RELATION OF CELL METABOLISM TO INFECTION WITH RICKETTSIAL AND BACTERIAL AGENTS

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The metabolic events which influence the invasion and subsequent multiplication of microorganisms constitute a complex array of often subtle interactions between the parasite and a variety of body cells. Although this subject has been the focus of interest for some time, it has not yet been possible to synthesize the data into a coherent system. In part, this is a function of the diverse properties of the parasites, host cells, and the environments in which they interact. Furthermore, the determinants of the various stages of the infectious process may differ one from the other. Many examples can be given (1) in which generalized metabolic disturbances, e.g., diabetes mellitus or starvation, markedly influence the outcome of infections. In general, the site, or more likely sites, at which these derangements affect resistance mechanisms are speculative.

Instead of reviewing this interesting body of information attention will be focused on two specific examples in which metabolic changes in either host cell or parasite result in alterations in activity. The first deals with an obligate intracellular parasite (rickettsia) and host cells which support its multiplication. The second deals with what might be termed an “obligate extracellular parasite” (bacteria) and a cell (leucocyte) which destroys it upon ingestion. Both interactions will be illustrated with systems in vitro in which it has been possible to isolate a portion of the infectious process and to study the influence of both parasite and host. In the first case evidence will be presented which suggests that the metabolic integrity of the rickettsiae plays an important role in the invasion of host cells, whereas the second demonstrates the importance of the leucocyte’s metabolism on the ingestion of bacteria.

RICKETTSIAE

General Considerations

In the past decade a number of studies have appeared which have served to distinguish rickettsiae from other microorganisms. Table 1 outlines certain of the properties of rickettsiae, bacteria, and the smaller animal viruses.

The rickettsiae are infectious agents which produce disease in man and which parasitize a wide range of animals. All are considered to be obligate intracellular parasites, and none have been cultivated on cell-free media. Morphologically they are coccobacillar or bacillar in shape and are visible with the light microscope when stained by the Giemsa or Macchiavello procedures. The electron microscope (2) has demonstrated a limiting membrane and little in the way of cytoplasmic structure. The early studies of Wolbach (3) suggested that the rickettsiae divide by a process analogous to binary fission. This has more recently been unequivocally demonstrated by Schaechter et al. (4) who, by means of phase contrast microscopy, observed rickettsiae dividing in tissue culture cells. In terms of chemical, morphological, and antigenic composition, they are complex organisms which differ markedly from the smaller animal viruses. The studies of Schaechter et al. (5) on the isolation and characterization of a rickettsial “cell wall” have indicated that the limiting envelope of Rickettsia typhi (Rickettsia mooseri) is composed of polypeptide, lipid, and polysaccharide moieties, whose general composition resembles that found in bacteria. Both type and group specific antigens are demonstrable and one of these, the erythrocyte sensitizing substance, appears to be localized in the cell wall. Studies on the nucleic acid composition of R. typhi (6) have shown both deoxyribonucleic and ribonucleic acids in a ratio of approximately 3:1, similar to that of the bacteria.

A feature which has so far set them apart from the viruses is an autonomous metabolism. As first revealed by Bovarnick and Synder (7), highly purified suspensions of typhus rickettsiae are able to oxidize a number of the intermediates of the Krebs citric acid cycle, the most actively utilizable substrate being L-glutamic acid. Other intermediates such as succinate, oxaloacetate, pyruvate, and α-ketoglutarate are oxidized to a lesser extent, and this is thought to be related to
TABLE I

<table>
<thead>
<tr>
<th>Property</th>
<th>Rickettsiae</th>
<th>Bacteria</th>
<th>Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode of multiplication</td>
<td>Binary fission</td>
<td>Binary fission</td>
<td>Synthesis de novo</td>
</tr>
<tr>
<td>Intracellular parasitism</td>
<td>Obligate</td>
<td>Faculative</td>
<td>Obligate</td>
</tr>
<tr>
<td>Growth in cell-free media</td>
<td>0*</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Complexity:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Structural</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Chemical</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Antigenic</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Autonomous metabolism:</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Energy yielding</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Synthetic</td>
<td>?</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Nucleic acids (3:1)</td>
<td>RNA + DNA</td>
<td>RNA + DNA</td>
<td>RNA or DNA</td>
</tr>
<tr>
<td>Antibiotic sensitivity</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>“Eclipse” phase in growth cycle</td>
<td>0</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Site of in vivo multiplication</td>
<td>Endothelial cells</td>
<td>Phagocytic cells or extracellularly</td>
<td>Wide range of parenchymal cells</td>
</tr>
<tr>
<td>Nutritional requirements for in vitro growth</td>
<td>Complex</td>
<td></td>
<td>Less complex than rickettsiae (?)</td>
</tr>
<tr>
<td>Rate of intracellular growth in vitro</td>
<td>Slow</td>
<td>Variable</td>
<td>Rapid</td>
</tr>
<tr>
<td>“Viability” associated with biological</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>properties</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ = Demonstrated; 0 = not demonstrated; RNA = ribonucleic acid; and DNA = deoxyribonucleic acid.

Their inability to penetrate the rickettsial cell membrane (8). Similar findings have been reported by Price (9) for members of the Rocky Mountain spotted fever group. The terminal respiratory pathway of R. typhi has been studied by Hayes et al. (10) with sensitive spectrophotometric methods and was found to be mediated through a flavin enzyme-iron-cytochrome system which probably includes cytochromes A₁ and B₁. This finding is in keeping with the large quantities of riboflavin which are extractable from this organism (11). The esterification of phosphate into high energy compounds such as adenosine triphosphate was demonstrated by Bovarnick (12). A preliminary investigation of the acid-soluble nucleotides of R. typhi has revealed adenosine monophosphate, adenosine diphosphate, and diphosphopyridine nucleotide as well as at least two other ultraviolet-absorbing compounds which are as yet unidentified (6).

It seems by inference, therefore, that although none of the enzymes catalyzing the above reactions has been demonstrated in cell-free preparations, the rickettsiae contain relatively complete energy-yielding systems. As yet none of the steps in the Embden-Meyerhof cycle has been demonstrated and there is no utilization of glucose by these organisms. In view of adenosine triphosphate synthesis, it has been of interest to examine whether these organisms can carry out the synthesis of macromolecules in vitro. Early attempts by Hoppes et al. (13) showed neither the incorporation of glutamic acid-C₁⁴ into rickettsial protein, nor the incorporation of P³⁸ into nucleic acids. However, Bovarnick (14), employing a specific set of conditions which maintained rickettsial viability in vitro, detected a small but constant incorporation of methionine-S³⁴ into rickettsial protein. The well-known sensitivity to the rickettsiae to broad spectrum antibiotics such as chloramphenicol is pertinent in this regard, since one of the important actions of this compound is the inhibition of protein synthesis.

From the preceding characteristics of the rickettsiae it appears that they resemble fastidious bacteria-like agents. Further modifications in the medium in which the purified organisms are suspended will no doubt result in the detection of new metabolic properties—the goal of all these studies being extracellular propagation. One of the problems in metabolic studies of purified rickettsiae is their permeability, which results in
the loss of important coenzymes and even nucleic acids upon incubation in isotonic media (6, 14). The loss of coenzymes has striking effects on the biological activities of the rickettsiae, as we shall see somewhat later.

Developments in tissue culture methodology have made it possible to investigate the interactions between various rickettsiae and homogeneous host-cell populations. It has been possible to study quantitatively the growth rates of these organisms, as well as to investigate their nutritional requirements for intracellular multiplication. Employing the MB III strain of mouse lymphoblast, Bozeman et al. (15) have shown that Rickettsia tsutsugamushi, the agent of scrub typhus, multiplies at a rather slow rate, with a 3-fold increase in 24 hr. This rate is essentially the same in a number of cell lines (16). It is similar to the growth rate of certain mycobacteria in tissue culture cells (17), and much slower than that of the animal viruses (18). Cells which are infected with rickettsiae show little in the way of cytopathic changes (19), and are capable of mitosis and cell division. Destruction of the host cells seems to occur only when the cytoplasm is engorged with the organisms. Infected tissue culture cells show no striking changes in either respiration or glycolysis (15). Recently published experiments by Hoppes et al. (20) suggest that the intracellular growth requirements for R. tsutsugamushi are complex and are intimately related to the requirements of the host cell. Conditions which favor the multiplication of the particular host cell also favor the growth of rickettsiae. These findings are in contrast to some of the earlier studies of Zinsser and Schoenbach (21), in which nonviable cells were thought to represent a better growth medium for the rickettsiae. The studies of Hoppes et al. also suggest differences in the requirements of rickettsiae and animal viruses, in that there are a number of examples in which tissue culture cells support the propagation of viruses in simple media (22, 23). These experiments are of considerable theoretical interest, since if we assume that rickettsiae are capable of carrying out independent metabolic processes, then what function does the host cell play in their nutrition? Perhaps the intracellular environment supplies a constant flow of cofactors and complex substrates, as well as constituting a physiochemical milieu which controls rickettsial permeability. This is merely speculative at the moment but represents a field in which further investigation is required.

There are two biological properties of rickettsiae which deserve special mention: first, their ability to lyse mammalian erythrocytes both in vivo (24) and in vitro (25); and second, their acute toxicity following intravenous injection into small rodents. Although both these properties have counterparts in the bacteria, e.g., hemolysins of streptococci and lipopolysaccharide endotoxins of gram-negative bacilli, there are distinguishing features which set them apart from the phenomena as seen with the rickettsiae. The most important distinction is that both hemolysis and toxicity are detectable only with intact, viable rickettsiae.

The toxicity of rickettsiae was first noted by Gildemeister and Haagen in 1940 (26) and has subsequently been described for R. prowazekii, R. rickettsii (9), and R. tsutsugamushi (27). This effect can be demonstrated in mice, rats, and rabbits. Initial pathophysiological studies by Neva and Snyder (28) revealed that a characteristic series of events occurs in mice and rats following the intravenous injection of typhus rickettsiae, in which there is progressive hemoconcentration, followed by terminal hypotension and death. Adrenocorticotropic hormone and cortisone have no influence on the toxic symptoms, and no circulating permeability or toxic factor has been uncovered. Wattenberg et al. (29) have also reported the marked changes in the permeability of the smaller blood vessels. More recently Greisman and Wisseman (30), employing the rat mesappendicular vascular bed, have described a specific type of increase in capillary permeability, which is qualitatively different from that produced either by the endotoxin of Salmonella typhosa or acute blood loss shock. From their results it appears that viable rickettsiae have a direct injurious effect upon the vascular, perhaps specifically upon the endothelial cell. This is certainly consistent with the pathology of the rickettsioses, in which the site of multiplication in man is chiefly the endothelial cell, with subsequent vascular changes which can account for the majority of the signs and symptoms in these diseases.

The lysis of erythrocytes is another function of rickettsiae which is related to the viability and metabolic activity of the organism. Studies by Snyder et al. (31) in vitro have shown the im-
importance of glutamic acid for this phenomenon. There is evidence that metabolic inhibitors which block the utilization of this substrate by rickettsiae, inhibit the reaction.

Perhaps the most striking example of the association between the metabolic properties of rickettsiae and their biological attributes can be found in the reactivation studies of Bovarnick and Allen (32, 33). In brief, purified suspensions of typhus rickettsiae which have been allowed to incubate in the cold or have been frozen and thawed lose their ability to (a) infect eggs, (b) lyse erythrocytes, (c) produce death of mice upon intravenous injection, and (d) oxidize glutamic acid. Associated with this treatment is the loss of diphosphopyridine nucleotide from the rickettsiae into the medium. All of these activities can be restored by incubating the “inactivated” organisms with glutamate and diphosphopyridine nucleotide at higher temperatures (33°C). Although the loss of metabolic activity through the diffusion of a critical coenzyme is not too difficult to imagine, the loss of infectivity and toxicity and their reactivation with a specific coenzyme is unusual in microbiology. The experiments of Gilford and Price (34), in which avirulent-virulent conversions of R. rickettsii could be produced in vitro with large amounts of diphosphopyridine nucleotide, are somewhat analogous.

Penetration of R. tsutsugamushi into Mammalian Cells in Vitro

With these unique properties in mind, studies were conducted at the Walter Reed Army Institute of Research to investigate the requirements which governed the invasion of R. tsutsugamushi into mammalian epithelial cells in vitro (35). Employing the MB III cell line which grows as free-floating cells in the nutritive medium, the penetration of partially purified suspensions of rickettsiae was studied under various environmental conditions. Penetration was evaluated microscopically by the number of rickettsiae visualized within the cytoplasm of the MB III cell, characteristically in the juxtanuclear region. With this system, only the over-all penetration process was evaluated, so that factors related to the adsorption of rickettsiae could not be ascertained. When the complete medium for the propagation of the MB III cell was employed, containing Gey’s basic salt solution (BSS), horse serum, and beef embryo extract, the penetration of rickettsiae was rapid with 80 per cent of the organisms entering during the first 30 min. As the incubation progressed the penetration became asymptotic, until after 120 min no further invasion occurred. Penetration of the organism in BSS alone was 50 per cent of that in the complete medium. If one inactivated the rickettsiae, in reference to mouse infectivity, by a variety of means, e.g., heat, ultraviolet light, or formalin, the penetration index was reduced to less than 1 per cent of the control, which contained viable organisms. This finding differs markedly from that found with bacteria-leucocyte interactions in which the viability of the bacteria makes little difference to the ingestion process, and in fact may retard the process if phagocytosis-inhibiting substances are present on the bacterial surface.

Further experiments on the role of the medium in the penetration process revealed that the divergent cations are essential, and that in their absence, penetration is inhibited. This is similar to other host-parasite interactions in which the divergent cations are thought to be important in an initial electrostatic binding process. Ba++, Cd++, Ca++, Mn++, and Mg++ served equally well. The fact that higher indices were obtained with the complete medium than with BSS alone, suggested that other components either of the horse serum or embryo extract were involved. Additions of various materials to BSS showed that albumin in a concentration of 1 per cent enhanced penetration to values at high as with the complete medium. This effect of albumin is thought to be related to the protective effect it has on the viability of the rickettsiae, a finding which is similar to that observed in purified suspensions of the typhus group (36).

Various metabolites were next evaluated in terms of their ability to enhance penetration when added to BSS. Initial screening experiments with various amino acids and citric acid cycle intermediates had strikingly different effects. The most marked enhancement occurred with L-glutamic acid, and a small but reproducible enhancement occurred with succinate and diphosphopyridine nucleotide. None of the other compounds tested, including α-ketoglutarate, pyruvate, malate, citrate, aspartic acid, lysine, alanine, glycine, and arginine had any demonstrable influence. L-Glutamate, however, enhanced the process to values which occasionally exceeded those found in the complete medium.
which contained protein. It was effective at concentrations as low as 1 μg per ml. When the unnatural isomer, L-glutamic acid, a compound not utilized by rickettsiae, was employed, penetration was depressed below the control values. L-Glutamine appeared to work as well as L-glutamate and it was of interest that mixtures of α-ketoglutarate and aspartic acids, which when present singly had no effect, enhanced penetration. Both glutamine and mixtures of α-ketoglutarate and aspartate can be oxidized by typhus rickettsiae, the former by deamination and the latter through the formation of glutamate by transamination.

Experiments in which inactivated rickettsiae were employed showed that glutamate enhanced the penetration only of “viable” organisms. Other studies demonstrated that one of the actions of glutamate, perhaps the most important, was to maintain the viability of the organism during the course of the experiments. In addition, glutamate had no effect when the reaction was carried out at low temperature, nor did it act when one of a variety of enzymatic inhibitors which interfered with its subsequent utilization was added.

From these experiments, it was concluded that the “viability” of the rickettsiae, whatever this property entails, is the deciding factor in the penetration of the MB III cell. The mechanism by which rickettsiae enter the host cell is still unknown. It is possible that the “viable” organism facilitates penetration either by simply adhering to the cell surface or by effecting an actual change in the cell membrane of the host.

The relationship of these results in vitro to certain of the properties in vivo of these organisms is not clear at the present time. Perhaps in the case of toxicity only the viable organisms can invade the endothelial cell, thereby bringing about alterations in permeability of the smaller blood vessels. Further speculation is not warranted and requires a more detailed knowledge of the fate and localization of the rickettsiae in vivo.

INTERACTIONS BETWEEN POLYMORPHONUCLEAR LEUCOCYTES AND BACTERIA

Whereas an obligate intercellular parasite must enter a susceptible host cell to establish infection, the phagocytic cells of the body must ingest bacteria to control infection. In general the property of phagocytosis is considered to be one of the most important functions of the leucocyte in resistance mechanisms and one which precedes the exposure of the microorganism to the bactericidal substances in the leucocytic cytoplasm. A vast literature exists on the factors which influence the phagocytosis of bacteria (37, 38). Such factors can be separated into three major groups: (a) the nature of the bacterial surface, (b) the presence of opsonizing materials in the blood and tissue fluids, and (c) those factors which influence the physiology of the leucocyte. The first two categories are intimately related to “specific immunity” and to the presence of opsonizing antibody. The last can perhaps better be discussed under “nonspecific factors” and is the one which will be reviewed in some detail.

The polymorphonuclear leucocyte has long been considered unique among mammalian cells on both morphological and metabolic grounds. It is a cell which, when mature, has a multilobed nucleus and a cytoplasm containing large numbers of characteristically staining granules. It is an end cell which has lost the ability to divide and is thought to have a transient life span in the circulation (39). Associated with the maturity of the granulocyte are the properties of motility and the ability to ingest particles. Metabolically, it is characterized by a high aerobic glycolytic rate and the presence of large amounts of glycogen (40, 41). A variety of enzymatic reactions have been demonstrated with leucocyte preparations (42) and some have shown variations which are indicative of generalized metabolic disturbances (43, 44).

In collaboration with Dr. Stephen Morse, a study has been made of the localization and fate of various bacterial species in homogeneous suspensions of rabbit peritoneal exudate leucocytes. The system employed to study the interactions was patterned after one described by Maaløe (45) and utilizes low speed centrifugation to separate the free bacterial and leucocyte populations. This technique allows a sequential analysis of (a) the rate of phagocytosis over prolonged periods of time, as measured by the reduction in the number of viable extracellular bacteria remaining in the supernatant fluid after low speed centrifugation, and (b) the fate of the ingested bacteria, as estimated from plate counts of the disrupted leucocytes. With this system to assay phagocytosis and intracellular inactivation, we have more recently been concerned with the inter-relationships between metabolic and functional properties, particularly (a) the metabolic requirements for
the phagocytic process, (b) the influence of particle ingestion on the functional and metabolic properties of the leucocyte, and (c) the role of certain bacterial products such as a lipopolysaccharide endotoxin.

Experiments with strains of *Staphylococcus epidermidis* (Staphylococcus albus) (46) revealed that in a medium composed of 10 per cent fresh rabbit serum, balanced salt solution, and 0.1 per cent glucose, with air as the gas phase, these organisms were rapidly killed in suspensions of polymorphonuclear leucocytes. The rate of phagocytosis was found to be the rate limiting step in the reduction of viable organisms. During a 180-min incubation period approximately 99 per cent of the bacteria were killed, and there was no increase in the number of viable leucocyte-associated bacteria. This indicated that efficient ingestion and rapid intraleucocytic destruction was taking place.

If the medium was altered, rather striking changes occurred in the fate of the staphylococci. The omission of rabbit serum or its inactivation at 56°C for 30 min markedly reduced the rate of killing. Fresh serum was therefore required for phagocytosis and subsequent inactivation. The presence or absence of exogenous substrate (glucose) made little difference in the absence of serum. If the experiment was conducted in the presence of constant amounts of serum but at varying glucose concentrations, a reduction of the phagocytic process occurred at limiting substrate concentrations. With 10 mg of glucose per 100 ml, a quantity which is utilized within 60 min, the rate of phagocytosis remained constant for approximately 90 min and then stopped quite abruptly. Since the exogenous supply of glucose was consumed prior to the cessation of phagocytosis, it appears likely that the leucocytes utilized a portion of their glycogen stores to carry out the process. A reduction of glycogen could be demonstrated but glycogenolysis was not capable of maintaining the phagocytic process for prolonged periods of time.

The addition of a variety of metabolic inhibitors to the leucocyte-bacteria suspensions resulted in marked changes in both the metabolic activities of the leucocytes and their ability to kill the staphylococci. Potassium cyanide, which reduced the respiration of leucocytes to approximately one third of the control, had little or no effect on the rate of killing. In contrast, arsenite and iodoacetate, both of which effectively inhibited glucose utilization and lactic acid formation, produced a marked reduction on the killing of the staphylococci. This resulted from an inhibition of phagocytosis rather than from phagocytosis followed by intracellular survival. Dinitrophenol at similar concentrations allowed phagocytosis to proceed normally for approximately 60 min, during which time 90 per cent of the bacteria were ingested and killed. Thereafter, the phagocytic process stopped, and little or no ingestion occurred for the remaining 120 min of the experiment.

From these experiments it was concluded tentatively that phagocytosis is an energy-requiring process which needs a constant supply of utilisable substrate. The substrate requirements of the system in vitro can be met either by exogenous glucose or, for a more limited time, by glycogen stores. Phagocytosis can proceed normally for prolonged periods when leucocyte respiration is inhibited. This finding is in keeping with the earlier studies of Nungester (47), who showed that anaerobiosis as well as cyanide were not inhibitory to particle ingestion. Glycolysis, however, seems to be of prime importance for the phagocytic process, and its inhibition results in decreased activity. It is of interest that the inhibition of leucocyte glycolysis by large amounts of influenza virus also decreases the phagocytic activity of these cells (48). Many of these results are analogous to those reported by Sbarra and Karnovsky, who employed guinea pig leucocytes and polystyrene particles (49).

The phagocytosis of particles also results in certain quantitative changes in the metabolism of the phagocyte. A number of investigators have demonstrated increases in oxygen consumption (50, 51) as well as in the quantity of glucose utilized via the hexose monophosphate shunt (52). Our own experiments employing heat-killed bacteria as well as other inert particles showed increases in respiration under conditions in which phagocytosis took place. In addition, there were increases in the utilization of glucose and a concomitant stimulation of lactic acid production under the same conditions. The per cent increase in respiration and glycolysis was related to the number of particles ingested. These changes were most striking during a period when the majority of particles were being ingested, although still demonstrable after the period of maximum phagocytosis. Changes also occurred in the glycogen content of phagocytizing leucocytes. Normal rabbit polymorphonuclear leucocytes in the pres-
ence of 0.1 per cent glucose show a relatively slow synthesis of glycogen during the first hour of incubation. Thereafter the rate rises and is most rapid during the third hour. During the 180-min experimental period there is a 60 per cent increase in the glycogen content of the leucocytes. During phagocytosis there is an initial decrease in total glycogen amounting to approximately 30 per cent of the original value. This loss is shortly followed by rapid glycogen synthesis, the rate of which exceeds that seen with the normal cell.

The functional activities of leucocytes undergoing these metabolic alterations were next examined. A comparison was made between cells which had ingested an inoculum of heat-killed bacteria and control cells in terms of their ability to kill a live inoculum of staphylococci. The results indicated that leucocytes which had already phagocytized dead bacteria subsequently killed live bacteria to a greater extent. The increase in killing rate was demonstrable early in the experimental period, and at 180 min the pretreated cells had killed 10 times as many bacteria. The pretreatment could be performed with a variety of gram-positive and gram-negative bacteria, all of which were readily ingested in the presence of normal rabbit serum. If the leucocytes were exposed to the same number of dead bacteria in the absence of serum, an environment in which phagocytosis does not occur, their activity was the same as the untreated control. Similarly, if an organism which was not ingested by the leucocytes was employed, no change in activity was noted. Further studies showed that the increased killing of the live inoculum was a consequence of an increased rate of phagocytosis, rather than the release of opsonizing or bactericidal substances into the medium.

Further experiments were conducted to examine what effect pretreatment of the leucocytes would have on the fate of microorganisms that survive intracellularly, i.e., mycobacteria. No change in the fate of these organisms was noted and the acid-fast bacilli survived in both the normal and pretreated cells. However, the ingestion of the mycobacteria was stimulated, as indicated by the more rapid fall in the number of extracellular bacteria.

During the course of these studies it was found that highly purified preparations of a lipopolysaccharide (LP) from Salmonella abortus equi, obtained through the kindness of Dr. Otto Westphal, also stimulated the phagocytic and glycolytic activities of leucocytes. These findings are in many ways similar to those obtained by the ingestion of heat-killed bacteria and may be summarized as follows.

A. Concentrations of LP ranging from 0.01 to 50.0 μg per ml enhance phagocytosis in the presence of rabbit serum. The greatest enhancement occurs with the lower concentrations of the compound. When low serum concentrations are employed (1 per cent), doses of 10.0 to 50.0 μg per ml inhibit phagocytosis and allow bacteria such as S. epidermidis to multiply in the presence of leucocytes. LP enhances the ingestion of mycobacteria but does not influence their intracellular fate. When phagocytosis-resistant organisms are employed, i.e., the Smith strain of Staphylococcus aureus, the enhancing effect of LP can be demonstrated only in the presence of specific antibody.

B. Metabolic studies showed that LP at the foregoing concentrations stimulates glucose utilization and lactic acid production, whereas respiration remains unaffected. The effects on glycolysis are maximal at lower concentrations of LP (0.1 to 1.0 μg per ml).

C. LP enhances the metabolic and phagocytic activities of leucocytes in the absence of serum.

From the results presented it appears that both the ingestion of particles and the presence of minute quantities of a lipopolysaccharide endotoxin influence the metabolism and phagocytic activity of the polymorphonuclear leucocyte. It is of interest in this regard that Meier and Schär (53) have demonstrated increased motility of leucocytes in the presence of lipopolysaccharides. In both cases an increase in glycolysis is associated with increased phagocytic activity. Whether this relationship is one of cause and effect or is a less direct association is not known. In part the interpretation of these data is made more difficult by our lack of knowledge concerning the basic mechanisms which control the activity and properties of the leucocyte membrane, e.g., the conversion of energy into membrane activity. From the biochemical results it appears that more substrate is utilized by the cell that has phagocytized. In other mammalian cell systems, it is thought that the rate limiting step in glucose utilization is the penetration of this substrate into the cell (54). Perhaps, during phagocytosis and endotoxin treatment, the permeability of the leucocyte membrane is altered, allowing a greater supply of substrate to enter the cell. This would then be
metabolized by the cell's normal enzymatic pathways, e.g., Embden-Meyerhof pathway and hexose monophosphate shunt. A number of recent findings seem to support such a conclusion. Leucocytes which have phagocytized become permeable to certain enzymatic inhibitors (55) and phosphorylated substrates (Z. A. Cohn and S. I. Morse, unpublished data) which had no influence on the normal cell. Such permeability effects may also have actions in the reverse direction, since we have observed a rapid release of lysozyme from such cells. A similar effect has been reported with an endotoxin (57). Possibly other substances are also released. In a sense, the entire process may be considered as a type of "cell injury" which is perhaps nonspecific in etiology but which nevertheless activates important functional properties of the leucocytes.

What is the relationship between serum factors and the metabolic determinants of the phagocytic process? At the moment it appears that both are required for efficient phagocytosis to take place, and that the importance of each depends upon the particular environment in which the leucocyte-bacteria interaction takes place. It seems that serum factors are in some way required to prepare the particle for ingestion and that this phenomenon in many cases masks the underlying metabolic requirements.

As yet it has not been possible to alter the fate of intracellular bacteria by any of the previously described manipulations. In each instance phagocytosis has been rate limiting and has controlled the fate of intrinsically susceptible organisms. Consequently, it has not as yet been possible to examine the influence of leucocyte metabolic activity on the intracellular destruction of bacteria. Whether or not it will be possible to alter the bactericidal properties of the leucocytic cytoplasm represents an important area for future investigation.

REFERENCES

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DISCUSSION

Although preparation of diabetic rabbits has been difficult, a few experiments have shown that leucocytes from ketotic animals or animals with chronic diabetes show decreased glucose utilization and diminution of phagocytic activity. However, restoration of the cell's glycolytic function is correspondingly associated with restoration of phagocytic function (Cohn, New York).

Most influenza viruses inhibit anaerobic glycolysis and phagocytosis of polymorphonuclear leucocytes when glucose or glucose 6-phosphate is employed as substrate. These effects of influenza virus could not be demonstrated when fructose 6-phosphate or fructose 1,6-phosphate was used as substrate (Fisher, T. N. and Ginsberg, H. S., 1956. The reaction of influenza viruses with guinea pig polymorphonuclear leucocytes II and III. Virology, 2, 637–655 and 656–664).