were spun down at low speed (<100 g), washed, and then suspended in Krebs-Ringer phosphate at a suitable concentration. The exudates from several animals were routinely collected in this way and pooled. During the process of collection from the animals the harvested cells were maintained in an ice bath. Counts were made in a hemocytometer to determine the number of cells present.

3. *Mononuclear Leukocytes*

Various substances were tested for their ability to elicit mononuclear leukocytes. Glycogen has been extensively used in the past (*e.g.*, 10) but in our experience does not provide optimal yields of cells. In some studies light mineral oil, warmed to body temperature, has been used as an agent for eliciting a mononuclear response (*e.g.*, 11). The exudate obtained after a few days was indeed found to consist almost entirely of mononuclear cells. However, these cells, in our experience, were full of large vacuoles containing mineral oil and were accompanied by a considerable amount of cellular debris. Although these oil-elicited cells respired normally, they did not show any respiratory increase when particles were offered to them and it is our opinion that they are not capable of further normal phagocytic activity.

The best method for obtaining mononuclear cells in our laboratory has been found to be through the use of 1.2 per cent sterile casein. This was injected as described above for polymorphonuclear leukocytes but an interim period of 3 to 4 days between administration of the eliciting agent and harvesting of the cells was allowed. Such exudates consisted mostly of mononuclear cells. Higher concentrations of casein consistently gave mononuclear preparations after 3 days, or longer periods, that were grossly contaminated with polymorphonuclear leukocytes, presumably because casein was present in the peritoneal cavity throughout a longer period after injection. The initial inflammatory reaction was thus of prolonged duration, with new polymorphonuclear cells constantly being elicited.

4. *Lung Macrophages*

Myrvik *et al.* (12) have described a method by which macrophages may be obtained from the lungs of rabbits. Some difficulty was experienced in obtaining these cells from smaller animals, such as guinea pigs, mice, or rats, by this method. Modifica-

tions in the method were developed to obtain lung macrophages from such animals.³

Guinea pigs were killed by injecting air into the heart. Animals which had been injected with casein for either of the above preparations or fresh guinea pigs were used. The lungs were removed and rinsed in saline; the upper part of the trachea was excised and the lung tissue was cut into very small pieces (2 to 3 mm) with dissecting scissors. Two or three cut-up lungs were suspended in 20 ml of Krebs-Ringer phosphate medium which contained 10 per cent of fresh guinea pig serum and 0.005 per cent heparin. The lung tissue was then agitated with a homogenizer (Multimixer, Lourdes Instrument Corporation, Brooklyn) in a 75 ml vessel coated on the inside with Teflon. The 6 cutting blades of the homogenizer were about 25 mm in diameter, and a speed of about 750 RPM was maintained for 1 to 2 minutes. It was found helpful to switch the machine on and off at 15-second intervals, in order to liberate an optimal number of cells. The suspension was filtered through a nylon gauze (100 mesh) in order to remove tissue fragments. A cell count of the filtered suspension above was made in a hemocytometer and the suspension diluted with 10 per cent serum, as required, to give an optimal cell concentration. The adjusted suspension was divided between stoppered tubes or flasks (Warburg flasks or plastic tissue culture flasks; see section 6 below) according to the needs of the experiment. It was found that, for an area of 10 cm², 2 to 2.5 ml fluid containing about 2.5×10^6 cells was optimal. The vessels were closed and incubated for 1 hour. During the incubation the vessels were kept tightly stoppered since a small amount of CO₂ is apparently necessary to ensure proper sticking of the cells to the surface. Each vessel was gently agitated every 15 minutes during this incubation period, to ensure that an even layer of cells adhered to the bottom. At the end of this time the excess fluid containing tissue fragments, those cells which had not stuck, and red blood cells were removed by aspiration, after carefully tilting the incubation vessels. The cell monolayer was gently washed twice with Krebs-Ringer phosphate medium, each washing equal in volume to the suspension that had been pipetted into the vessel.

By these manipulations, a monolayer was obtained consisting of *ca.* 90 per cent lung macrophages. A few polymorphonuclear leukocytes could be seen. After the final washing of the monolayer, a suitable medium was introduced into the vessel and the experiments described below performed. Lung macrophages adhering to glass or plastic surfaces

³The basis of this method was largely worked out in the Laboratory of the Israeli Institute for Biological Research, Rehovoth, Israel.

and maintained in tissue culture media, described by Braun *et al.* (13), preserved their phagocytic activity for 1 month.³

5. Quantification of cells

As mentioned above, cells were counted in a hemocytometer. Cellular protein was measured on aliquots of the polymorphonuclear leukocyte suspensions or monocyte suspensions in Krebs-Ringer phosphate.

In the case of the alveolar macrophages it was necessary to prepare triplicate flasks for protein and phosphorus determinations in addition to those that were to be used in the metabolic and phagocytic experiments. After the monolayer of cells had been washed in Krebs-Ringer phosphate twice, and this fluid carefully removed, the cells were digested in 0.5 N NaOH and the protein was measured on aliquots. If phosphorus measurements were to be made, the monolayer was thoroughly washed with isotonic saline instead of Krebs-Ringer phosphate solution before digestion in NaOH solution. In those metabolic experiments in which no serum was used in the final incubating medium it was possible to add, at the end of the incubation, enough NaOH solution of normality greater than 0.5 N to bring the final concentration of NaOH to 0.5 N. The cells were allowed to digest in the alkali overnight and the precipitated magnesium and calcium salts were removed by centrifugation. Aliquots could then be analyzed for protein. The methods used for all cells were those of Lowry *et al.* (14) for protein and of King (15) for phosphorus.

6. Respiratory Experiments

Measurements of respiration were carried out using the Warburg microrespirometer. The experiments with PMN were described previously (2), and those with MN and AM were performed in a similar fashion. The values for the monocytes were obtained using flasks with a capacity of 7 ml containing a total of 1.1 ml of fluid. The center-wells contained 0.1 ml 20 per cent KOH. The cells were in suspension as indicated above.

The experiments with the alveolar macrophages were performed with 15 ml Warburg flasks, the bottoms of which were coated with a monolayer of cells as described above. It should be mentioned that numerous Warburg flasks were tested, and a selection made of those with the flattest bottoms for use in these experiments. It was necessary for the flasks to be scrupulously clean. The final volume of the system was 3.2 ml. The center-wells contained 0.2 ml 20 per cent KOH.

The sidearms in these experiments contained

³R. Oren, unpublished observations.

glucose and, where the cells were to phagocytize, starch particles or polystyrene spherules (2 to 2.5 mg/ml, final concentration). When starch particles were to be ingested the medium also contained 30 per cent serum, but no serum was needed in the case of the polystyrene spherules (2). All measurements were made at 37°C.

7. Experiments with Specifically Labeled Glucose as Substrate

In the case of PMN and MN the experiments were performed in Warburg respirometric flasks, as has been previously described (2) (6 above), and respiration was followed. The conversion of glucose carbon-1 or glucose carbon-6 to $C^{14}O_2$ was determined by collecting the contents of the center-well and precipitating the expired CO_2 as $BaCO_3$ (16) at the termination of the experiments in which oxygen uptake was measured (2).

In the case of the alveolar macrophages, separate experiments were performed for measurement of respiration and of glucose oxidation. The experiments using glucose-1- C^{14} or glucose-6- C^{14} and alveolar macrophages were performed in tissue culture flasks made of plastic and with a volume of 30 ml (Falcon Plastics Company, Los Angeles). These flasks were used here rather than Warburg flasks because one flat side of these vessels provided a surface area larger than that possible with Warburg flasks. Thus, a larger number of cells could be used. Five ml of a suspension containing 1.5×10^6 cells/ml was pipetted into each flask. The cells were permitted to adhere to the plastic surface, as described above, and washed. After the introduction of the final medium containing the glucose labeled in carbon-1 or carbon-6, particles were introduced. The flasks were stoppered with a boiled rubber stopper carrying a reservoir with 0.1 ml 20 per cent KOH for collection of CO_2 . The arrangement is illustrated in Fig. 1. Each flask contained a final volume of 2 ml of fluid, made up as follows: 1.7 ml of Krebs-Ringer phosphate medium containing 30 per cent serum; 0.1 ml of glucose-1- C^{14} or glucose-6- C^{14} ; 0.2 ml of a suspension of starch containing 25 mg/ml (or in control flasks, 0.2 ml Krebs-Ringer phosphate). The flasks were agitated gently every 10 minutes in order to mix contents. One hour after the stoppers had been put in place the experiment was terminated. The alkali reservoir was removed and placed in a small test tube containing 1.5 ml of distilled water. The reservoir was washed four times with fresh aliquots of distilled water and the carbonate was finally precipitated from the pooled alkali and washings, as previously described (2). All radioactive measurements were performed as described by Karnovsky *et al.* (16).

8. The Effect of Various Metabolic Inhibitors on Phagocytosis

In the case of monocytes, the effect of metabolic inhibitors on particle uptake was determined by examining aliquots of a cell suspension after incubation. The experiments were performed in small Erlenmeyer flasks on the Dubnoff incubation bath. The techniques followed were identical with those reported previously for polymorphonuclear leukocytes (2).

In the case of the alveolar macrophages the cells were allowed to adhere in tubes (150 × 17 mm),

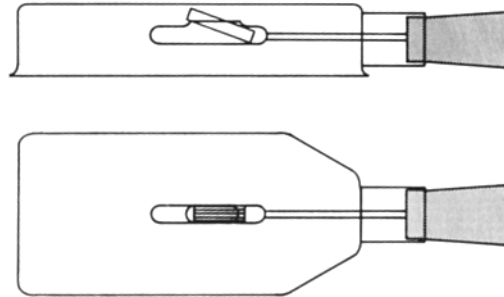


FIGURE 1

Tissue culture flask equipped for collection of respiratory CO_2 . A boiled rubber bung carries a rod terminating in a small reservoir. A filter paper fan may be placed in the reservoir to increase surface area of the 20 per cent KOH solution (0.1 ml) which is placed in the reservoir. Top, side view; Bottom, view from above. The cells are allowed to adhere to the bottom of the flask, and are gently washed. When everything is ready for the experimental period, the KOH well is inserted (see text).

in the manner indicated above. It was found satisfactory to use a final volume of 1 ml of medium and about 1×10^6 cells in each tube. Inhibitors and starch or polystyrene spheres were added at the beginning of the experimental period; *i.e.*, after preparation of the monolayer and addition of the final medium. After 1 hour of incubation, during which the system was gently agitated, the cells were removed from the tube with the aid of a glass rod and stained on a slide with Wright's stain. The number of engulfed particles was determined as previously described (2). As a check on these procedures, experiments were made using the crude suspension obtained after homogenizing and passing the chopped-up lung suspension through the nylon gauze. The suspension was incubated in flasks which were gently shaken on the Dubnoff metabolic incubator so that the cells did not adhere to the glass surface. Experiments with particles and in-

hibitors were performed on these suspensions in the same way as with the monolayers. The results with respect to the macrophages examined under the microscope, in these experiments, were not different from those obtained with adhering cells.

When it was wished to study the effect of anaerobic conditions, the tubes or small Erlenmeyer flasks were gassed with nitrogen (<0.01 per cent O₂) for 5 minutes, then stoppered and the incubation continued. The particles for engulfment were added just prior to removal of the gassing tubes, and flasks or tubes were immediately stoppered.

9. Experiments on the Incorporation of Labeled Substrates into Lipids

The incorporation of uniformly labeled glucose carbon, or of phosphate-P³², into lipids by cells at rest or during phagocytosis was carried out for cells in suspension, as described previously (17). When cells were used as a monolayer on glass, the following technique was employed: large glass tissue culture bottles, 150 × 45 × 45 mm, were used to provide a very large surface to which as many cells as possible might adhere. The cell monolayer was prepared as described above. Experiments were of 30 minutes' duration after addition of radioactive substrate and starch particles. The bottles were agitated gently every 10 minutes. The final fluid volume was 4.5 ml, consisting of 3.6 ml Krebs-Ringer phosphate medium containing 30 per cent serum, 0.2 ml glucose (from 2 to 20 μmoles in various experiments; 1.5 to 9 μc in experiments with C¹⁴); 0.2 ml starch suspension (5 mg) and 0.5 ml Krebs-Ringer phosphate. In experiments on the incorporation of P³², the last mentioned addition contained 10 μc phosphate-P³².

The experiment was terminated, and lipids extracted as follows: the medium was removed by suction and the cells washed twice with Krebs-Ringer solution (5 ml); 3 ml chloroform-methanol (2.1 v/v) was then added (18) and the flasks stoppered and kept at 55°C for 4 hours. The solvent was changed every hour, pooled, and retained. Finally 5 ml of solvent was added and the flasks left at room temperature overnight. The total extract was combined in 50-ml centrifuge tubes, processed, and counted as described before (17, 19).

RESULTS

1. Characteristics of PMN, MN, and AM

The suspensions of polymorphonuclear cells were 85 per cent PMN, contaminated with about 12% MN. The remaining cells were a few lymphocytes and eosinophiles. The MN preparations contained 90 per cent MN. Most of the contaminating

cells were PMN, and a few lymphocytes were seen. These data are in accord with those attained previously (10).

The preparations of alveolar macrophages were about 90 per cent pure. The remaining cells were mostly PMN. The predominant cells were typical macrophages that tended to flatten against the supporting surface, exhibiting undulating membranes as a "halo" around the cells when fresh preparations were examined by phase microscopy.

In Table I are summarized some of the chemical constants for polymorphonuclear leukocytes, peritoneal monocytes, and alveolar macrophages. It appears that the alveolar macrophages are about twice the size of the peritoneal monocytes on the basis of protein content of the cells. The peritoneal monocytes are in turn about 50 per cent larger, on this basis, than polymorphonuclear leukocytes. Further, the polymorphonuclear leukocytes contain less total phosphorus per cell than the other two types of cells.

2. Some General Observations on the

Metabolism of PMN, MN, and AM

The most noteworthy metabolic observation in Table I is the fact that the alveolar macrophages have a respiratory activity which is almost an order of magnitude greater than that of the polymorphonuclear leukocytes and three times that of the monocytes. This respiratory activity is rather high, even compared with values in the literature for such tissues as liver and kidney, and approaches those that have been obtained with slices of brain. The glucose oxidized by PMN and MN, particularly, is quite small, compared with the amount of glucose consumed, most of which is converted to lactate. The data for the polymorphonuclear leukocytes are taken from prior studies (2, 20) but similar measurements made during the course of the present work were in agreement with the previously obtained figures.

It was of interest to determine whether the very high respiratory activity of the alveolar macrophages was due to the fact that they were adhering to a surface; consequently peritoneal monocytes were allowed to adhere to the bottom of Warburg flasks as a monolayer and the respiration determined. The Q_{O₂} value for monocytes in this experiment was as follows: 8.9 ± 1.5 μl O₂/hour and milligram protein. The Q_{O₂} of the monocytes

adhering to a surface was thus not significantly different from that obtained with cells in suspension (see Table I).

The value given for the Q_{O_2} of alveolar macrophages in Table I was obtained in the presence of 20 per cent guinea pig serum, and excess glucose. Some aspects of the effects of serum on these cells are shown in Fig. 2. Omission of the serum caused a decrease in oxygen uptake of about 25 per cent (Fig. 2, panel A). The use of dialyzed serum indicated that only a part of the stimulatory effect of serum was due to glucose. In experiments with a

single batch of guinea pig serum, a sample of which had been dialyzed and another not, the respiration in the presence of the dialyzed serum plus glucose was 10 to 30 per cent lower than that in the presence of fresh serum plus glucose. The respiration of PMN and MN has usually been found to be somewhat stimulated by serum (2, 10).

The effect of glucose on the AM is different from that on either polymorphonuclear leukocytes or monocytes. Both of the latter types exhibit the Crabtree effect; *i.e.*, depression of respiration by glucose (2). Glucose depresses the respiration of

TABLE I
Some Chemical Characteristics of Three Types of Phagocytic Cell from Guinea Pigs*

Cells	Polymorphonuclear leukocytes (PMN)	Monocytes (MN)	Alveolar macrophages (AM)
Protein ‡	150	200	400
Phosphorus ‡	2	4.5	8
Protein §	7.5 ± 0.3	4.6 ± 0.1	4.0 ± 0.1
phosphorus	(16)	(13)	(17)
Glucose used	430 ± 30	280 ± 20	460 ± 30
	(4)	(4)	(8)
Lactate produced	690 ± 48	500 ± 0	740 ± 10
	(4)	(4)	(8)
Glucose-C-1 → CO ₂	8.4 ± 0.7	26.1 ± 2.7	217.6 ± 18.4
	(25)	(10)	(9)
Glucose-C-6 → CO ₂	1.0 ± 0.2	1.3 ± 0.2	36.9 ± 1.2
	(25)	(10)	(6)
Q_{O_2} ¶	2.5 ± 0.1	7.0 ± 0.3	20.4 ± 0.1
	(34)	(11)	(12)

* The data are given as means and, where proper, the standard errors of the means. The numbers of determinations are quoted in parentheses. The figures for polymorphonuclear leukocytes were mostly derived from previous publications from this laboratory (2, 20).

‡ microgram per 1×10^6 cells. These figures are approximate and no indication of variability is given because of the relative uncertainty of the counting procedure compared with the chemical analyses.

§ milligram protein per 100 μ g cell phosphorus. The values for protein and phosphorus were obtained by analysis of different aliquots of the same cell suspension. The standard error of the means of the ratios of these chemical determinations is thus given, since the data did not involve determination of the number of cells.

|| millimicromoles per milligram protein and hour. The concentration of glucose in incubation media for the PMN ranged from 5 mM to 10 mM. In the case of the MN, it was from 7.5 to 15 mM. In the experiments with alveolar macrophages it was 7.5 mM or 10 mM. Glucose was always in considerable excess.

¶ microliter O₂ per milligram protein and hour.

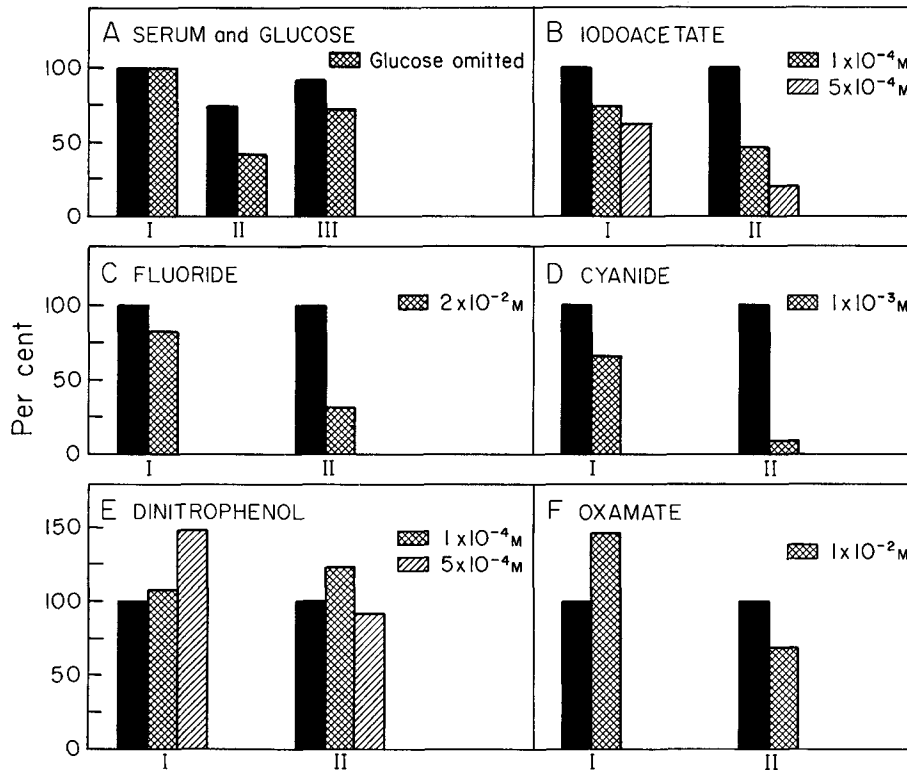


FIGURE 2

Effect of various substances on respiration of alveolar macrophages. Krebs-Ringer phosphate solution constituted the medium, and I, II, and III refer, respectively, to the presence of 20 per cent fresh serum (I), no serum (II), or the addition of 20 per cent serum dialyzed exhaustively against physiological saline (III). The concentration of glucose was 5 mM unless otherwise indicated. The respiratory activity of the control is always set at 100. In panel A, this is the system with fresh serum and glucose. In panels B through F, the control (100 per cent) is the system without the metabolic inhibitors studied and the numerals I or II refer to the presence or absence of fresh serum.

PMN by about 50 per cent (2); the figure for monocytes, obtained in the present study (compare reference 10), is about 40 per cent. The alveolar macrophages show a notable stimulation of respiration by glucose; thus, in the absence of serum (Fig. 2, panel A, II), 5 mM glucose stimulated respiration by about 80 per cent; in the presence of 20 per cent (exhaustively dialyzed) serum, addition of the glucose caused a 30 per cent increase in respiratory activity (Fig. 2, panel A, III).

Some experiments were carried out to determine whether all three cell types exhibited a Pasteur effect. This has already been shown to be so for PMN (2, 10); the absence of oxygen caused a 50 per cent increase in lactate production.

In the case of the peritoneal monocytes in the

presence of glucose (5 mM), an increase in lactate production (20 to 90 per cent) was noted under anaerobic conditions compared with aerobic conditions.

Alveolar macrophages showed a very dramatic Pasteur effect, up to a fourfold increase in lactate production in the presence of exogenous glucose, when they were deprived of oxygen. This result was found when the lactate was measured specifically by chemical means, or less specifically by manometric means; *i.e.*, by expulsion of CO₂ from a bicarbonate buffer.

3. Changes in Metabolism during

Phagocytosis

When the three types of cell were offered particles which they ingested, metabolism was stimulated

as shown in Table II. Ingestion was monitored microscopically. Respiration during phagocytosis has previously been shown to be more than doubled in the case of polymorphonuclear leukocytes (2). This effect was even more pronounced with the monocytic cells. However, in the case of the alveolar macrophages, there was only a small increment of about 25 per cent in the respiratory activity when particles were ingested.

In Table II it may also be seen that the phagocytic event was accompanied in monocytic cells by a tremendous increase in the oxidation of glucose carbon-1 to CO_2 . This result is comparable to that previously noted for polymorphonuclear leukocytes (2). However, the increase in conversion of glucose carbon-6 to CO_2 in the monocytes, in contrast to PMN, was of a magnitude comparable

to that of carbon-1; *i.e.*, there was approximately a tenfold increase. During particle uptake in polymorphonuclear leukocytes the increased oxidation of glucose carbon-6 was much less marked than that with respect to carbon-1. Consequently, whereas the ratio: carbon-1 converted to CO_2 to carbon-6 converted to CO_2 , decreased somewhat in the case of monocytes during phagocytosis, as compared with the values at rest, the same ratios for polymorphonuclear leukocytes have previously been shown to increase considerably during phagocytosis (2). In the case of the alveolar macrophages, there was but a comparatively small increase in the conversion in the oxidation of both glucose carbon-1 and carbon-6 during the phagocytic event.

TABLE II
*Metabolism of Phagocytizing Cells**
Expressed as percentages of resting values.

	PMN	MN	AM
(a) Oxygen uptake	244 ± 2	352 ± 5	127 ± 10
Experiments	34	11	5
<i>P</i>	<0.001	<0.001	0.05
(b) Glucose-C-1 → CO_2	666 ± 13	915 ± 32	132 ± 6
Experiments	25	10	7
<i>P</i>	<0.001	<0.001	<0.01
(c) Glucose-C-6 → CO_2	250 ± 27	1036 ± 22	119 ± 8
Experiments	25	10	5
<i>P</i>	<0.001	<0.001	0.05
(d) Ratio C-1/C-6 (→ CO_2)	267 ± 2	88 ± 2	107 ± 14
Experiments	25	10	3
<i>P</i>	<0.001	<0.001	N.S.
(e) Lactate formed‡	104 ± 28	108 ± 14	100 ± 3§
Experiments	7	6	3
<i>P</i>	N.S.	N.S.	N.S.

* The data are given as the mean ± SE and are expressed as a percentage of the value obtained with resting cells (see Table I for absolute values for resting cells). All data were taken at 60 minutes from the time of adding the particles.

‡ Although the data do not show an increase in lactate production at 60 minutes, it has been shown clearly with PMN that significant increases may be detected at earlier times. The increases are rather small compared with the resting values, and become more and more difficult to detect with certainty as the baseline values increase with time (2, 20).

§ These values are more strictly "total acid production" and were obtained from measurements of CO_2 displacement from a Krebs-Ringer bicarbonate medium under air-5 per cent CO_2 .

4. *The Effect of Metabolic Inhibitors on Particle Uptake*

In previous studies (1, 2) the use of conventional metabolic inhibitors has been very helpful in assessing the importance of various routes of carbohydrate metabolism in providing energy for the phagocytic process. Similar studies have been carried out here and some of the data obtained are illustrated in Fig. 3 for monocytes and in

lism, the energy for the process presumably being provided by glycolysis. Interference with the latter process blocks particle ingestion, as may be seen in Figs. 3 and 5.

In the case of the alveolar macrophages, however, the data are different, and are presented in Fig. 4. Here interference with aerobic metabolism or oxidative phosphorylation had a definite depressive effect on particle uptake. Inhibition of

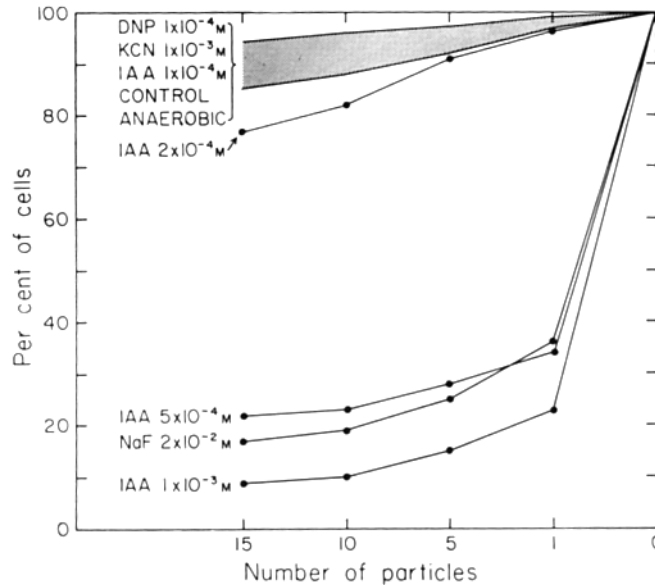


FIGURE 3

Effect of metabolic inhibitors on the ingestion of particles by monocytes. The abscissa lists a lower limit of particles present in cells; the ordinate lists the percentage of cells containing that number of particles, or more than that number, after one hour. For example, all cells (100 per cent) contain 0 or more particles; 21 per cent of cells contain 1 or more particles in the presence of 1×10^{-3} M iodoacetate; 20 per cent of cells contain 10 or more particles in the presence of NaF (2×10^{-2} M). The values within the dark area are not significantly different. DNP refers to 2,4-dinitrophenol; IAA denotes iodoacetate; KCN is potassium cyanide and NaF is sodium fluoride.

Fig. 4 for alveolar macrophages. For reference a summary of some previously obtained data for polymorphonuclear cells is given in Fig. 5 (2).

It may be noted that the monocytes display the same patterns that were seen in the case of the polymorphonuclear leukocytes with respect to the ability of various inhibitors, or anaerobiosis, to affect particle ingestion (2). Monocytes apparently require larger concentrations of iodoacetate than do PMN to inhibit particle uptake. Both types of cell perform their physiological function perfectly efficiently without aerobic metabo-

glycolysis is even more damaging to the phagocytic ability, indicating perhaps that the cells are not entirely dependent on oxidative metabolism for particle ingestion but can perform the phagocytic function to some extent with energy provided by glycolysis.

5. *Effects of Inhibitors on the Metabolism of Resting and Phagocytizing PMN and MN*

In the case of polymorphonuclear leukocytes a detailed examination of particle uptake in the

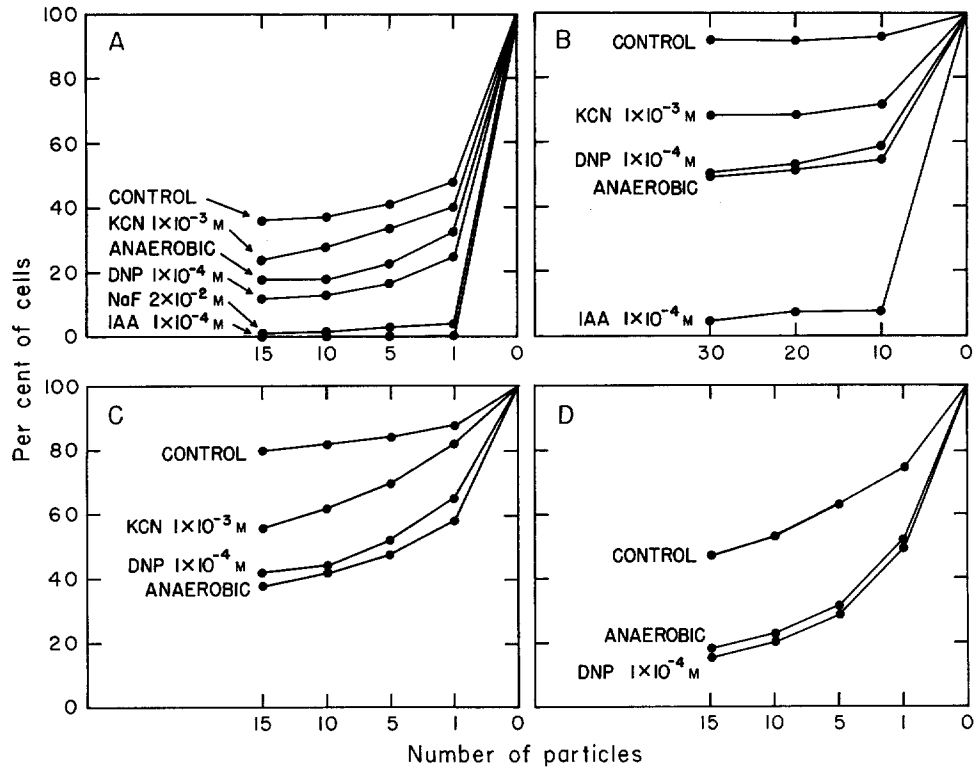


FIGURE 4

Effect of metabolic inhibitors on particle uptake by alveolar macrophages (for mode of expression see legend to Fig. 2).

[A] and [D] Alveolar macrophages in suspension. Polystyrene particles. No serum present. (D is a duplicate experiment of A, but focusses only on effects under anaerobic conditions and in the presence of DNP) dinitrophenol.

[B] Alveolar macrophages adhering to glass. Polystyrene particles. No serum.

[C] Alveolar macrophages adhering to glass. Particles: starch. 20 per cent serum present.

presence of metabolic inhibitors has been carried out, with simultaneous measurements of the changes in metabolism. It was found by Sbarra and Karnovsky (2) that in the presence of those substances that affect respiratory events (such as cyanide, antimycin A, dinitrophenol) there was no suppression of particle uptake (Fig. 5) and the metabolic concomitants of phagocytosis (Table II) were superimposed on the metabolic changes caused by the inhibitor (2). For example, cyanide depressed oxygen uptake of resting cells, but when particles were added to the system the usual increase in oxygen uptake appeared. Again, dinitrophenol caused an increase in the respiration of resting cells, and some increase in the oxidation of glucose C-1. Upon the addition of particles, phagocytosis occurred normally and

the usual increases in the two metabolic activities mentioned were seen.

On the other hand, in the presence of substances that inhibit glycolysis and particle ingestion, the metabolic changes usually associated with the phagocytic event were not superimposed on the effect of the inhibitor itself on the metabolic pattern. For example, sodium fluoride or iodoacetate suppressed particle ingestion (Fig. 5) and, when particles were added to the cells in the presence of these substances, there was *no* large increase in oxygen uptake or conversion of glucose carbon-1 to CO_2 ; *i.e.*, no increases in metabolism usually associated in these cells with phagocytic activity (Table II).

Similar measurements were made in the present study on monocytes, whose metabolic responses

during phagocytosis are very large (Table II), and these are presented in Table III. It may be noted that dinitrophenol and cyanide, which elevate or suppress respiration, respectively, permit particle uptake (Fig. 3) and an increment in respiration is seen to accompany particle uptake although the former is apparently impaired. The very large increased conversion of glucose carbon-1 to CO_2 during particle uptake is unimpaired by these inhibitors. Cyanide grossly suppresses the conversion of carbon-6 to CO_2 in phagocytizing cells. Dinitrophenol raises this function in resting

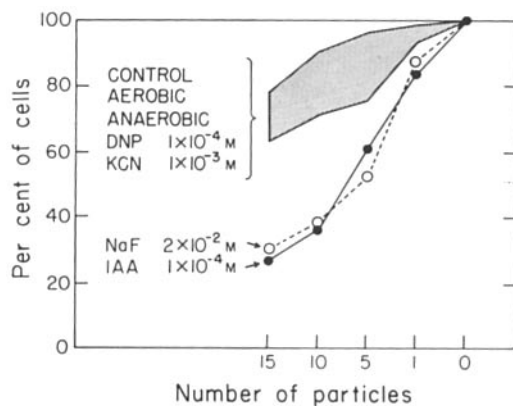


FIGURE 5

Effect of metabolic inhibitors on particle uptake by polymorphonuclear leukocytes. The data are plotted as in Figs. 3 and 4. Four different experiments are represented in this figure. The dark area represents the spread of control data in these experiments, and there is no significant effect of anaerobiosis, dinitrophenol, or cyanide.

cells, and particle uptake is accompanied by no further effect. The effect of iodoacetate must be considered only partial in these experiments since they were carried out before it was recognized that the monocytic cells were less sensitive than polymorphonuclear leukocytes to this agent, in so far as particle uptake is concerned. A major difference between PMN and MN lies in the response to NaF. This inhibitor blocks phagocytosis and the associated metabolic changes in both types of cell. In the case of PMN, it causes a very large increase in the level of respiration at rest (2- to 3-fold) and a doubling of conversion of glucose carbon-1 to CO_2 (2). These phenomena are not seen in the case of MN.

The values for the lactate determinations in Table III are offered only because they illustrate

the effects of the inhibitors on this variable. Changes in lactate production due to phagocytosis are, as mentioned above, detectable only at time intervals shorter than 1 hour (2).

6. Metabolic Responses of Alveolar Macrophages to Inhibitors

Because of the very small metabolic responses of alveolar macrophages to the phagocytic event (Table II) it was not feasible to carry out the same type of study as described for PMN and MN on the simultaneous effects of inhibitors and particles on metabolic levels. An examination was made, however, of the influence of a number of factors on the respiratory activity of resting alveolar macrophages. These experiments are summarized in Fig. 2.

Iodoacetate ($1-5 \times 10^{-4}$ M), fluoride (2×10^{-2} M), and cyanide (1×10^{-3} M) depressed respiration. Dinitrophenol (1×10^{-4} M) caused a definite stimulation. The effect of serum on the action of these substances was interesting in that it decreased the effect of iodoacetate, fluoride, and cyanide, apparently rendering the cells less sensitive to these inhibitors (Fig. 2). For example, concentrations of 5×10^{-4} M iodoacetate, which caused a depression of 80 per cent in the absence of serum, caused only a 40 per cent depression in its presence. On the other hand, serum markedly enhanced the stimulatory effect of higher concentrations of dinitrophenol presumably by decreasing the toxicity of this substance. At a concentration of 1×10^{-4} M, DNP caused a stimulation of 24 per cent in the absence of serum. DNP at 5×10^{-4} M under these conditions depressed respiration by 10 per cent. The addition of serum reduced the effect of the lower concentration of DNP to less than a 10 per cent stimulation, but permitted the higher concentration to exhibit a stimulation of 50 per cent.

Oxamate (1×10^{-2} M), an inhibitor of lactic dehydrogenase, had a depressive effect (35 per cent) in the absence of serum, but caused a significant increase (20 to 70 per cent) in respiration in its presence.

7. The Effect of Phagocytosis on the Incorporation of Radioactivity into Cellular Lipids

It has been shown that the phagocytic event in polymorphonuclear cells causes a marked increase

TABLE III
Effect of Inhibitors on Metabolism of Monocytes Alone or in the Presence of Polystyrene Particles*

	Q _{O₂}		CO ₂ (c.p.m.)						Lactate	
	-	+	-	+	-	+	-	+	-	+
Normal ²	7.4	18.5	43.5	323.9	2.2	9.0	1675	1554		
Iodoacetate (1 × 10 ⁻⁴ M)	6.5 ± 0.2	13.8 ± 1.3	17.0 ± 4.4	106.5 ± 16.1	3.9 ± 1.2	15.0 ± 3.1	428 ± 46	581 ± 171		
Normal	6.3 ± 1.6	22.9 ± 9.7	27.6 ± 4.2	245.1 ± 115.7	1.7 ± 0.0	13.1 ± 7.3	1269 ± 208	1676 ± 246		
2 × 10 ⁻² M NaF	6.9 ± 1.5	9.5 ± 3.0	22.8 ± 7.1	40.5 ± 19.7	2.9 ± 1.2	7.1 ± 3.4	324 ± 66	355 ± 61		
Normal	6.7 ± 0.8	17.8 ± 4.9	20.1 ± 4.9	132.2 ± 43.2	0.9 ± 0.2	8.8 ± 3.9	1503†	1915†		
1 × 10 ⁻⁴ M DNP	13.8 ± 2.7	19.5 ± 4.5	32.3 ± 3.9	149.5 ± 36.9	14.1 ± 3.9	11.1 ± 2.7	3011 ± 434	2925 ± 139		
Normal	6.5 ± 0.9	28.2 ± 1.0	18.5 ± 2.4	286.3†	0.9†	11.9†	1220 ± 62	1473†		
1 × 10 ⁻³ M KCN	2.4 ± 0.9	10.4 ± 0.9	16.5 ± 4.2	216.4 ± 6.8	0.5 ± 0.2	0.5 ± 0.2	2610 ± 206	2356 ± 188		

* - signifies absence and + presence of particles. Q_{O₂} expressed as microliters O₂ per milligram protein and hour. C-1 and C-6 represent the CO₂ derived from glucose-1-C¹⁴ and glucose-6-C¹⁴, respectively. The data are given as millimicromoles per milligram protein and hour calculated from the specific activity of the substrate glucose. The data for lactate are given as millimicromoles per milligram protein and hour.

† These values determined in only one experiment. All other data are expressed as the mean ± range for two experiments. Values within each experiment were obtained from triplicate flasks.

in the conversion of glucose carbon or acetate carbon into lipids. The same phenomenon was observed in the case of phosphate- P^{32} (17, 19). A number of experiments was performed with alveolar macrophages and monocytes. In order to determine the effect on this phenomenon of adhesion to a surface, experiments were also performed with polymorphonuclear leukocytes and peritoneal monocytes adhering to a glass surface. The data for phosphate- P^{32} are sum-

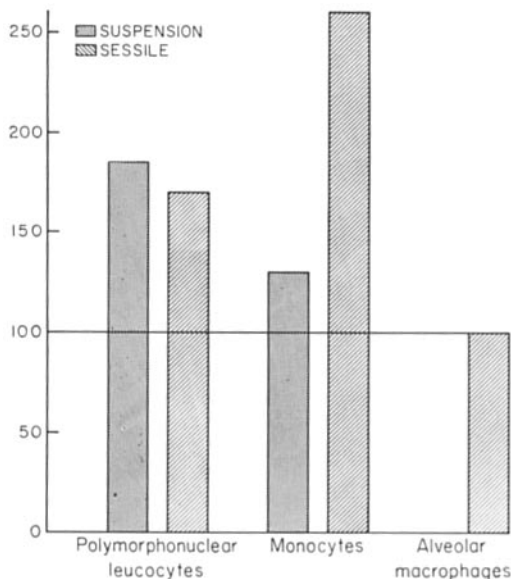


FIGURE 6

Effect of phagocytosis on the incorporation of inorganic phosphate- P^{32} into lipids. The specific activities of the lipids of resting cells are set at 100 per cent, and all other values expressed as percentages of resting values (ordinate). Experiments were performed with suspended and adhering cells in the case of PMN and MN. In the case of AM only adherent cells were used.

marized in Fig. 6. There was no increase in incorporation of inorganic phosphate- P^{32} into the lipids of phagocytizing alveolar macrophages over that observed with cells at rest. Similarly, no stimulation of incorporation of glucose carbon into lipids of alveolar macrophages was detected during ingestion of particles.

In the case of monocytes a definite increase in incorporation of P^{32} into phosphatides was detected when the cells were free-swimming in suspension and also when they were adhering to a glass surface. The results in the case of poly-

morphonuclear leukocytes adhering to glass conformed to those previously determined on suspended cells.

DISCUSSION

The reasons for the very large increase in oxygen uptake of polymorphonuclear leukocytes during phagocytosis, and the associated stimulation of conversion of glucose carbon-1 to CO_2 , have been explored by Evans and Karnovsky (20, 21). These changes have been shown to be due to the presence of a cyanide-insensitive oxidase for reduced pyridine nucleotides. This enzyme favors DPNH over TPNH by a factor of 10 and is activated under conditions of low pH. Such conditions are thought to prevail in the cell after the burst of glycolysis which accompanies particle ingestion. The second key enzyme in this context is thought to be a TPNH-linked lactic dehydrogenase which is also activated at low pH and which serves to increase the provision of TPN through the conversion of excess pyruvate (resulting from reoxidation of DPNH by the oxidase above) to lactate. The increased availability of TPN in turn stimulates the direct oxidative pathway for glucose-6-phosphate. Most of the increase in oxygen uptake during phagocytosis apparently does not yield energy for the ingestion process and may be regarded as a concomitant rather than an essential metabolic change (20).

Whether similar mechanisms operate in the stimulation of respiration of phagocytizing monocytic cells is not yet clear. As shown in Table I, however, these cells are considerably more active with respect to their respiratory processes than are the polymorphonuclear leukocytes. From the data given in Table III it is manifest that, as with polymorphonuclear cells, at least a part of the increase in oxygen uptake that accompanies phagocytosis is cyanide-insensitive and that the large increase in the conversion of glucose carbon-1 to CO_2 is similarly cyanide-insensitive. On the other hand, in contrast to PMN, phagocytosis by monocytes stimulates the conversion of glucose carbon-6 to CO_2 to a greater degree than that of carbon-1. The pattern of metabolism found in phagocytizing monocytes is however, on the whole, quite similar to that of polymorphonuclear leukocytes.

The alveolar macrophages differ from both polymorphonuclear leukocytes and monocytes in that the resting respiration of the alveolar macro-

phages is high and phagocytosis causes only a small increase in oxygen uptake and in glucose oxidation. As judged from the data obtained with metabolic inhibitors, these cells appear to depend heavily on cytochrome-linked respiration, and energy provided by oxidative phosphorylation, for optimal particle ingestion.

The greater efficiency of aerobic cells able to derive energy by oxidative phosphorylation, in terms of the yield of metabolic energy per mole of glucose used, may be reflected in the very small changes in metabolism that accompany particle ingestion by the alveolar macrophage. Aerobic metabolism of glucose and electron transport *via* a cytochrome-linked system (*i.e.* a cyanide-sensitive system) would yield 38 high energy phosphate bonds per mole of glucose oxidised to CO₂. By comparison, a less efficient cell that obtains its energy mainly by glycolysis would have a net yield of 2 high energy phosphate bonds per mole of glucose utilized. It might be unnecessary for the efficient cells (alveolar macrophages) to increase their metabolic activity to any great degree in order to perform the additional work of particle ingestion. It is teleologically of some interest to note that alveolar macrophages, which presumably are not called upon to function under conditions of low oxygen tension, appear to be dependent on oxygen for efficient particle uptake. Polymorphonuclear leukocytes and monocytes, on the other hand, are able to carry out the task just as well under anaerobic as under aerobic circumstances. This observation is interesting in that the two latter types of cell may be found in regions where low oxygen tensions prevail (*e.g.*, areas of inflammation).

It has been noted previously that the stimulation of labeling of lipids, particularly phosphatides, in phagocytizing polymorphonuclear leukocytes, is most notable in the case of phosphatidic acid, phosphatidyl serine, and inositol phosphatide (19). These findings have been considered in relation to the formation and budding off of phagocytic vesicles, and the internalization of parts of the

plasma membrane of the cell. This sequence of thought stems from the fact that biological membranes are rich in complex lipids. A comparison has been drawn with secreting cells, such as the acinar cells of the pancreas, where the obverse process (egestion) occurs, and similar stimulation of specific phosphatides was noted (22, 23).

The failure to detect an increased labeling of lipids in the case of alveolar macrophages when they are engulfing particles is puzzling. These cells exhibit the same morphologically observable pattern of ingestion; *i.e.*, formation of pseudopodia, engulfment of particles, and movement of vesicles bounded by membrane into the cell (5). It is conceivable that the failure thus far to detect stimulation of incorporation of phosphate-P³² into lipids during phagocytosis is due to the greater rate of conversion of inorganic phosphate to ATP in these cells and the rapid equilibration of inorganic, ester, and anhydride phosphates. This possibility would be consistent with their higher metabolic efficiency, due to their greater capacity for oxidative phosphorylation. Although techniques have not yet been completely worked out for following these phenomena in more detail with the rather limited amounts of cells available, it would seem important to examine the reason for this apparent discrepancy between those cells that do not depend upon the availability of oxygen for optimal particle uptake (polymorphonuclear leukocytes and peritoneal monocytes) and those that do (macrophages from the lung alveoli). Since only the total phosphatides have been examined so far, it may also be that changes in specific types of phosphatide are obscured.

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