The Biochemical Basis of Phagocytosis

I. METABOLIC CHANGES DURING THE INGESTION OF PARTICLES BY POLYMORPHONUCLEAR LEUKOCYTES*

ANTHONY J. SHARIA AND MANFRED L. KARNOVSKY†

From the Departments of Bacteriology and Biological Chemistry, and the Biophysical Laboratory, Harvard Medical School, Boston, Massachusetts

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For many decades the phenomenon of phagocytosis has been of prime interest to medical scientists as an important factor in the defense of the host in infectious disease (1, 2). As a manifestation of cellular activity, it has also held the interest of biologists in general. However, the biochemical mechanisms that underlie the ingestion of relatively large particles by phagocytes have not been systematically studied.

Occasional reports have appeared in the literature which indicated a transient increase in oxygen uptake by leukocytes during phagocytosis (3-5). During studies of the host-parasite relationship in our laboratory (6-8), a dramatic increase in the oxygen uptake rates of polymorphonuclear leukocytes or monocytes was observed while tubercle bacilli were being ingested. Such observations might be interpreted as offering evidence contrary to widely held opinions that phagocytosis is a process which does not require a specific expenditure of energy on the part of the phagocytizing cell (9, 10). The present paper contains results of a study of some changes in the metabolic patterns of leukocytes which occur during the phagocytic event. In particular the relationship between glucose metabolism and phagocytosis, and the influence of various inhibitors on both processes, have been examined. Preliminary reports have appeared previously (11-13).

EXPERIMENTAL

Guinea pigs weighing 300 to 400 gm. were used in all experiments. The animals, usually males, were purchased from outside dealers and were fed with pelleted, water ad libitum, and cabbage.

Suspensions of Polymorphonuclear Leukocytes—The preparation of suspensions rich in polymorphonuclear leukocytes has previously been described (6). Sixteen to 18 hours after the intraperitoneal injection of a sterile 12 per cent solution of sodium caseinate, an exudate rich in polymorphonuclear leukocytes (80 to 90 per cent) was collected by washing the peritoneal cavity with 40 to 50 ml. of physiological sodium chloride solution containing 0.005 per cent heparin. The cells were collected in 50 ml. cellloid tubes and were lightly centrifuged and resuspended in phosphate-free Krebs-Ringer solution. The cells were maintained in the cold (0-4°) after removal from the animals. Total cellular phosphorus of this suspension was measured on aliquots. Such determinations were found to be a more satisfactory measure of cellular protoplasm than conventional counting methods; 100 μg. of cellular phosphorus represents 5.53 × 10⁶ cells. Phosphate buffer was now added to the suspension to yield a suspension of cells in the usual Krebs-Ringer phosphate medium buffered at pH 7.4.

Inert Particles—Two types of inert particle were used for phagocytosis experiments: polystyrene latex spherules, usually 1.17 μ in diameter, and insoluble starch granules of Amaranthus cruentus (14). These were suspended in the buffered medium. The authors are deeply indebted to Dr. J. W. Vanderhoff and the Dow Chemical Company for a generous supply of polystyrene particles, and to the Northern Regional Research Laboratories for a gift of starch particles.

Metabolic Inhibitors—All of the common inhibitors were of analytical reagent grade. Antimycin A was obtained from the Wisconsin Alumni Research Foundation. It was dissolved in a small quantity of ethanol and brought to volume with Krebs-Ringer phosphate medium. In control experiments the relevant amount of alcohol was included.

Radioactive Substrates—Uniformly labeled glucose-C³⁴, glucose-1-C³⁴ and glucose-6-C³⁴ were procured from the Nuclear-Chicago Instrument and Chemical Company. Glucose-1-C³⁴ and glucose-6-C³⁴ samples were also obtained through the courtesy of Dr. H. Isbell of the National Bureau of Standards.

Phagocytosis-Promoting Factors—It has previously been reported that phagocytosis by leukocytes occurs maximally only in the presence of serum. This is usually regarded as being due to the presence of serum complement. Fresh guinea pig serum or stored horse serum were used in early experiments to promote phagocytosis. When it was necessary to avoid complications in metabolic measurements due to the presence of small organic molecules in serum, a solution of a thoroughly dialyzed protein fraction of human plasma was substituted for serum. This protein fraction was kindly provided by Drs. James Tullis and D. Surgenor of the Protein Foundation, and is referred to by those workers as “Phagocytosis-Promoting Factor” (15). It is a mixture of α-1 and β-globulins, and was supplied as a frozen-dried powder containing NaCl.

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† Work carried out during the tenure of a Lederle Medical Faculty Award.

1 “Phagocytosis” in this paper is used to describe the process of ingestion of particles by cells. The fate of the particles ingested is not considered here.
General Experimental Procedure  The phagocytosis experiments were carried out in Warburg flasks at 37° in the following way. The main compartment contained the leukocyte suspension (about 5.5 x 10⁶ cells; 100 μg. of cellular phosphorus) in 1 ml.; serum (1.0 ml.) or phagocytosis-promoting factor (286 μg. of protein N); the final volume in this compartment was 2.6 ml. The side arm contained 0.2 ml. of glucose (30 μmoles) and 0.2 ml. of particles (10 mg.; about 2 x 10¹⁹ particles in the case of polystyrene or about 4.5 x 10¹⁷ particles in the case of the starch). In control flasks, the side arm contained 0.2 ml. of medium. Where metabolic inhibitors were used, these were also placed in the side arm. The center well contained 0.2 ml. of 20 per cent KOH solution and a filter paper fan or, in the case of experiments with KCN as inhibitor, KOH-KCN mixtures as described by Umbreit et al. (16). After equilibration of the flasks the side arms were tipped, and measurements of oxygen uptake made for a suitable period.

Chemical Determinations—When chemical analysis of the flask contents was desired, phagocytosis was stopped by adding 0.5 ml. of 0.1 N iodoacetate to each flask and immediately transferring the contents of each flask quantitatively to centrifuge tubes held at ice-bath temperature. The cells were then centrifuged and washed twice. The supernatant fluids were collected, brought to volume, and analyzed. The cells were digested and their glycogen content was determined. Glucose and glycogen analyses were determined by the methods of Mendel et al. (17) and Kemp et al. (18). Mr. Warren Evans of these laboratories, with the use of guinea pig exudates, has compared these methods with the more conventional methods of Nelson (19) and Good et al. (20) and has found them to yield identical results. Lactic acid was determined by the method of Barker and Summerson (21).

Determinations of Radioactivity—At the conclusion of the incubation the contents of the center well were quantitatively recovered, and the carbonate precipitated as BaCO₃ (22). The activities of glucose samples used as substrate were determined on their osazones. All radioactive measurements were carried out as previously described (23).

Counting of Engulfed Particles—At the conclusion of many experiments, phagocytosis was measured by counting the number of leukocytes showing engulfment according to the method of Hamburger (24) under the phase microscope.

RESULTS

Effect of Particle Concentration on Respiration During Phagocytosis—The extent of the increased oxygen consumption when polymorphonuclear leukocytes were allowed to engulf particles is exemplified in Fig. 1. Respiration of phagocytizing cells was more than double that of "resting" cells during the first hour after tipping in the particles, and later declined. It was important to discover whether this effect was related to the number of particles engulfed, and experiments were thus carried out in which the concentration of particles offered to a constant number of leukocytes was varied. The results of a typical experiment are shown in Fig. 2. The increment in oxygen uptake increased with the number of particles available for engulfment until a maximal value was reached.

Requirement of Serum in Phagocytosis—The influence of serum concentration in the presence of an optimal concentration of available particles was studied. The results of such experiments are presented in Fig. 3. The increment in oxygen uptake during phagocytosis was found to vary with the concentration of serum added. At very low serum levels the oxygen uptake of cells in the presence of particles was only slightly higher than the resting level. Maximal stimulation occurred when serum levels reached about 30 per cent of the medium.

In all the experiments mentioned above it was found that particle uptake, determined by direct count under the phase microscope correlated well with the increment in oxygen uptake. Thus, cells which had exhibited considerably increased respiration always contained numerous particles, whereas those whose respiratory level was close to the resting level contained few particles; for example, without added serum about 30 per cent of the...
cells contained 1 to 10 particles per cell. In the presence of 66 per cent serum in the medium, 91 per cent of the cells contained particles, usually more than 10 particles per cell. A point of considerable importance was the fact that serum heated at 56° for 30 minutes was inactive in stimulating phagocytosis as measured by direct count. Only 13 per cent of the cells contained particles. There was also no increase in oxygen uptake. This conforms with previous observations by others on the phagocytosis-promoting role of serum (25), and emphasizes the correlation between increased respiration and particle uptake under aerobic conditions. The limitations of the method of direct counting of engulfed particles as a measure of phagocytosis will be discussed later. Application of this method in a few key experiments is presented in Table I.

In ensuing experiments aimed at obtaining information on metabolic changes during phagocytosis, it was desirable to eliminate serum from the medium since serum contains various small molecules which might obscure the picture. Consequently the phagocytosis-promoting factor of Tullis and Surgeor (15) was substituted for serum, at a level equivalent to 30 per cent serum in the medium. This protein fraction was as effective in permitting phagocytosis as serum, by the criteria of direct count. Only 13 per cent of the cells contained particles.

Glucose Utilization and Lactate Production During Phagocytosis Under Aerobic and Anaerobic Conditions—The cells used in these experiments exhibit a marked Pasteur effect as may be seen in Table I below. Further, they show a definite Crabtree effect. In previous work these cells were incubated in the absence of exogenous glucose, and showed a \( Q_{O_2} \) (P) of 36.5 \( \pm \) 2.5 \( \mu \)l in the absence of glucose and 38.3 \( \pm \) 2.0 \( \mu \)l in the presence of glucose, for a given concentration of particles in the medium.

In order to obtain maximal differences in glucose utilization and lactate production between resting and phagocytizing cells, the exposure of leukocytes to particles was restricted to a period of only 30 minutes. The results of these experiments are shown in Table II. It should be mentioned that phagocytosis, determined by direct count, proceeded just as well in the absence of oxygen as in its presence (see Table I). Glucose consumption and lactate production by resting cells under anaerobic conditions were, as expected, considerably greater than under aerobic conditions. The values of 25.6 and 29.2 were obtained in the absence of glucose. Further, they show a definite Crabtree effect.

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Pathways of Glucose Utilization During Phagocytosis—It has been previously observed in these laboratories that phagocytosis induced a great increase in $CO_2$ production from glucose carbon 1 relative to glucose carbon 6 (8). In those experiments the medium included fresh guinea pig serum (2 per cent) which created some difficulty in establishing the quantitative significance of the observations. This difficulty was eliminated in the present experiments, as has been mentioned above, by substituting a phagocytizing factor for serum. In Table III are given the results of experiments carried out with glucose-l-$C^4$ and glucose-6-$C^4$, with resting and phagocytizing cells. It may be seen that while oxygen uptake and conversion of glucose carbon 6 to $CO_2$ was enhanced almost to the same degree as was respiration. In the presence of particles, there appeared to be a further increase in oxygen uptake, but no significant change in the conversion of glucose carbon 1 to $CO_2$. Again, phagocytosis was depressed to the minimal level.

The effects observed with potassium cyanide and antimycin A were very similar. In both cases active phagocytosis was manifested although the respiration of resting cells was greatly diminished. The phagocytic event, however, was accompanied by a real increment in oxygen uptake which, at least in the case of antimycin A, was not different from the increment obtained during phagocytosis in the absence of the inhibitor. The appearance of glucose carbon 1 as $CO_2$ was enhanced almost to the same degree as was respiration. In the presence of particles, there appeared to be a further increase in oxygen uptake, but no significant change in the conversion of glucose carbon 1 to $CO_2$. Again, phagocytosis was depressed to the minimal level.

The experiments were carried out at several concentrations of each inhibitor, but the tables quote results for only those concentrations at which the most important results were obtained. Sodium iodoacetate decreased glycolysis, as was expected, and depressed phagocytosis to a minimal level. No changes in the pattern of glucose utilization were observed in the presence of particles as compared with resting cells. In the case of sodium fluoride, respiration of resting cells was greatly increased and lactate production diminished. The appearance of glucose carbon 1 as $CO_2$ was enhanced almost to the same degree as was respiration. In the presence of particles, there appeared to be a further increase in oxygen uptake, but no significant change in the conversion of glucose carbon 1 to $CO_2$. Again, phagocytosis was depressed to the minimal level.

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### Table IV

Influence of iodoacetate on respiration and glucose utilization, and phagocytosis*

<table>
<thead>
<tr>
<th>Particles</th>
<th>Activity in CO₂</th>
<th>Lactate</th>
<th>Phagocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QO₂ (P)</td>
<td>C₁₁</td>
<td>C₆₁</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td>µ₄</td>
</tr>
<tr>
<td></td>
<td>µ₄</td>
<td>µ₅</td>
<td>µ₆</td>
</tr>
<tr>
<td>1 × 10⁻⁴ m iodoacetate</td>
<td>16.9 ± 2.0 43.9 ± 2.3</td>
<td>284 ± 25 2191 ± 70</td>
<td>55 ± 2 306 ± 16</td>
</tr>
<tr>
<td>p ↓</td>
<td>&lt;0.5</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Experiments</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

* All results are given on the basis of 100 µg. of cellular phosphorus per hour. Radioactivity data are normalized to 1 × 10⁵ c.p.m. added to the flasks as glucose.

† Glucose labeled in C₁ or C₆ as substrate.

‡ Refers to probability values for differences due to the inhibitor. The normal (control) values (top line) are given only for experiments concerning this inhibitor. For all normal values obtained see Table III.

§ Refers to probability values for differences caused by the presence of particles when the inhibitor was present.

### Table V

Influence of NaF on respiration and glucose utilization, and phagocytosis*

<table>
<thead>
<tr>
<th>Particles</th>
<th>Activity in CO₂</th>
<th>Lactate</th>
<th>Phagocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QO₂ (P)</td>
<td>C₁₁</td>
<td>C₆₁</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td>µ₄</td>
</tr>
<tr>
<td></td>
<td>µ₄</td>
<td>µ₅</td>
<td>µ₆</td>
</tr>
<tr>
<td>2 × 10⁻² m NaF</td>
<td>45.6 ± 2.2 56.2 ± 3.6</td>
<td>459 ± 37 506 ± 71</td>
<td>26 ± 6 36 ± 11</td>
</tr>
<tr>
<td>p ↓</td>
<td>&lt;0.01</td>
<td>&lt;0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>p →→</td>
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<td>&lt;0.3</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Experiments</td>
<td>6</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

* The results are expressed on the same basis as in Table IV.

### Table VI

Influence of KCN on respiration and glucose utilization during phagocytosis*

<table>
<thead>
<tr>
<th>Particles</th>
<th>Activity in CO₂</th>
<th>Lactate</th>
<th>Phagocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QO₂ (P)</td>
<td>C₁₁</td>
<td>C₆₁</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td>µ₄</td>
</tr>
<tr>
<td></td>
<td>µ₄</td>
<td>µ₅</td>
<td>µ₆</td>
</tr>
<tr>
<td>1 × 10⁻³ m KCN</td>
<td>16.6 ± 1.9 45.9 ± 3.7</td>
<td>229 ± 29 1294 ± 336</td>
<td>56 ± 6 52 ± 17</td>
</tr>
<tr>
<td>p ↓</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.5</td>
</tr>
<tr>
<td>Experiments</td>
<td>6</td>
<td>6</td>
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</tbody>
</table>

* All results are expressed on the same basis as Table IV.

### Table VII

Influence of antimycin A on respiration and glucose utilization during phagocytosis*

<table>
<thead>
<tr>
<th>Particles</th>
<th>Activity in CO₂</th>
<th>Lactate</th>
<th>Phagocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QO₂ (P)</td>
<td>C₁₁</td>
<td>C₆₁</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td>µ₄</td>
</tr>
<tr>
<td></td>
<td>µ₄</td>
<td>µ₅</td>
<td>µ₆</td>
</tr>
<tr>
<td>3.3 µg./ml. anti-</td>
<td>10.3 ± 1.4 44.8 ± 13.0</td>
<td>217 ± 66 1304 ± 386</td>
<td>10 ± 10.27 ± 7</td>
</tr>
<tr>
<td>mycin A</td>
<td>p ↓</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Experiments</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

* All results are expressed on the same basis as Table IV.
result was that the C-1:C-6 ratios during phagocytosis in the presence of these inhibitors achieved levels of between 50 and 56.

When the effect of 2,4-dinitrophenol was studied, the results were of great interest. This substance caused a considerable (2-fold) increase in the resting level of oxygen uptake. Phagocytosis was unaffected, and the increment in oxygen uptake which accompanies the phenomenon was noted over and above the oxygen uptake already stimulated by dinitrophenol. This substance stimulated appearance of both glucose carbons 1 and 6 as CO₂ in resting cells. Only the conversion of glucose carbon 1 to CO₂ was further increased by the phagocytic event.

DISCUSSION

Serum Factors and Nature of Particle—The factors involved in phagocytosis have been studied for many years, and numerous claims have been made concerning substances promoting or inhibiting the process (27-28). These studies have been greatly impeded by the lack of rapid sensitive methods for evaluating the degree of phagocytosis in an accurate way. Most studies have depended on direct count of particles ingested (29), a process both tedious and only approximate. The inadequacy of such methods is brought out in the paper of Smith and Wood (30).

Thus, the counting data of Table I can be accepted as roughly indicative and do not present an adequate reflection of the great differences in appearance between cell suspensions which have phagocytized normally and those which have been inhibited. A gross excess of particles has always been used in these experiments to obtain maximal metabolic responses. The upper end of the scale in direct counting methods is thus obscured due to the extreme difficulty of determining the numbers of engulfed particles, such as increased oxygen uptake, during the process of engulfment might provide suitable techniques for precise studies. Increased oxygen uptake during phagocytic activity has now been demonstrated with two types of inert particle (insoluble starch and polystyrene) and with at least five different bacteria [Mycobacterium tuberculosis R, (6, 7); Bacillus subtilis (7); Micrococcus pyogenes var. aureus (5); Sarcina lutea (3); and Diplococcus pneumoniae (4)]. It has also been demonstrated with three types of phagocytic cells, polymorphonuclear leukocytes, monocytes (7), and mouse ascites tumor cells.³

³ Unpublished experiments with Dr. D. Wallach of this institution.

The nature of the surface of the particles offered to the cells has long been considered to be of primary importance (31). That the nature of the particle surface might also determine the nature of serum factors necessary for engulfment might be illustrated by the following points. It was shown in early experiments (Fig. 3) that the presence of serum was essential for satisfactory uptake of starch particles by leukocytes. As has been mentioned, the serum could be replaced by a fraction of human plasma (15). Later, it was found that in the case of polystyrene particles no serum or phagocytosis-promoting factor appeared to be necessary for phagocytosis. This situation might stem from the difference between the hydrophilic nature of the surface of starch particles and the lipophilic nature of the surface of polystyrene particles, and might be construed as giving support to some of the views expressed by Fenn (9) and Ponder (10) in which the surface forces involved are considered to be of prime importance in permitting phagocytosis. The formulation of the above authors, however, postulates that no special expenditure of energy on the part of the cell is necessary to accomplish the engulfment of particles. As is seen under "Results" considerable metabolic changes have consistently accompanied phagocytosis in the present work. Although the possibility has not yet been excluded that these changes may be sequela of particle ingestion, it is our view that the evidence presented here is in accord with the postulate that the cell does perform work requiring metabolic energy during the accumulation of particles. This is further discussed below.

Metabolic Changes During Phagocytosis—The metabolic patterns of the polymorphonuclear leukocytes obtained from the peritoneal cavity of the guinea pig and used in this study, are not dissimilar to the patterns exhibited by mixtures of human white cells. For example, Beck (32) has shown that leukocytes obtained from human blood show a very high aerobic glycolysis, and that about 20 times more glucose carbon is metabolized to lactate than is converted to CO₂. Calculations for resting guinea pig leukocytes based on results of Table II and measurements of the conversion of uniformly labeled glucose-C¹⁴ to CO₂, indicate that under aerobic conditions 12 times more glucose carbon appears as lactate than as CO₂. In experiments lasting for 1 hour in which phagocytosis occurred under aerobic conditions only twice as much glucose carbon appears as lactate compared with CO₂. The assumption has been made that all of the lactate formed comes from glucose (32). Computations carried out according to Beck (33) based on lactate production and on the appearance as CO₂ of the C¹⁴ from glucose-1-C¹⁴, glucose-6-C¹⁴, and uniformly

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**Table VIII**

Influence of dinitrophenol on respiration and glucose utilization during phagocytosis*

<table>
<thead>
<tr>
<th>Activity in CO₂</th>
<th>Lactate</th>
<th>Phagocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>C-6</td>
<td>C-1:C-6</td>
</tr>
<tr>
<td>Normal</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>1 X 10⁻⁴m dinitrophenol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>p &lt; 0.05</td>
<td>&gt;0.1</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Experiments</td>
<td>7</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* All results are expressed on the same basis as Table IV.
labeled glucose-C\textsuperscript{14}, would further indicate that in resting cells (polymorphonuclear leukocytes) only 3 to 6 per cent of the amount of glucose converted to lactate is metabolized by the direct oxidative pathway. In experiments of 1 hour duration, where phagocytosis was proceeding this figure rose to 20 to 40 per cent depending on whether the conversion of glucose-1-C\textsuperscript{14} or uniformly labeled glucose-C\textsuperscript{14} to C\textsubscript{14}O\textsubscript{2} was used in the calculation. The validity of such calculations is open to some question, since account is taken only of CO\textsubscript{2} which is trapped in the center well and CO\textsubscript{2} fixation is ignored (8).

The observations of an increment in oxygen uptake during phagocytosis provided the original indication that the cells expend metabolic energy in accumulating particles. The data obtained under anaerobic conditions, and under aerobic conditions in the presence of dinitrophenol indicate, however, that oxidative phosphorylation is not essential for maintenance of phagocytic activity in these cells, and the data with KCN and antimycin A show clearly that most of the oxygen uptake, especially during phagocytosis, is not cytochrome linked. The major part of the increment in oxygen consumption and the simultaneous great increase in the appearance of glucose carbon 1 as CO\textsubscript{2} would thus appear to be only concomitants of particle intake and perhaps not of prime importance in the process. It is, however, noteworthy that under aerobic conditions no situation has so far been uncovered in which phagocytosis occurs without these changes in metabolism. These data are of interest when considered in the light of the observations of Kaplan et al. (34) concerning TPNH oxidation which was accompanied by little or no oxidative phosphorylation. The question of the mechanism for reoxidation of TPNH in polymorphonuclear leukocytes, especially during phagocytosis, and the possible role of transhydrogenase, remains to be explored.

It might appear from the present data that glycolysis provides the initial energy for the phagocytic act in polymorphonuclear leukocytes, although the alternative possibility cannot be overlooked that it might furnish some substance needed for successful phagocytosis. In support of the conclusion concerning the essentiality of glycolysis the work of Fisher and Ginsberg (35; cf. 36) may be cited. These authors, with the use of guinea pig polymorphonuclear leukocytes, showed that during phagocytosis, is not cytochrome linked. The major part of the increment in oxygen consumption and the simultaneous great increase in the appearance of glucose carbon 1 as CO\textsubscript{2} would thus appear to be only concomitants of particle intake and perhaps not of prime importance in the process. It is, however, noteworthy that under aerobic conditions no situation has so far been uncovered in which phagocytosis occurs without these changes in metabolism. These data are of interest when considered in the light of the observations of Kaplan et al. (34) concerning TPNH oxidation which was accompanied by little or no oxidative phosphorylation. The question of the mechanism for reoxidation of TPNH in polymorphonuclear leukocytes, especially during phagocytosis, and the possible role of transhydrogenase, remains to be explored.

A point of interest concerns reutilization of lactate by leukocytes, mentioned by Beek (32). It may be noted that in short term experiments (30 minutes) anaerobically or aerobically, phagocytosis causes a definite increase in lactate production (Table II). If the aerobic experiments are protracted (1 hour) (Tables III through VIII) no excess production of lactate was noted due to phagocytosis (right hand columns of Tables III through VIII). It would appear that a situation similar to an oxygen debt in muscle pertains. Under the dramatic demand for an immediate energy supply, it might be postulated, glycolysis proceeds rapidly and lactate accumulates. As phagocytosis is completed and the immediate energy demand decreases, glycolysis is reversed. Presumably oxidative phosphorylation catches up due to the operation of the tricarboxylic acid cycle, which apparently has a low capacity in these cells.

The present study represents an attempt to investigate the metabolic processes which underlie an easily invoked function of a free swimming mammalian cell. The conclusions that have been drawn from the experiments described are that active glycolysis is essential for phagocytosis and probably provides the immediate energy supply required. Further, the process, if carried out under aerobic conditions, is accompanied by a dramatic increase in oxygen uptake most of which is not cytochrome-linked, and by a considerable stimulation of the direct oxidative pathway for glucose metabolism. The significance of these metabolic changes is as yet not fully understood, and remains under investigation.

**SUMMARY**

A study has been made of certain metabolic changes that occur in guinea pig polymorphonuclear leukocytes during the ingestion of inert particles. These cells can phagocytize several types of inert particles under suitable conditions. Polystyrene particles are particularly suitable. Phagocytosis occurred equally well under anaerobic or aerobic conditions.

During the uptake of particles under aerobic conditions the following metabolic changes were observed: (a) increased lactate production; (b) increased oxygen uptake; (c) increased appearance of C-1 of glucose as CO\textsubscript{2} relative to C-6 of glucose, i.e. C-1: C-6 ratio rose from 0.6 to 9.1 for resting cells to 21.6 for phagocytizing cells. In addition, under anaerobic conditions a significant increase in glycerol and glucose utilization and lactate production was observed during phagocytosis.

Interference with glycolysis by iodonate and fluoride inhibited phagocytosis. Antimycin A, cyanide, and dinitrophenol had no effect on phagocytosis, although the metabolic patterns of the cells were considerably altered.

It has previously been postulated by others that phagocytosis is a process not requiring a specific expenditure of metabolic energy, but the present experiments indicate that active glycolysis is essential for the occurrence of the process in polymorphonuclear leukocytes, and that increased metabolism is involved in particle uptake.

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