INTERACTION BETWEEN PHAGOCYTES AND PATHOGENIC MICROORGANISMS

EMANUEL SUTER[1]

Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts[2]

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[1] Markle Scholar in Medical Science.

[2] Present address: Department of Microbiology, College of Medicine, University of Florida, Gainesville, Florida.

I. INTRODUCTION

When pathogenic microorganisms gain access to tissues, the connective tissue serves at times as an intermediary in the host-parasite relation. Since the connective tissue responds to the presence of foreign material with a sequence of reactions leading to various forms of inflammation, any consideration of interaction between host and pathogens has to take into account the components of the inflammatory exudate. Three main elements in inflammation play a major role in host-parasite relations: phagocytes (219), bacteriostatic and bactericidal mechanisms of plasma and exudate fluid (250, 251), and the changing chemical environment at the site of connective tissue reaction (78). The relative concentration of these components in the inflammatory exudate and the susceptibility of the pathogen to them determines to a great extent the course of any infection. The composition of the exudate varies from host to host, from one site to another in the same host, and is influenced by previous immunization or sensitization. This variability provides a basis for a wide spectrum of types of interactions between parasites and potential hosts. In contrast, the susceptibility of one given pathogen to the products of inflammation is relatively constant.

A. General Function of Phagocytes

The elimination of pathogenic microorganisms by phagocytes represents the development of a specialized function in higher animals and man. The ability to engulf and digest particles and to eject indigestible products is an essential nutritional mechanism in protozoa and primitive metazoa. Many amebae are known to obtain nutrient material from digestion of engulfed microorganisms which, if not digested, can cause fatal infections of the same cell. A relationship between the degree of digestive capacity and the resistance to infection has been established in some cases (216). Indigestible organisms are frequently egested. Evidently, nutrition and destruction or elimination of potentially harmful microorganisms are inseparable functions within a single cell. Some specialization is found in colonial protozoa (*Protospongia*) in which two types of cells can be distinguished; an outer layer of flagellated cells serving locomotion and an inner core of amebic cells with phagocytic capacity. Specialization is not irreversible, the cells retaining the ability to transform into each other. The next step in differentiation is exemplified by location and function of phagocytic cells in sponges, in which two types of nutritional phagocytes are distinguishable. Particles flowing through the canalicular system are captured out of the water by endodermal phagocytes and are passed on to mesodermal phagocytes, which either digest them or reject them towards the outside. Larger objects are embedded in syncytial masses formed by the fusion of several cells (218). In the absence of hydrolytic enzymes in the tract phagocytosis of food particles by endodermal cells lining the digestive canal is essential. With the development of the ability to secrete digestive enzymes, phagocytosis is no longer necessary, and these cells are not phagocytic in higher animals.

The role of phagocytes in the internal economy of animals and man under normal and pathological conditions is further indication of the many functions performed by these cells. Short-lived cells such as erythrocytes, polymorphonuclear leukocytes, and others are taken up and digested by phagocytes. Some chemical constituents of these cells are reintroduced into the economy of the organism and others are eliminated (116). Large molecules, especially lipides, are also handled by fixed phagocytes (148). Metamorphosis of invertebrates and lower vertebrates, during which whole organs are destroyed and rebuilt within an astonishingly short time, depends on phagocytic function. It is obvious that the animal depends on an economic system for reutilization of tissue components (354).

B. Special Role in Infection

The development of the concept that phagocytes are of importance for resistance against infections was gradual (43, 257, 365). The evidence for this role of phagocytosis is circumstantial and has accumulated as the result of a great number of independent observations, some of which are: (a) A direct correlation exists between the degree of phagocytosis and resistance as in the host-parasite relation between *Daphnia* and *Monospora bicuspidata*. If spores that have gained access to the body cavity of this crustacean are phagocytized, no infection occurs. If, however, some escape engulfment, multiplication of the parasite and death of the host will follow (219). (b) Phagocytic activity can be re-

duced by artificial means and the effect on resistance measured. Paralysis of the phagocytes by general anesthesia or by lowering the body temperature below normal or raising it above normal results in a decrease of natural resistance (341). Similarly, depletion of polymorphonuclear leukocytes in animals by chemical means (264), or by blockade of the reticulo-endothelial system and splenectomy, can be detrimental to resistance (248). (c) Frequently the progress of an infection depends on the site of inoculation, and the results can be correlated with local differences in mobilization of phagocytes. Thus, mice are much more resistant to infection with pneumococci by the intravenous and intramuscular routes than by the intraperitoneal (82). (d) For some pathogenic organisms it has been established that surface components which are essential for virulence are also anti-phagocytic (75).

Besides phagocytes, the bactericidal power of serum and tissue fluids contributes independently to the destruction of microorganisms in the tissues (250). The relative importance of phagocytes and humoral factors differs with the species of the host and with the pathogen. The interaction between phagocytes and pathogens is essentially similar to the interaction between phagocytes and any foreign particles which have gained access to tissues or blood. It includes migration, phagocytosis and attempts at intracellular digestion. Any deviation from this basic pattern must be explained by peculiarities of the pathogen, the phagocyte, or the environment in which the interaction takes place. Environmental influences can be nonspecific or specific, and affect the viability of the pathogen or its ingestion. The resistance of the pathogen to the intracellular environment determines its fate after it has been ingested.

II. ANATOMY AND PHYSIOLOGY OF THE PHAGOCYTIC SYSTEM

A. *The Elements*

The phagocytic system represents a functional unit composed of widely dispersed and morphologically distinct cell types. These cells, specialized in phagocytosis, derive from the mesodermal layer. Two methods have mainly been used to identify the various elements of the system: provocation of a cellular reaction by applying an irritating agent to the tissues, and vital and supravital staining (330).

1. *Polymorphonuclear leukocytes.* The cells accumulating during the acute phase of the inflammatory response are mostly polymorphonuclear leukocytes (PMN) which long have been known to migrate from the capillaries into irritated tissues. Direct observation under the warm stage and vital staining established their main activities, migration and phagocytosis (55, 287). A second cell type, mononuclear phagocytes (MN) or macrophages, supersedes the PMN during the subacute and chronic phases of inflammation or when healing takes place (218). The relative proportions of PMN and MN in the inflammatory exudate vary depending on animal species, tissues, duration of response and the irritating agent.

2. *The reticulo-endothelial system* (RES), which is a functional unit of the phagocytic system can be demonstrated by vital staining. Its elements are also made manifest when particulate matter, such as india ink, injected into the circulation is eliminated and accumulates intracellularly. The cells of the RES are found mainly in the endothelium of the sinusoids of various organs, such as liver, spleen, lymph nodes and bone marrow (10, 148), but also in the connective tissue of these and other organs (151). Various types of cells are included in the RES, depending on the amount and type of dye injected (261). Silver staining of tissue sections identifies the so-called argyrophilic or metallophilic cells which are essentially the cells of the RES (200). In general, they are "those mononuclear cells, wherever they may be, lining vascular channels, resident in the connective tissues or entirely free, whose protoplasm constitutes a physical system characterized above all by its response to finely particulate matter" (90), *i. e.*: (a) monocytes of the circulating blood; (b) sessile cells within the endothelium lining the sinusoids of liver, spleen, bone marrow and lymph nodes, sometimes also of adrenal cortex and anterior lobe of the pituitary; (c) sessile or resting wandering cells of the connective tissues in various organs (histiocytes, clasmatocytes, macrophages); (d) microglial cells.

Considerable discrepancy of opinion exists as to whether lymphocytes should be classified in the RES or not (257), whereas there is no doubt that the alveolar cells belong to this category (200, 265).

3. *Potentiality, development and interrelations*

between the various types of phagocytes have been the subject of much controversy. On the basis of function and morphology the PMN can be distinguished from the cells of the RES. With full maturation the PMN loses the ability for further differentiation or for mitotic division, presumably because of the production of a mitotic inhibitor by mature cells (244). Estimations of the life-span of mature PMN in man range from 4.5 to 13 days (164, 245). The hematological state of the PMN is a variable and dynamic equilibrium between production and delivery of leukocytes on one hand and destruction on the other. Several factors, such as physical activity, emotional stress, and a great number of pathological states, influence the level of PMN in the peripheral blood. Tissue necrosis and inflammation, regardless of their cause, result in leukocytosis. A substance which elicits leukocytosis upon injection in man and animals has been isolated from inflammatory exudates. This leukocytosis-promoting factor (LPF), found in the pseudoglobulin fraction of exudates, could well be a general inducer of leukocytosis which follows injury of tissues (214). Very little is known about the regulatory mechanisms which maintain the level of leukocytes, especially of PMN, in the periphery, but there are indications of a complex system including the hypothalamus, the anterior lobe of the pituitary and the liver (18, 113, 271).

The elements of the RES, especially MN, macrophages and histiocytes, are morphologically and functionally distinct forms of the same cell type under different environmental conditions, and these cells are endowed with the potentiality for further development and for changes in their enzymatic activities (51, 85, 330, 352). Although it has been shown that the source of serum and not of the cells used determines the morphology of macrophages in tissue culture (45), little is known of the exact conditions governing these processes. Probably because of the adaptability of these cells and their response to environmental changes, several problems have remained unsolved. Thus, many observations indicate that lymphocytes can transform into macrophages (33, 257), but a more definite distinction between the two cell-types is also justified (94). A conclusive answer to this problem cannot be expected unless more objective means for differentiation of these two cell types are found. Furthermore, it is difficult to decide whether macrophages are capable of active proliferation *in vivo* or *in vitro*. There are numerous claims in the affirmative (4, 33, 39, 147, 206, 330), but it may be that the tissue cultures of macrophages are contaminated with fibroblasts which actually are the multiplying cells (194). However, there is little doubt that proliferative potentialities are stimulated by pathologic conditions—wound healing (4, 9), the granulomatous response to bacterial and viral infections, and tumors of unknown origin, such as sarcoid tumors, lymphogranulomatosis and reticuloendotheliosis (313). Proliferative response of the RES is obtained with repeated injections of trypan blue (300), with a lipide component of *Listeria monocytogenes* (81) and with certain components of tubercle bacilli (279, 319). It is possible that the regeneration of the RES after intensive blockade, as well as compensatory changes of the RES after splenectomy (248), are due to a similar proliferative process (20). All these observations indicate that the cells of the RES are capable of proliferation either in the mature form or in a less advanced stage of development.

In considering the interaction between phagocytes and pathogens the potentialities and especially the adaptability of the RES have to be kept in mind. The nature of the lesion is not only due to the specificity of some component of the parasite but also to the functional flexibility of the host cells. The response of PMN is more uniform, probably because of the irreversible differentiation of these cells.

B. Functions

1. *Motility and chemotaxis.* Motility of PMN and MN is essential for the cellular response in inflammation. Several techniques have been developed for the observation of random movement of PMN. If a drop of a leukocyte suspension in whole blood or in exudate fluid is placed on a slide, covered with a coverslip and sealed, the movement of leukocytes can be followed under the microscope and recorded (189). To obtain clearer recordings, washed leukocytes which adhere to the coverslip after it is kept in blood for 30 minutes are used. The coverslip with the leukocytes is then inverted on a slide on which a drop of plasma has been placed. The tracks of the movements of the leukocytes are recorded, using dark-ground illumination (125).

A second method for observing motility is to follow the emigration of leukocytes from a buffy coat. A piece of buffy coat is placed in plasma in a Carrel flask and the zone of emigration, visible as a halo around the explant, is recorded (208); or whole blood is centrifuged in a flat slide chamber formed by a microscope slide and a coverslip, and the migration of cells from the buffy coat into the clear zone of plasma is followed under the microscope (205).

The speed of migration of PMN is approximately 29 to 34 μ/min, determined either as random movement on a coverslip (189) or as emigration from a buffy coat (162, 205). Several observations show that random motility is determined by some property of the PMN, and especially by certain components of the medium in which the determination is made. Ca^{++} and complement in the medium increase motility (66). Citrated plasma supports emigration much less than heparinized plasma, even after recalcification (5). The component missing in recalcified citrated plasma has been found to be associated with the γ-globulin fraction (6). These findings have been confirmed by the observations that the migration enhancing factor of plasma of certain human beings is found in fraction II (γ-globulin), while an inhibitory factor resides in fraction III (β-lipoprotein and ceruloplasmin) (162). The relative quantities of these two fractions in the plasma, rather than a property of the PMN themselves, determine the motility of PMN in the test. Anesthetics and metabolic blocking agents inhibit migration. Of the latter, those inhibitors which also reduce aerobic respiration of PMN are most effective, suggesting that aerobic oxidative metabolism supports migration. It is interesting that these inhibitors frequently have no influence on phagocytosis (170).

Chemotaxis, the directed migration of PMN and MN under the influence of a concentration gradient of certain substances, has been widely explored *in vivo* and *in vitro*. By injecting substances under the skin or into the peritoneal cavity the quality and quantity of the inflammatory exudate can be determined, but no precise information is obtained on the influence exerted on phagocytes alone. The foregoing described *in vitro* methods (see page 97) for study of migration have all been used for chemotactic measurements. Bacteria or crystals are placed under a coverslip with the cell suspension; fluids are deposited on a slide in a drop of clotted plasma which is then covered by the coverslip. Alternatively, the bacteria or crystals are placed at a distance from the explant of the buffy coat in plasma; fluids, sealed in a capillary tube, open at the end pointing towards the buffy coat, are placed in the plasma. The speed of random locomotion is not increased by any chemotactic agent tested (71). The greatest difficulty in these tests seems to be the localized application of the substance in order to obtain concentration gradients, and this may well explain the conflicting results obtained with the same substances in different laboratories. Detailed accounts on chemotaxis can be found in recent reviews (127, 190).

Of the many substances with a positive chemotactic effect only a few will be mentioned. Various sugars and polysaccharides liberated from tissues have been found to be active (47). Other components released from an inflamed area may have systemic and local effects on leukocytes. For example, the LPF discussed above induces leukocytosis when injected into animals or man, and leukotaxin isolated from inflammatory exudate causes increased permeability and diapedesis of leukocytes at the site of injection (240). The validity of the evidence for the presence of leukotaxin in sterile turpentine exudates has been challenged recently (125, 127), although others have confirmed the production of leukotaxin and LPF in inflammatory exudates (364). Difficulties of interpretation of results obtained with different animals in different laboratories could well be the cause of this discrepancy (214). It is interesting that injured muscle releases a leukotaxin-like substance active *in vivo* (see 214), a finding which could explain leukocytosis caused by aseptic necrosis, such as infarction of the myocardium.

A number of microorganisms exert a positive chemotactic effect, such as *Bacillus anthracis*, *Corynebacterium diphtheriae*, *Diplococcus pneumoniae*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Salmonella typhi*, *Micrococcus pyogenes* var. *albus*, and *Streptococcus pyogenes* (125, 190, 208). The activity seems to reside in a polysaccharide fraction of these organisms. These fractions are active *in vitro* at dilutions of 10^{-7} and 10^{-8} (209). Unlike leukotaxin, these fractions affect only leukocyte migration and do not

increase capillary permeability (211). The lipopolysaccharide and the phosphatides of the tubercle bacillus are inactive (209, 346), whereas protein and polysaccharide of the same organisms are very active (67). Other organisms have no influence on leukocyte migration (125). Toxins of some anaerobic bacteria are strong inhibitors of migration. Thus, washed organisms of *Clostridium tetani* and of *C. chauwoei* are rapidly phagocytized, while unwashed toxin containing cultures are not (171).

The fact that pneumococci and streptococci have a chemotactic effect on PMN indicates that chemotaxis alone does not bring about phagocytosis. Most experiments were done using PMN, and what little information was available on the response of macrophages to chemotactic stimuli was negative. But it has now been shown that macrophages do display positive chemotaxis to microorganisms such as *Micrococcus pyogenes* var. *aureus* and *albus*, *Streptococcus pyogenes*, *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Corynebacterium diphtheriae*, *Salmonella typhi* and *Bacillus anthracis* (126), and chemical agents like starch, glucosamine and arabinose (168). The monocytes show slow random migration. It is possible that results of earlier experiments were negative because the tests were done over a period of only 24 hours, whereas the more recently published experiments were performed on monocytes cultured for 7 days prior to the test (126). During this period many changes can occur in these cells (352).

Chemotaxis probably increases the chances of contact between phagocytes and microorganisms in the tissues by directing the movement of the phagocytes and by increasing the number of leukocytes emigrating into the area. So far, no satisfactory theory for the mechanism of chemotaxis has been formulated. It has recently been postulated that chemotaxis is exerted by particles through their property to adsorb from the medium components which stimulate migration, and thus create a concentration gradient by which the leukocytes are attracted (191). It is of interest that precipitated antigen-antibody complexes have a chemotactic effect which could be mediated through a similar mechanism (210).

2. Phagocytosis. Contact between phagocyte and particle is a necessary condition for successful phagocytosis *in vivo* or *in vitro*. The chances for contact are increased *in vitro* by shaking mixtures of cells and particles in a liquid medium. Chemotaxis similarly increases contacts between phagocytes and particles on solid surfaces under natural and experimental conditions. The possibility of contact is also greater if the density of the cell population increases, as it does at sites of inflammation. It has been shown that quantitative analysis of phagocytosis requires careful definition of the population density (122).

Phagocytosis depends not only upon contact of the particles with the surface of the phagocyte but also upon a certain degree of adhesion. Deposition of protein on the surface of the particle has this effect. The serum of animals vaccinated with bacteria, besides increasing phagocytosis of these bacteria, causes several alterations of their surface properties, such as increased cohesiveness, decrease in surface electric potential differences, and decrease in wettability by oil (179). The difference between the physicochemical changes due to specific and nonspecific opsonization seems to be quantitative rather than qualitative (23, 229, 344). Whether the coating is sufficient for phagocytosis depends on the physical conditions under which the process is observed. In liquid media maximum coating is necessary, whereas rough surfaces allow phagocytosis with little opsonization. This effect of surfaces, termed surface-phagocytosis, was observed *in vitro* and *in vivo* with organisms which require specific antibody for successful phagocytosis when in suspension (358). The phagocyte is able to trap the particle between its cytoplasm and the surface and to engulf it without opsonization. Similarly, phagocytosis by MN is stimulated by opsonization (180) and on surfaces (285).

The mechanism of phagocytosis has not been elucidated, although models have been constructed and hypotheses formulated. The various aspects of phagocytosis have been discussed in earlier reviews (23, 229, 239, 363).

The fixed cells lining the sinusoids of various organs engulf particles under different conditions. The efficiency of the RES in removing particles from the circulation was found to be due to the deposition of fibrin on the surface of particles, which then tend to stick to the reticuloendothelial cells in the sinusoids. Particles which are not coated in the circulation are only slowly phagocytized, as has been demonstrated by si-

multaneous injection of two types of particles, such as india ink which is coated and cream droplets which are not coated. Heparin, administered prior to the injection of particles, inhibits the coating and phagocytosis, whereas phagocytosis is not prevented by heparin after coating has taken place (166). It was also shown that the speed of removal of india ink depended on the number of particles injected. Above a certain quantity of india ink, a sharp increase of the rate of removal was noticed (120). The increased rate of removal was due to the deposition of fibrin on the particles as shown by the fact that fibrinogen in blood was lowered after the injection of a high dose of india ink and that thrombokinase and thrombin enhanced removal of particles from a smaller dose. Heparin, by contrast, prevented the deposition of fibrin (27). Fibrin-coated particles are caught by the cells of the RES. It is very likely that similar coating assists in the phagocytosis of pathogens by the RES.

Phagocytosis determines whether an intracellular relationship between phagocytes and pathogens is established. In the non-immune host those pathogenic organisms are taken up which do not produce substances paralyzing leukocytic motion and which can be phagocytized in the absence of antibody; but in the immune animal most of them are phagocytized. This difference is stressed because in the former case extracellular factors are more important for resistance, whereas in the latter it is the intracellular location of the pathogen which determines the subsequent course of events. Egestion of phagocytized microorganisms, as observed with streptococci, may allow some pathogens to escape intracellular destruction (355).

3. Enzymatic equipment and metabolism. Leukocytes are a convenient tool for metabolic studies as they can be obtained repeatedly from the same source in free-floating suspension without disruptive measures. Because of the ease with which they are obtained as biopsy material from man, attempts have been made to find metabolic differences between leukocytes from healthy and sick individuals. Attention has further been focussed on leukocytes because of their cardinal role in infectious diseases.

a. Cellular constituents: Phagocytes contain all the known structural and chemical protoplasmic constituents of cells in general. Particular attention has been paid to the nature of the granules of PMN which take up lipide stains (356) and to glycogen present in large amounts in PMN. [The values vary from 2 to 15 μg per 10^6 cells depending on the source of the cells and the nutritional state of the animal (334, 342)]. MN do not contain stainable lipides nor glycogen when taken from blood, but they have been found to accumulate lipide material during cultivation *in vitro* (352). This is also true for the fixed cells of the RES in certain metabolic diseases such as Niemann-Pick's and Gaucher's diseases. PMN have a relatively high content of histamine amounting to 0.0196 μg per 10^6 cells (335).

b. Enzymatic equipment: The phagocytes contain a large number of enzymes necessary for the performance of various metabolic functions. Table 1 lists enzyme functions that have been determined in leukocytes. In this list many enzymes are missing which are essential for the metabolism of the cell. It can be inferred from over-all metabolic studies that many of these must be present, but they have not been studied in detail. This is true for the enzymes of the Embden-Meyerhof scheme as well as of the tricarboxylic acid cycle. The simultaneous activity of aerobic oxidative mechanisms yielding CO_2 and aerobic glycolysis yielding lactic acid is a common feature of leukocytes, tumor cells and embryonic tissues.

The variety of enzymes observed indicates that leukocytes are able to handle carbohydrates, proteins, lipides and nucleic acids. Very little is known about their synthetic abilities. One can assume that some functions have been lost, since the mature forms are no longer capable of mitotic division.

c. Respiration and glycolysis:[3] One of the features of the carbohydrate metabolism of leukocytes is their aerobic glycolysis, which is influenced by many environmental conditions besides oxygen tension. Attention has been given to respiration of blood leukocytes from man in the hope that differences between the carbohydrate metabolism of normal and leukemic leukocytes will provide an understanding of the nature of leukemia. If leukemic cells are neoplastic in

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TABLE 1

Enzymes found in phagocytes

Enzyme	Cell Type and Source	Substrate	Remarks	References*
I. Hydrolytic Esterases				
Simple esterases	PMN† exudate; blood MN‡ tuberculous lesions	Methyl butyrate esters of polyglycol	Epithelioid and giant cells (normal MN negative)	38 20, 21, 47
Lipases	PMN pus; blood MN lymph node	Fats, tributyrate Stearic esters of glycerol	Mainly lymphocytes	3, 6, 13, 31, 38 31, 38
Choline esterase	PMN blood		Not confirmed	3, 57
Alkaline phosphatase	PMN blood	Glycerophosphate and diNa-phenylphosphate	PMN inflammation	22, 36, 45, 8, 44, 48, 21
	MN blood negative		cultivation *in vitro*	52
Acid phosphatase	PMN blood; exudate; lymphocyte blood	As above	Very low or negative	8, 56, 22
	MN tuberculous lesion; tissue culture		In lesions or after some days of cultivation	21, 52
ATP-ase	PMN exudates	Adenosinetriphosphate		30
Nucleases	PMN blood	Ribonucleic and deoxyribonucleic acids		3, 12
Nucleotidases	PMN blood	Thymus nucleic acid	Optimum at pH 4 very high activity	11, 42
II. Carbohydrases				
Polysaccharidases	PMN blood	Glycogen: alpha- and beta-amylases	See respiration and glycolysis	53
Nucleosidase	PMN blood	Adenosine	Biological activity test	3
III. Peptidases Dipeptidases and tripeptidases	PMN	Dipeptides and tripeptides	Activated by Ca^{++}	41, 15
IV. Proteinases				
Peptic	MN	Gelatin, peptones	Inhibitors in exudates	3, 10, 33, 53, 54, 51
Tryptic	PMN		Supernatants	10
Catheptic				37
Phosphorylases	PMN blood			
V. Oxydation-reduction				
Dehydrogenases	PMN exudate; blood	Acceptors: methylene blue, tetrazolium salts	See also glycolysis	32, 49
Oxydases				
Phenyloxydase	PMN blood; pus	Dimethyl-*p*-phenyldiamine		50, 55
	MN blood	Alpha-naphthol		
Peroxydases	PMN blood MN blood	Benzidine	Strongly positive, weakly positive, or negative	
VI. Hydrases				
Glyoxalase	PMN blood	Methyl glyoxal	PMN 735× as active as erythrocytes	26
Beta-glucuronidase	PMN blood		Increased in leukemia	17
Zinc-protein	PMN blood		Enzyme activity unknown	46

* Numbers refer to a list of references a copy of which can be obtained from the author. Because of limitation of space it is not possible to include these references in this review.

† Polymorphonuclear leukocytes.

‡ Mononuclear leukocytes.

TABLE 2

Average and range of values for respiration and aerobic and anaerobic glycolysis of leukocytes from different sources

Source	Q_{O_2}*	$Q_{Lactate}$†	$Q^{N_2}_{CO_2}$†	Glycolysis/Respiration	Inhibition of Glycolysis by Air	References§
					Per cent	
Human blood leukocytes	6.33 [7]¶ 1.5–20	16 [4] 6–34	28 [2] 11–72	2.5	63	4, 5, 24, 29, 35, 39, 43
Rabbit peritoneal exudates	4.6 [6] 0.4–5.5	9.6 [6] 4.8–17	14 [6] 5–24	2.1	32	1, 2, 7, 14, 25, 27, 34
Guinea pig peritoneal exudates	3.3 [6] 1.7–6.8	9.3 [3] 6–13	15.4 [2] 16	2.8	40	1, 9, 23, 28, 40
Rat blood leukocytes	9.4 [3] 9.2–9.8	3.0 [3] 2.6–3.7	20.6 [3] 20.2–21.1	0.32	85	15, 19

* Q_{O_2} = μl O_2 consumed per 1 mg dry weight per hr.

† $Q_{Lactate}$ = lactic acid converted to μl CO_2 produced per mg dry weight and per hr.

‡ $Q^{N_2}_{CO_2}$ = μl CO_2 evolved in an atmosphere of nitrogen per mg dry weight and per hr.

Because some data were calculated per cell number and others per dry weight, the following conversion factors were used:

Human: 1 mg dry weight = 5.1×10^6 leukocytes.

Rabbit, guinea pig, rat: 1 mg dry weight = 7.1×10^6 leukocytes.

§ Numbers refer to a list of references a copy of which can be obtained from the author. Because of limitation of space it is not possible to include these references in this review.

¶ Number of determinations.

nature, higher aerobic glycolysis would be expected than with normal leukocytes. That this is not so has been reported repeatedly, and it is generally agreed that high aerobic glycolysis is typical for normal mature PMN (15, 157). Myeloid bone marrow shows similarly high aerobic glycolysis (345). The literature on carbohydrate metabolism of leukocytes has been reviewed recently (17, 169). Table 2 represents some of the available data on respiration, aerobic and anaerobic glycolysis of leukocytes of various species in the form of mean values. All the values have been recalculated for μl O_2 or CO_2 consumed or produced by 1 mg dry weight of cells per hr, or in other words Q_{O_2}, $Q^{O_2}_{lactate}$ and $Q^{N_2}_{CO_2}$ assuming that all the CO_2 released under anaerobic conditions is due to lactic acid production which, however, may not be so (28). As can be seen, the Q_{O_2} of blood leukocytes of man as well as that of the rat is higher than that of exudate leukocytes from rabbit or guinea pigs. It has been suggested that exudate leukocytes are damaged cells, a fact which should account for their lower rate of respiration. This is unlikely, since it is known that damage to leukocytes as well as to other cells causes not only reduced respiration but also increased aerobic glycolysis resulting in a higher ratio for glycolysis:respiration (100, 203). But this is not true of the examples quoted in the table. It is further evident from the table that the results obtained by different investigators do not show good agreement. The experimental sources for these discrepancies will be discussed *in extenso* elsewhere (311); it suffices to list here briefly some of them.

(i) Animal species (see table 2).

(ii) Estimation of the amount of cellular protoplasm (cell number, dry weight, nitrogen).

(iii) Eliciting agent used to obtain exudates.

(iv) Relative proportion of the various cell types, especially PMN and MN: based on phosphorus determination for the quantification of protoplasm, the respiration of MN is about twice that of PMN (311).

(v) Physical integrity of the cells: leukocytes are extremely susceptible to damage by centrifugation, pipetting, etc. Delay in processing can cause the accumulation of acid (203).

(vi) Number of cells in the reaction vessel (130, 308), especially if respiration is observed over a prolonged period. Crowding and accumulation of metabolic products influence the rate of respiration.

(vii) Oxygen tension: lowering of the oxygen

tension reduces respiratory activity and increases glycolysis (311).

(viii) Buffering capacity of the mixture and initial pH.

(ix) Substrates: recent experiments have shown that various substrates influence respiration and glycolysis (16, 203). Thus, glucose and fructose increase aerobic glycolysis of intact and disintegrated cells, whereas succinate greatly enhances respiration of disintegrated cells. This indicates that the ratio glycolysis:respiration will vary depending on what substrates are present in the suspension fluid. It is of interest that leukocytes from diabetic patients show lower lactate production and less glucose utilization than those from healthy individuals unless insulin is added to the mixture (204).

(x) Period of time over which readings are made influences the results because activity drops off after an initial high rate (156, 260, 311).

It is evident that the carbohydrate metabolism of leukocytes is extremely labile and easily influenced by environmental conditions. This is especially important in considering metabolic activity at the site of inflammation. It has been well established that inflammation represents a continuously changing environment with respect to the concentrations of glucose, lactate, HCO_3^-, CO_2, O_2, H^+ and probably many other substances which have not as yet been identified (78, 158, 214). The source for lactate production by PMN is glycogen, of which they contain a considerable amount. Glucose has a sparing effect on the utilization of glycogen. Similarly, monocytes produce lactic acid under aerobic conditions (311), but only in presence of glucose, since their content in glycogen is very low or almost undetectable.

Respiration has been found to increase during phagocytosis (12, 199, 311) but this stimulation is not always concomitant with phagocytosis. Phagocytosis is not dependent on aerobic processes, as the inhibition of respiration by KCN does not reduce phagocytosis. Anaerobic glycolysis is even decreased when particles are phagocytized (2), and the intracellular glycogen diminishes proportionally to the degree of phagocytosis (14). PMN contain a highly active adenosine triphosphatase which splits ATP into ADP and inorganic phosphate. The energy liberated is approximately 11,000 calories per mole ATP. Substances such as ascorbic acid, stimulating phagocytosis, enhance the activity of this enzyme, whereas those impeding phagocytosis inhibit the enzyme (230). Apparently, phagocytosis depends on stored energy and whether energy-yielding metabolic processes are stimulated or not is of secondary importance. This is in contrast to motility, which is reduced in presence of respiratory inhibitors (170).

III. FORMS OF INTERACTION BETWEEN PHAGOCYTES AND PATHOGENS

Convincing evidence has left no doubt that phagocytes, by their detrimental effect on microorganisms, play a dominant role in resistance to many bacterial infections; yet, unprejudiced observers have noticed that in some infections phagocytes do not harm the ingested organisms but indeed allow proliferation within their cytoplasm. Histologic and bacteriologic studies have revealed a series of possible interrelations between pathogens and phagocytes, namely, extracellular, facultative intracellular, and obligate intracellular parasitism (111, 246). To these three groups a fourth category of organisms should be added: those organisms which under normal conditions are not able to establish a foothold in the tissues of the host although they are living in close contact with the host either on the skin or on the surface of the mucous membranes of open cavities. Many of these organisms, called saprophytes or commensals, are potential parasites insofar as they are able to invade the host and to cause disease under conditions which, from within or without, impair the physiology of the host. These organisms are not able to survive or to multiply in an extracellular or intracellular environment within the tissues, whereas all the pathogenic organisms find suitable conditions in either or in both of those.

This classification is arbitrary but it serves as a framework for the subsequent discussion. Some pathogens do not fit into any category, mainly because of incomplete information. Most observations have been made with PMN and MN, and relatively little is known about the fixed cells of the RES. Despite this lack of knowledge it is assumed that the classification holds for all elements of the phagocytic system.

A. Obligate Extracellular Pathogens

Most organisms causing acute bacterial infections belong to this category. Phagocytosis reduces the chances for the pathogens to survive

or to proliferate in the host tissue. Therefore, the virulence of these organisms depends to some extent on their resistance to phagocytosis, if the extracellular environment provides satisfactory nutritional conditions. The resistance of the host is based on mechanisms increasing the effectiveness of phagocytes. The part played by extracellular humoral components in the destruction of the pathogen varies widely. In some cases there exists very little or no bactericidal activity of serum or tissue fluids; in others this activity plays a major role.

1. Factors reducing phagocytic effectiveness. Obligate extracellular parasites may remain extracellular in the presence of phagocytes by means of a surface structure which renders ingestion impossible; or they may reduce the detrimental consequences of phagocytosis by producing substances which damage the phagocytes. In some infections one of these factors prevails, in others there is a combination of both.

a. Surface structures. Many pathogens contain as part of their surface a component or components which render phagocytosis difficult. This is especially true with pneumococci, streptococci, and *Pasteurella pestis*. These antiphagocytic substances have been called aggressins or antiphagins. It has long been recognized that sera from immune animals and man contain antibodies which neutralize the activity of these aggressins by acting not on the phagocytes but on the microorganisms (37, 236, 270).

Pneumococcus: Most complete information on the relation between surface structure and virulence has been obtained for the pneumococcus. These findings have been reviewed repeatedly (103, 361, 365) and leave no doubt as to the significance of the capsular carbohydrate in determining the relation between pneumococci and phagocytes. However, they do not explain the pathogenic effect of pneumococci. The production of the lesion may to some extent be induced by the chemotactic effect of the multiplying organisms, but other components, which unlike the soluble specific substance are present in virulent and avirulent strains, facilitate the establishment of pneumonia (247).

Group A streptococcus: The virulence of group A streptococci is closely correlated with their ability to produce a protein as part of their surface structure. The M-protein induces type-specific immunity in mice and rabbits which can be transferred passively to non-immunized animals (142). The virulent form also resists phagocytosis by whole blood and escapes its bactericidal effect (274); the protective antibody enhances phagocytosis (142, 274). The M-protein, easily distinguished from the T-protein by physicochemical and serological methods, is part of the cell-wall structure or at least remains associated with it after mechanical disruption of the cell (282). Although the opsonic activity of protective sera is the most striking effect (37, 68, 188), bacteriostatic or bactericidal activity can be detected in sera from immunized animals (68) or in sera from patients in the acute phase of other infections (327). This activity is abolished under anaerobic conditions or in presence of strong reducing agents (327), a finding which is compatible with the observation that the reducing power of serum is inversely proportional to its bactericidal effect on pneumococci (135).

In addition to the M-protein, the formation of capsular material consisting of hyaluronic acid can contribute to antiphagocytic resistance. This latter property is less important in the case of group A streptococci (155, 274), but it plays a part in determining the virulence of group C streptococci (292). The hyaluronic acid from both groups is the same and is identical with that from animal tissue. Intraperitoneal treatment of susceptible animals with hyaluronidase increases their resistance substantially against intraperitoneal infection with group C but only slightly against group A streptococci. The enzyme does not destroy the viability of the organisms of either group (155, 274). The same difference in importance of M-protein and hyaluronic acid for group A and group C streptococci is demonstrable by testing the influence of proteolytic enzyme and hyaluronidase on phagocytosis *in vitro* (228). Thus, in streptococci, antiphagocytic activity is connected with two components which differ chemically and in their localization. The M-protein, linked somehow to the cell wall, probably is an indispensable part of the bacterial surface, whereas hyaluronic acid appears as a secretion product forming a dispensable capsule.

Pasteurella pestis: Natural resistance and acquired immunity against the plague bacillus depend to a large extent on the phagocytic capacity of PMN, MN and the RES (221). Antiphagocytic activity of *P. pestis* is related to the

presence of a component, fraction I, obtainable in crystalline form. Two subfractions have been isolated from it, fraction IA—a protein-carbohydrate complex, and fraction IB—a protein (11). The amount of these fractions contained in plague antigenic preparations determines their immunizing potency. Fraction I is easily extracted in water, is located on the surface as a slimy envelope embedding a number of organisms (88), and is not part of the capsule. Virulence is correlated with the amount of fraction I produced but not with the quantity of toxic material (water soluble and water insoluble antigens) which is present in virulent and avirulent strains (87). *P. pestis* grown *in vitro* seems to differ from organisms growing in the peritoneal cavity: if virulent and avirulent organisms, grown *in vitro*, are injected intraperitoneally into guinea pigs and phagocytosis is followed, it is observed that both types are phagocytized to the same extent during the first hour. In the case of the avirulent type, phagocytosis continues after the first hour at the same rate. On the other hand, no phagocytosis of the virulent form occurs after the first hour, but there is extracellular multiplication. If these virulent organisms are now injected into fresh guinea pigs, no phagocytosis occurs, indicating that some component was produced by the virulent strain in the peritoneal cavity which was not present in the first culture. Phagocytes from the cavity which no longer take up virulent forms are also unable to ingest the avirulent type of *Micrococcus pyogenes* var. *albus*. This indicates some damage although no morphological signs of degeneration can be observed. A mutant which is deficient in hypoxanthine, and which cannot multiply in the peritoneal cavity unless hypoxanthine is provided, has been used to demonstrate that growth of the virulent organism must occur *in vivo* before it becomes antiphagocytic. Without hypoxanthine it behaves towards phagocytes like an avirulent culture but in the presence of it, like a virulent one. Thus, one has to assume two properties in which the virulent forms differ from the avirulent ones: ability to survive and to multiply in presence of PMN, and capacity to produce an antiphagocytic factor. The latter might be related to the somatic antigen.

The change from the phagocytosis-sensitive to the phagocytosis-resistant virulent type has been studied further. Virulent organisms harvested from tryptic meat-agar slants are sensitive to phagocytosis but become highly resistant when incubated from 1–3 hours in tryptic meat broth or in a glucose-ammonia-phosphate-cystine medium. The change shows no correlation with encapsulation, which takes place simultaneously, and no chemical basis for it has as yet been established. A close relationship was found between virulence and the ability to convert to the phagocytosis-resistant form under the conditions described above (42).

The intracellular fate of *Hemophilus influenzae* is less predictable. Phagocytosis is slow in the normal animal or man and is greatly accelerated in immunized animals. The type-specific antibody, which is an anti-carbohydrate, causes capsular swelling, agglutination and opsonization (3). Immune serum also has a bactericidal effect on both the virulent and avirulent variants, whereas normal serum is active against the avirulent non-encapsulated strain only (522). Phagocytosis does not necessarily result in the loss of viability of the pathogen, as washed phagocytes from cerebrospinal fluid contain viable organisms (96). Recent observations in mice indicate that phagocytized *H. influenzae* transform into L-forms which are able to survive the intracellular environment. In immunized mice phagocytosis and appearance of L-forms is more rapid (357). L-forms generally are more resistant to unfavorable environmental conditions *in vitro* and *in vivo*.

Members of the genus *Neisseria* are found frequently within PMN, either in the cerebrospinal fluid or in secretions from the urethra. Viable gonococci have been found in pus containing no extracellular forms, but there is no evidence that they are able to multiply intracellularly (92). Immune sera have bactericidal and bacteriolytic effect. The bactericidal effect of whole blood does not depend on the number of phagocytes present nor on the degree of phagocytosis, but on a system requiring complement (310). However, the findings that blockade increases susceptibility of mice to meningococcal infection, and that the virulence of meningococci can be enhanced by growing them in leukocyte extracts, suggest that phagocytes may play a part in this infection (231).

The virulent form of *Micrococcus pyogenes* var. *aureus* is able to survive and to multiply within leukocytes and to escape after destruction of the

leukocyte (187, 269, 336). Nevertheless, pathogenic strains possess a mechanism by which phagocytosis is reduced, as compared to nonpathogenic strains. Virulent, coagulase positive organisms prevent phagocytosis, provided the plasma contains the necessary activators for the conversion of inactive coagulase into a thrombin-like substance. The deposition of fibrin resulting in clumping of cocci and PMN prevents phagocytosis. Animals, including man, susceptible to staphylococcal infection have a high content of activator in their plasma, whereas resistant animals have a low content. Staphylococci cannot coagulate the plasma of the latter group and are thus easily phagocytized. The fact that most tests for phagocytosis have been done in a fibrinogen-free system, or in the presence of citrate or heparin, might explain the general finding that staphylococci are easily phagocytized. The addition of coagulable plasma to staphylococci increases their virulence for resistant animals (307). Likewise, the plasma of mice of an unusually high susceptibility to staphylococcal infection was found coagulable by staphylococci (115). This mechanism of prevention of phagocytosis by fibrin deposition is unusual and contrary to the observation that a network of fibrin enables PMN to engulf encapsulated organisms in absence of antibody (358).

b. Leukocidin. Damage to leukocytes results in morphologic changes (pycnosis and other signs of cellular degeneration) and in a loss of certain functions. Thus, hydrogen-transfer capacity is impaired as measured by tests using methylene blue or tetrazolium as hydrogen acceptors (235), and motility is reduced as demonstrated by the lack of emigration of PMN from the buffy coat (202). Finally, it has been observed that damaged leukocytes release lysozyme-like substances which are detected by their ability to lyse *Micrococcus lysodeikticus* (161). Toxic substances for leukocytes produced and released into the culture medium by microorganisms were first observed with staphylococci and were called leukocidins (336). Virulence of staphylococci is closely connected with their production of leukocidin, as well as with many other properties, as shown by a correlation between their mouse pathogenicity and the release of a leukocyte inhibiting substance (315). It remains yet to be explored whether ability to survive phagocytosis and production of leukocidin are independent but essential properties of pathogenic staphylococci or whether they are correlated. Streptococci and pneumococci also produce leukocidins (105, 243). A crystallized hemolysin with leukocidin activity was obtained from streptococcal cultures; it can therefore be assumed that leukocidin is closely related to hemolysin (63).

The relationship between extracellular parasites and phagocytes is obviously dependent on many factors. This and virulence are almost exclusively determined by the capsular polysaccharide in the case of pneumococci, a fact further substantiated by the finding that virulence depends on a single genetic trait and can be transferred from virulent to avirulent strains by means of a pure preparation of DNA (143). The mechanisms of interaction are more complicated in staphylococcal and streptococcal infections. This might contribute to the great variation found in duration and complexity of infections due to these organisms.

The interaction between *Bacillus anthracis* and leukocytes appears more confusing. Originally it was suggested that phagocytosis played a major role in resistance to this infection (219), but it was soon found that leukocytes and tissues liberated an anthracidal substance which was essential for resistance and that phagocytosis was of secondary importance (253). This anthracidal substance was later characterized as a basic peptide (34). Fully virulent organisms appear to produce *in vivo* all of the following components, each of which has biologic activity: (a) A capsule essential for virulence. It is antiphagocytic and antagonizes the anthracidal substance in tissues. The capsular material is a polypeptide containing 40 to 50 glutamic acid molecules (145). When isolated from lesions it consists exclusively of D(−) glutamic acid and has a lesion-producing effect not present in the "*in vitro* capsule" which contains about 15 per cent L(+) glutamate (347). The component is antigenic, and the antibody protects mice but no other animals against a virulent infection. (b) Extracts of bacilli collected from infected animals contain an anticomplementary factor which seems firmly bound to the cell structure (159). (c) A protective antigen identified as protein was originally found only in lesions in which bacilli had grown (117, 316, 348). It was later isolated from cultures, especially of nonproteolytic strains, grown *in vitro* under carefully controlled conditions (19, 110,

360). This antigen is also found in a fraction isolated from plasma of infected guinea pigs. It produces edema and shock when injected into animals, and is highly antiphagocytic (304). Injection of the nontoxic protective antigen obtained from *in vitro* cultures results in production of an antibody which protects against subsequent infection and which neutralizes the toxic fraction obtained from plasma (304). Products of *in vitro* grown organisms do not show any toxicity when injected into animals. This difference between organisms grown *in vitro* and *in vivo* is important, and the suspicion arises that similar differences could be found with other pathogenic microorganisms.

2. Enhancement of phagocytosis. The infected host is able to mobilize several mechanisms which increase the effectiveness of phagocytes and neutralize toxic products of the pathogens.

a. The component of the surface of the pathogen responsible for prevention of phagocytosis generally is *antigenic* (11, 142, 208) and stimulates the production of an *antibody* which enhances phagocytosis of these organisms by leukocytes (36, 236) or by the RES (160, 361). Likewise, antibodies neutralizing the effect of leukocidin have been found (69, 187, 235).

b. A characteristic of recovery from pneumococcal or streptococcal pneumonia, in addition to the type-specific antibody response, is an intense *macrophage reaction* (267, 349), which results in an increase of nonspecific resistance. Repeated preliminary injections of aleuronate into the pleural cavity of a dog before infection with streptococci or pneumococci cause a rapid mobilization of macrophages at the site of injection with resulting increased resistance (50). The local macrophage reaction contributes cells which require less opsonization for phagocytosis, which have a greater proteolytic power at low pH than PMN (266), and which are more resistant to leukocidins (105, 243). In man this reaction begins on the fifth day after onset and reaches its maximum on the tenth day (267). In repeated, experimentally produced attacks of pneumococcal pneumonia in the dog, the macrophage reaction is more rapid and more intense if reinfection is at the same site. The acquired resistance is not correlated with the presence of specific antibody in the serum (54). The importance of the macrophages and the RES has been recognized in other infections, such as experimental infections with *E. coli* and *M. pyogenes* var. *aureus* (234) or with *P. pestis* (25). A very extensive macrophage reaction occurs in the spleen as a result of plasmodial infections (320).

c. Recent findings indicate that even without specific opsonization phagocytes are capable of engulfing encapsulated organisms when a proper surface is available. Surface phagocytosis (358) helps to explain the many observations of phagocytosis of encapsulated organisms occurring *in vivo* even in the absence of antibodies. Surface phagocytosis of encapsulated pneumococci or *Klebsiella pneumoniae* in the absence of specific antibody has been observed under various conditions: on filter paper or in formalin-fixed lung, intercellularly in concentrated suspensions of phagocytes, and within strands of fibrin in the plasma clot (358). During the evolution of the pneumonial lesion, conditions favorable for surface phagocytosis develop gradually. In *early edema*, when the phagocytes and cocci are suspended in the alveolar exudate, little phagocytosis takes place in the absence of antibody. The crowding of leukocytes and the deposition of fibrin in red and gray hepatization are conducive to surface phagocytosis. The macrophage reaction during resolution causes the accumulation of cells which are able to phagocytize either on surfaces (285) or with little opsonization (266). However, even surface phagocytosis may be prevented by the heavy, slimy layer of a young culture of pneumococcus type III and enhanced by the specific antibody (358), showing that the difference between surface and ordinary phagocytosis is quantitative rather than qualitative.

Conditions which favor surface phagocytosis through crowding of PMN and formation of a fibrin net prevail in the inflamed lymph node. The difference in filtering capacity between normal and inflamed lymph nodes can be measured by the degree of bacteremia developing after injection of pneumococci into an afferent lymphatic: heavy bacteremia occurs with a normal lymph node and is absent with an inflamed one (305). Surface phagocytosis has been found effective also in the case of *B. anthracis* (273).

3. Intracellular destruction of pathogens. Extracellular parasitic existence is essential for some pathogens because the intracellular environment is unfavorable for their survival and propagation. The multiplicity of enzymes found

in phagocytes was discussed in an earlier paragraph. However, considering the wide biological range of phagocytosis and the individuality of the surfaces of microorganisms, it is very unlikely that phagocytes contain enzymatic systems specifically designed for the degradation of microorganisms. Death and lysis of bacteria appears to be initiated by a variety of mechanisms.

A number of substances with *bactericidal effect* has been found in phagocytes, especially in PMN. Some early workers showed that extracts from leukocytes had a wide range of activity (165), while others reported that the range of bactericidal activity was limited to the one found for lysozyme (13). The fact that crude extracts were used containing from 10 μg to 10 mg of cell material might explain some of the discrepancies. One of the active substances is a basic polypeptide isolated from PMN and other organs. This peptide protects mice against infection with *B. anthracis* but is also effective *in vitro* against other organisms (34). Basic proteins such as protamine and histone also exhibit bactericidal activity (222). Further exploration of antibacterial activity of cellular proteins and polypeptides seems indicated.* It has also been suggested that lactic acid, which inhibits growth of tubercle bacilli and staphylococci *in vitro*, is responsible for the antibacterial activity of inflammatory exudates containing phagocytes (78). The accumulation of lactic acid in areas of inflammation is well known (99, 158). Low oxygen tension and increase of acidity enhance (76), and ketone bodies antagonize (77), the bactericidal effect of lactic acid. Such an exploration of metabolic interactions between pathogens and phagocytes or tissues has only begun. In further experiments attention should be given to the relative concentration of these inhibitory and antagonistic substances in the normal and inflamed tissues. The irregular distribution of such inhibitors within various organs of a host could well be the basis for differential resistance and susceptibility (78).

Intracellular bacteriolysis can be the consequence of autolysis or of the action of an enzyme present in phagocytes. A combination of mechanisms may be implicated when pneumococci are phagocytized (74, 79), whereas a lysozyme-like enzyme causes lysis of some gram-positive cocci and bacilli (13). Lysozyme is widely distributed in nature and contained in almost all cells and tissue fluids including serum and secretions of many glands (326). The substrate for the enzyme is a highly polymerized mucopolysaccharide which is present in cell wall preparations of *M. lysodeicticus* and other bacteria (283, 328). It is most active against saprophytic gram-positive cocci and bacilli and to a limited extent against some strains of *E. coli* and of pathogenic organisms such as staphylococci, streptococci and tubercle bacilli (13, 232). These pathogens are not completely lysed by lysozyme, a fact which suggests that their ultimate lysis depends on additional mechanisms. An explanation might be given by the fact that lysozyme is inhibited by some acidic polymers derived from pathogenic bacteria (302). MN contain little or no lysozyme (165, 233).

It is evident that no single enzyme or metabolic condition is responsible for intracellular digestion of microorganisms. The latter depends on a sequence of reactions catalysed by enzymes which are provided by the parasite and the host cells. The intracellular environment, including the digestive vacuole formed around ingested particles, may serve as a trigger mechanism for this process. It is tempting to speculate that one of the attributes of microbial saprophytes of higher animals is their susceptibility to lysozyme. The possibility that phagocytes are capable of producing adaptive enzymes in response to foreign material has also to be considered.

Chronic infections can be caused by extracellular parasites, such as staphylococci and streptococci. Theoretically, these pathogens can proliferate only if phagocytes have no access to the lesion and the parasites. This is true in necrotic and to some extent in fibrotic lesions. Before these conditions have developed in the lesion, it is likely that survival within phagocytes is sufficient for the maintenance of the infection. Egestion of phagocytized microorganisms

* New work on the extraction of a bactericidal substance from exudate leukocytes has just been published (HIRSCH, J. G., Phagocytin: a bactericidal substance from polymorphonuclear leukocytes. J. Exptl. Med., **103**, No. 5, 589–611; Studies of the bactericidal action of phagocytin. *Ibid.* **103**, No. 5, 613–621.). Phagocytin, exhibiting properties characteristic for globulin, is found almost exclusively in rabbit PMN and has greatest activity towards gram-negative enteric bacilli under acidic conditions.

as described for streptococci (355) may also favor survival of some pathogens.

Infections with species of *Treponema, Borrelia* or *Leptospira* demand special mention because they are difficult to classify. There is no direct evidence that phagocytosis contributes in any way to resistance against these organisms, and it is doubtful whether live spirochetes are phagocytized at all (312, 365). Indirectly, by splenectomy and blockade, it has been shown that the RES probably plays some role in resistance (8, 176), but the mechanism is unknown. The location of the organisms during the latent phase of these diseases is not known. A coccoidal form which escapes identification and resists destruction in tissues might exist since treponemata pass through relatively complex growth cycles (64).

B. Facultative Intracellular Parasites

The organisms classified in this group can, during certain stages of the host-parasite relationship, survive or proliferate within phagocytes or in extracellular spaces. This property, which enables them to find a suitable environment either extracellularly or intracellularly, permits survival for a considerable length of time in the host and can result in subacute and chronic infections. Consequently, the cellular reaction of the host to these organisms is different from that to extracellular parasites and usually results in an infectious granuloma (148). Granulomatous reactions are characterized histologically by the accumulation of elements of the RES. They include endothelial cells, macrophages, either as large, round cells or epithelioid cells, and giant cells of the foreign body or Langhans type. In the periphery of the granuloma a lymphoid and fibrocytic reaction is found. In the center of the lesion necrosis often develops which leads to infiltration of PMN. The granulomatous response to some stimuli is specific and has diagnostic value. Some of the factors causing granuloma formation are: (a) the pathogens resist destruction by PMN and cause a macrophage reaction following the acute PMN phase; (b) bacillary components, such as lipides, are released and stimulate the accumulation of macrophages; and (c) focal necrosis is induced by the action of an endotoxin and secondarily invaded by macrophages.

Most of these infections begin as an acute phase with free proliferation and spread of the causative organisms; many diseases such as tularemia and typhoid fever usually terminate soon after this phase. Other infections continue into a chronic stage which can be interrupted at any time by an acute relapse. Some infections, such as brucellosis, eventually die out and others, *e.g.* tuberculosis, may persist for life. Evidence of intracellular survival and proliferation has been obtained mainly from: (a) results of histological findings from either human cases or experimentally infected animals; (b) bacteriological results obtained by culturing lesions in which the bacteria were microscopically located intracellularly; and (c) tissue-culture studies of infected cells. Since all the pathogens in this group can also multiply outside of cells and in cell-free media, the interpretation of the results is sometimes difficult.

1. Bacterium tularense (Pasteurella tularensis), Salmonella typhi and Brucella spp. Infections with these parasites have many aspects in common and will be discussed first.

Bacterium tularense. Intracellular location of *B. tularense* is characteristic of the lesions caused by this organism (41, 111). The cells invaded are mainly ectodermal and hepatic cells (41), with some involvement of Kupffer cells and MN. The pleomorphism observed *in vitro* of *B. tularense* suggests that other than bacillary forms may occur in tissues (137). This could account for the difficulty in identifying the agent in sections and isolating it from lesions (174). Whether primary necrosis or specific bacterial components are the cause of the reticuloendothelial hyperplasia characteristic of this disease is questionable (83).

Salmonella typhi. Intracellular location of typhoid bacilli and granulomatous lesions in tissues are a constant finding in infections with this pathogen.

Typical cells, presumably called forth in response to the bacilli, are found along the route of their spread in the intestinal wall, in the lymphatics, and in the lymph nodes (182), and the bacilli are usually found in plasma cells (1). It is not known whether in man the bacilli multiply intracellularly in lesions, although they have been shown to multiply within the endothelial cells of the chick embryo (112). Even if the bacilli do not multiply extensively within cells, intracellular survival suffices for a continuation of the host-parasite relationship, since intra-

cellular bacilli are protected against the bacteriolytic activity of serum (275).

The granulomatous lesions are most frequently found in the liver, and agreement is general that they originate from local cells of the RES, mainly Kupffer cells. Typhoid bacilli are very rare in such lesions, and the granulomatosis is considered a secondary response to multiple foci of necrosis caused by bacillary products (148, 215, 219). Indeed, the somatic antigen causes widespread hemorrhagic and thrombotic lesions upon intravenous injection into rabbits (227).

The effect of *S. typhi* upon PMN could explain the pathogenesis of the granulomatous lesion. If typhoid bacilli or their somatic antigen are injected intravenously into rabbits, a sharp drop in the number of circulating PMN results, and a few minutes after injection the leukocytes are found in the capillaries of the lungs and the sinusoids of liver and spleen (268). The property of causing leukocyte-platelet thrombosis in capillaries, especially of the lungs in normal animals or at sites of primary preparation for the Shwartzman phenomenon, is characteristic of all substances which when injected intravenously into a previously prepared rabbit (314) elicit the local Shwartzman reaction. *S. typhi* was also found to inhibit migration of PMN (202). Other workers could not confirm this observation (24, 65), but found that after intravenous (i.v.) injection of *S. typhi* or its somatic antigen into rabbits the motility of PMN was impaired as measured *in vivo* by the number of cells in an inflammatory reaction (65) or by the extent of migration from a buffy-coat explant (24). This effect is demonstrable within 5 minutes after injection and lasts for 6–12 hours (24). This time relationship correlates well with the interval between primary leukopenia caused by glycogen and the reappearance of leukocytes due to their release from the capillary regions of the lungs, as demonstrated in heart-lung preparations (40).

One might assume that i.v. injection of somatic antigen causes a platelet-leukocyte aggregation *in vivo* which is responsible for (a) leukopenia, (b) the local Shwartzman in previously prepared animals, and (c) the "inhibition" of migration of PMN from the buffy coat or from the vessels. The formation of leukocyte-platelet aggregates may also occur in typhoid fever and cause focal necrosis due to reactions similar to the local Shwartzman phenomenon. This assumption is indirectly substantiated by the fact that streptococcal infections induce a state of preparation for local and general Shwartzman reactions (324), and it is further supported by the fact that the lesions occur in spite of the presence of antibodies, as antibodies do not inhibit the effect of the somatic antigen upon leukocytes (53, 226, 268). Heat-killed typhoid bacilli and their endotoxin when injected i.v. into mice reduce the ability of the RES to remove india ink from the circulation. This depression remains for about 24 hours. A second i.v. injection into the same animals, however, stimulates the activity of the RES, a fact which could explain the refractory state against endotoxin induced by repeated injections of an endotoxin (26).

Brucella spp. survive and presumably multiply within phagocytes. In the chick embryo the organisms are found in ectodermal and in endothelial cells, in fibroblasts and MN (38, 41, 46, 112). *B. suis* and *B. abortus* have a predilection for mesodermal cells, whereas *B. mellitensis* is frequently located in the ectoderm. The response to the presence of *Brucella* spp. in various tissues leads to reticulo-endothelial hyperplasia in the form of epithelioid granuloma with giant cells which seem to form around infected cells (46, 198). The cells containing the bacilli frequently have pycnotic nuclei and appear to be in a stage of degeneration (306), the degree of necrosis and suppuration of lesions being an indication of either the virulence of the culture or the susceptibility of the host (38). Thus, in chronic brucellosis the bacilli tend to disappear gradually from granulomatous lesions, whereas they persist in necrotic foci (38). Mild chronic infections in mice result in a diffuse histiocytic infiltration without necrosis.

Degree of phagocytosis of brucella by PMN depends on the content of opsonins in the serum. The opsonocytophagic index is based on the observation that opsonic titer of the blood rises during infection. The bactericidal activity of the blood is independent of phagocytosis, but the presence of formed elements seems important, and the strain of bacteria used as well as the species from which the blood is taken determine the outcome of the test (337, 338). PMN which have phagocytized virulent organisms show depressed migratory activity *in vitro* (86).

2. Bartonella and pathogenic fungi. A number of other organisms are frequently found intracellu-

larly. Infections due to organisms of the Bartonellaceae cause lesions by intracytoplasmatic development of masses of the pathogens within the endothelial cells of capillaries and lymphatics. The intracellular location of *Bartonella* is regular, but it is no proof for obligate intracellular parasitism, as some members of the group can be cultivated *in vitro* in cell-free media (249).

Pathogenic fungi are found in lesions intracellularly or extracellularly. The location depends on the causative agent and on the type of tissue response: if suppuration and abscess formation prevail the agent is usually extracellular, whereas in the granulomatous lesion the fungi are more frequently intracellular. Systemic mycotic infections usually result in a combination of the two reactions. The following pathogenic fungi are frequently found within epithelioid and giant cells: *Actinomyces* spp., *Blastomyces brasiliensis*, *Coccidioides immitis*, *Histoplasma capsulatum* and *Cryptococcus neoformans*. The latter organism produces an abundance of capsular material which causes very little tissue reaction, probably because of its inhibiting effect upon PMN (72).

H. capsulatum, which is found *in vivo* almost exclusively within macrophages and giant cells, can be cultivated in tissue culture of embryonic cells or of Earl's "L" strain and appears to multiply intracellularly (256). In infections with *C. immitis* sporangiospores or endospores alternate with spherules, and the changing tissue reaction, suppurative or granulomatous, can be correlated with the developmental form of the parasite. Thus, mycelial aggregates and young spherules attract PMN, resulting in suppuration, whereas mature spherules are surrounded by epithelioid cells. Release of endospores or young spherules will again cause PMN activity (95). It is possible to explain the alternating lesions on a chemical basis. The surface structures of sporangiospores and spherules differ chemically; the spherules are rich in phospholipids, whereas the sporangiospores possess a thick outer layer of mucopolysaccharide (322).

3. Mycobacterium tuberculosis. This organism elicits in tissues an accumulation of PMN followed by MN with the formation of characteristic granulomata which can undergo caseous necrosis (44). The tissue changes are accelerated and more intense in previously infected animals. This reaction is attributed to specific hypersensitivity (104, 178).

a. Tubercle bacilli and PMN. The primary reaction is a nonspecific inflammatory response with a preponderance of PMN. Tubercle bacilli are easily phagocytized by PMN *in vivo* and *in vitro* without specific opsonization, but serum from infected animals promotes phagocytosis to some extent (179). PMN containing large numbers of tubercle bacilli appear to be damaged (217, 343), as is indicated both *in vitro* and *in vivo* by impairment of their motility (5, 29). This inhibition of migration of PMN can be correlated with the virulence of the strain (205). A petroleum-ether extract prepared from virulent tubercle bacilli has a similar effect upon migration of PMN when *B. subtilis*, coated with this extract, is phagocytized by them (30, 43). This extract, the cord factor, has been purified and found to consist of two molecules of mycolic acid and one molecule of trehalose (trehalose dimycolate) (238). The cord factor is toxic when injected repeatedly into mice (30), and it promotes infection of mice with virulent tubercle bacilli when it is injected 1 day prior to the infection (32). Phagocytosis of tubercle bacilli by PMN and MN does not reduce to any observable extent respiration of the parasites; thus, when phagocytized in large numbers, oxygen uptake and CO_2 production of tubercle bacilli is the same as in absence of phagocytes (311).

b. Aging and degenerating PMN are phagocytized by MN, which play a major role throughout the tuberculous infection. Histologic findings (44, 119, 182) and tissue culture experiments leave no doubt that tubercle bacilli are able to multiply intracellularly within MN (39, 192, 317). In early tissue culture experiments difficulties arose because the extracellular spread of tubercle bacilli soon overgrew the culture of MN (39, 207). This complication could be eliminated by adding to the tissue culture medium enough streptomycin to prevent extracellular growth without interfering with intracellular proliferation (192, 317). Multiplying tubercle bacilli damage the host cells, and if a virulent strain is used most of the parasitized cells in a tissue culture will be destroyed (192, 207, 317). The component of tubercle bacilli responsible for the relationship between the virulence and the destructive effect upon MN is not known, but it can be assumed that the cord factor has some activity. Low toxicity of the tubercle bacilli for macrophages or high resistance of the cells are indis-

pensable for true symbiotic interaction which has been observed directly in tissue culture and deduced from observations *in vivo* (119, 207). In tissue culture, attenuated tubercle bacilli multiply more slowly in MN than virulent ones (194) and are less damaging to the cells (317). Furthermore, virulent tubercle bacilli require a longer lag phase before multiplying in lungs of highly resistant animals than in those of susceptible ones (186). Both these observations indicate that attenuation as well as native resistance find a quantitative expression in the interaction between cell and parasite.

There is no doubt that infection with tubercle bacilli results in the decrease of the number of bacilli during the primary infection or in the inhibition of growth of bacilli in reinfection (80, 183). Information on the mechanism of inhibition of tubercle bacilli as a result of immunization is incomplete and contradictory. The following possibilities are suggested:

(i) Many observations show that macrophages from infected animals destroy tubercle bacilli *in vivo* and *in vitro* (52, 173, 183, 217, 280). In addition, MN from immunized animals have been found to prevent intracellular multiplication of tubercle bacilli in the animal or in tissue culture (185, 255, 317). This inhibitory property of MN could not be observed by others (193). Tissues of infected human beings or animals may simultaneously harbor lesions in which the bacilli multiply actively and others in which only very few bacilli are found (44, 183). Apparently, the local environment, the quantitative relationship of bacteria and phagocytes, and other still unknown factors play an important role (318). More detailed information on the factors which influence the interaction between tubercle bacilli and MN from normal and immunized animals is required in order to interpret the conflicting observations. The mechanism of intracellular inhibition or lysis of tubercle bacilli is unknown. Enzymes have been found in tuberculous tissues which are not present in normal tissues (109, 118, 340), and it has been shown that metabolites accumulating in the inflammatory lesions may suppress the metabolism of tubercle bacilli (117). Indeed, tubercle bacilli recovered from chronic lesions in the mouse show a different pattern of respiratory metabolism than tubercle bacilli grown *in vitro* (293). Some importance is attributed to the lymphocytes, which contain highly active esterases (21), but the possibility of an autolytic mechanism of bacterial destruction within cells cannot be excluded. In addition, MN derived from tuberculous animals exhibit a nonspecific hyperactivity, such as increased phagocytosis of carbon particles and mobilization of cells upon irritation (184).

(ii) All attempts to demonstrate antibacterial activity of serum from immunized animals have so far failed (123, 163, 255). This lack of positive results regarding a component of antiserum inhibitory to tubercle bacilli should not be accepted as conclusive, especially since it is possible to transfer passively delayed hypersensitivity from sensitized to normal guinea pigs by means of plasma fraction IV-10. The active fraction contains all antibody to proteins of tubercle bacilli and has no transferring capacity in presence of fraction II containing antibody to tuberculopolysaccharide (56). It may be that the antibacterial activity of antiserum is masked by the presence of other inactive fractions.

(iii) Recent observations indicate that substances with tuberculostatic and tuberculocidal property, such as organic acids and CO_2, accumulate in the inflammatory lesion (76) or are present within tissue cells and are released when necrosis occurs. Some of the tuberculocidal compounds are spermine (140), a basic peptide isolated from thymus (141), and lysozyme (232, 233). Thus it appears that survival or death of tubercle bacilli in tissues depends on a delicate balance between growth-promoting and inhibitory substances. A local change in one component could easily alter the form of parasitism with important consequences to the entire host.

c. Cell response. The evolution of the *cell response* to the presence of bacilli follows the pattern of the formation of a granulomatous lesion. The lesion is not absolutely specific since very similar ones are found in tularemia, brucellosis and other granulomatous diseases of viral or unknown origin. Formation of epithelioid cells and giant cells in presence of tubercle bacilli has been observed *in vitro* using macrophages from normal animals, indicating that hypersensitivity is not essential for granuloma formation (207, 254, 362). Several components of the tubercle bacillus which elicit strong cellular reactions resembling those brought about by dead tubercle bacilli have been identified. The most active components in this respect are the phosphatide fraction and phthioic acid, which produce a reaction mainly of epithelioid and giant cells. In the previously infected animal the reaction to the phosphatide is much stronger and resembles a Koch phenomenon (279, 281, 397). To avoid the

possibility that some bacilli or fragments therefrom might be present in the material, synthetic fatty acids have been used in more recent studies (333). The reactions produced by these acids were degenerative and proliferative. Likewise the lipopolysaccharides isolated from tubercle bacilli elicit a granulomatous reaction in tissues (319). A correlation between the granuloma producing activity and the inhibitory effect upon migration of PMN by phthienoic acid and some derivatives has been reported (144). It must be kept in mind that other lipids, and especially long-chain fatty acids not related to tubercle bacilli, cause degeneration and granulomatous lesions in animals (108, 139). This form of lesion represents, then, a basic pattern of tissue response, especially of the macrophage, to more or less indigestible material. The specificity of the reaction can be very subtle and frequently is difficult to detect.

Caseation, that form of tissue necrosis characteristic for tuberculosis, can be induced by some of the lipide components of the tubercle bacillus (333), but large quantities of these substances are required for this effect. It is likely that tissue destruction during infection is mainly due to the state of hypersensitivity which renders the relatively innocuous tuberculoprotein capable of widespread tissue destruction (262). Spleen cells from tuberculous animals are damaged or killed when cultivated *in vitro* in the presence of tuberculin, which is harmless for cells from normal animals (263), and necrotic pneumonia can be induced in tuberculous animals by instillation of tuberculin into the bronchial tree (259). The real problem is the elucidation of the mechanism by which the necrotic tissue is converted into caseous material instead of being liquefied and resorbed. There is evidence that carbohydrates, proteins and phosphatides from tubercle bacilli inhibit cellular proteolytic enzymes and deoxyribonuclease present in caseous material (350). If inhibition of such enzymes of the dying tissue is responsible for caseation, then it has to be assumed that softening occurs when these inhibitors have been inactivated or when enzymes not susceptible to inhibitors have appeared, and indeed leukocytic proteolytic enzymes are capable of hydrolyzing caseous material (351). Tubercle bacilli are almost completely absent from caseous material but reappear upon softening (44, 262), and it is conceivable that inhibitors are liberated during caseation and removed as softening sets in. The very rapid reappearance of the tubercle bacilli indicates that caseous foci are not sterile.

The relation between tubercle bacilli and the cells of the host is reciprocal. The presence of the bacilli stimulates the formation of the granulomatous lesions which by cellular or biochemical activity brings about destruction of bacilli. The liberated components of the bacilli may contribute to caseous necrosis of tissue areas which, if softening occurs, harbor large numbers of bacilli. It is not surprising that numerous extraneous factors can alter such a complex system in favor of either the bacilli or the host tissues. ACTH or cortisone seem to exert such a double action; it is beneficial to the tissues by reducing the impact of the hypersensitivity reaction (259), but on the other hand it favors the bacilli by reducing the inhibitory power of the tissues and phagocytes (186).

C. Obligate Intracellular Parasites

Parasites which have never been successfully cultivated *in vitro* in cell-free media and which are found in intracellular locations *in vivo* are usually classified as obligate intracellular parasites. Viruses, rickettsiae, some parasitic protozoa and *Mycobacterium leprae* belong to this category. Whereas there is little doubt that the intracellular existence of the viruses, and to a lesser extent the rickettsiae, is dictated by dependence of the parasites on the enzymatic systems and intermediary metabolites of the host cell, the necessity for intracellular parasitism of *M. leprae* and of some protozoa is poorly understood.

1. Mycobacterium leprae. This is the only obligate intracellular animal parasite of the class *Schizomycetes*. Although its intracellular location had long been recognized, very little is known of the biology of this organism. *M. leprae* in man, or *M. lepraemurium* in rats, is found in lepra cells which mainly derive from histiocytes (242). There is histologic evidence that the bacilli partially disintegrate within the cells. In most properties *M. leprae* differs little from other pathogenic mycobacteria, but so far it has never been propagated *in vitro*, either in tissue culture or in cell-free media. *M. lepraemurium* also differs from other mycobacteria in its metabolic capacity: (a) substrates which enhance respiration and hydrogen transfer capacity of cultivable mycobacteria do not enhance either of

these properties in *M. lepraemurium*; (b) the endogenous metabolism of *M. lepraemurium* declines rapidly in contrast to endogenous respiration of other mycobacteria; (c) rat serum inhibits the metabolic activity of *M. lepraemurium* but enhances that of other mycobacteria. Albumin and yeast supplement protect *M. lepraemurium* against the harmful effect of serum as measured by metabolic activity and by infectiousness which is closely related to metabolic integrity (124). These findings might aid in understanding of the mechanism of intracellular parasitism of *M. leprae*. The suggestion has been made that intracellular location is the consequence of susceptibility to extracellular inhibitors rather than the consequence of a nutritional requirement for intracellular metabolites. No proof of this hypothesis is available, but it opens new ways towards a study of many problems of leprosy.

2. Pathogenic protozoa. Some pathogenic protozoa are facultative and others are obligate intracellular parasites. A few examples chosen arbitrarily will be cited here.

Trypanosomes have an alternate existence in a vertebrate and invertebrate host. They multiply intracellularly or extracellularly, some of the developmental forms having been propagated in cell-free cultures and others in tissue cultures. No specific cell tropism for intracellular proliferation has been observed (220) and the parasites multiply within macrophages (366), although in some locations they are destroyed in the same cells (321). Humoral immunity also plays an important role, either directly or through the mediation of phagocytes of the RES which destroy the parasites (70). Consequently, splenectomy has a profound influence on the resistance to protozoan infection (8, 175).

Plasmodia are mainly erythrocytic parasites. Survival and limited proliferation are possible in a cell-free system provided that erythrocyte extract and other cofactors are added (331). Exoerythrocytic forms, which naturally occur in the tissue phase during the infection, can be cultivated *in vitro* in tissue culture within macrophages, endothelial cells or fibroblasts (73) and can multiply in the RES *in vivo* (22). However, the most significant aspect of the interaction between RES and plasmodia is the destruction of the latter by cells of the RES in the naturally resistant or immunized animal (7). This has been shown by splenectomy of infected animals (91, 367) or by histologic follow-up of the evolution of the reaction of organs rich in RES tissue (301, 320). Increased resistance following infection is characterized by RES hyperplasia, accelerated phagocytosis and parasite destruction by cells of the RES. There is evidence for the presence of specific antibody, which enhances phagocytic activity by increasing the stickiness of the parasite (320).

Toxoplasma gondii is an obligate intracellular parasite. It is found in a great variety of tissue cells in lesions in man and animals and can be cultivated in tissue culture of cells from all three embryonic layers (48). The outcome of the interaction between macrophages and toxoplasma *in vitro* is determined in part by natural resistance or acquired immunity of the animal from which the cells are taken. Generally the parasites multiply intracellularly and are released by degeneration of the host cell. The rate of propagation of the parasite depends on the rate of multiplication and on the degree of cellular damage and subsequent release of parasites. Macrophages from resistant animals, *e.g.*, rats, appear to be able to confine within their cytoplasm a larger number of parasites than cells from more susceptible animals such as mice. This may result in a different rate of propagation in the two species due to innate resistance (339). Acquired immunity is largely ascribed to antibodies neutralizing the infectivity of the parasite (278), but in addition, the macrophages themselves have the power to restrain parasitic development to some extent (339). Toxoplasma can persist for a long period of time intracellularly within pseudocysts in many tissues, especially in the central nervous system (98).

Thus phagocytes, especially cells of the RES, play a dual role in protozoan infections. They sustain intracellular propagation and survival, but they also destroy parasites. In the immunized host, this destructive capacity is increased by antibody which reacts with the parasite, and by a reticulo-endothelial hyperplasia. It has been suggested that the cells themselves contain antibody or enzymes enhancing parasitic destruction (339).

IV. PROTECTION AFFORDED BY INTRACELLULAR LOCATION

An increasing number of observations indicate that an intracellular location protects facultative

or obligate intracellular parasites from agents which are bactericidal in the absence of cells.

1. Normal and immune sera. Lysis of typhoid bacilli or of erythrocytes by an immune serum is prevented when the bacteria or the red cells are phagocytized by PMN prior to the addition of the serum (275). Likewise, intracellular *Brucella* spp. are killed at a much slower rate by fresh rabbit or human serum than are extracellular organisms. After 24 hours' exposure to such serum no viable extracellular *Brucella* can be recovered, but 50 per cent of the organisms are still alive when phagocytized by PMN before exposure to the serum (411). It is possible that the killing effect of serum against intracellular organisms occurs only if the PMN begin to degenerate, which can be expected after 24 hours' incubation. For instance, vaccinia and Shope viruses, when fixed by living tissue cells are not neutralized in the presence of antibody, but this protection is not afforded by cells killed either by heat or by UV-light, although the virus is still fixed by them (276). More detailed analysis indicates that the "immunity" of virus against antibody results from the very close union of the virus with the cell after penetration, whereas superficially absorbed virus remains accessible to antibody (136). Although the protection afforded by cells against bactericidal components of normal and immune sera appears to be similar with bacteria and viruses, the mechanism may be quite different. With virus and a toxin, such as lecithinase of *Clostridium perfringens*, neutralizing antibody and some cellular constituent compete for the same active site on the agent, whereas in the case of bacteria, protection is due to the fact that antibody does not easily penetrate into the phagocytes.

2. Chemotherapeutic agents. The consequences of intracellular location for chemotherapy of infections with facultative and obligate intracellular parasites are of practical importance. Chemotherapeutic agents inactive against microorganisms within phagocytes *in vitro* have little or no *in vivo* therapeutic value against facultative or obligate intracellular parasites (192, 195, 295, 296). The various theoretical and practical aspects have been recently reviewed (317). However, chemotherapy with broad spectrum antibiotics is successful against some intracellular parasites such as rickettsiae.

Utilizing the fact that particulate matter such as carbon is taken up by the cells of the tuberculous granuloma, chemotherapy of tuberculosis with an antibiotic isolated from a micrococcus was attempted. This agent, called micrococcin, is almost insoluble in water and is readily ingested by phagocytes after intravenous injection. It has moderate antituberculous activity *in vitro*, and it was hoped that with it mononuclear phagocytes of tuberculous lesions might be selectively "fortified." This attempt was unsuccessful (131). Experiments on intracellular chemotherapy with a surface active agent triton WR 1339 (a condensation product of octylphenol and formaldehyde which is esterfied to 17 to 20 molecules of ethylene oxide) were more successful. This product suppresses acute tuberculosis in mice and produces appreciable regression of established tuberculosis in guinea pigs. To be active the condensation product must consist of more than three phenolic nuclei and must contain between 15 to 20 ethylene oxide units. The introduction of 25 to 30 units results in an inactive compound, and of 45 to 75 units in a compound enhancing significantly the tuberculous infection. Some of the more important findings with this material may be summarized as follows: (a) macrophages from animals treated with triton WR 1339 prevent intracellular multiplication of tubercle bacilli when cultivated *in vitro*; (b) the agent enters the MN *in vivo* as can be demonstrated with the help of victoria blue; and (c) the compounds active as antituberculous agents remove cholesterol from the surface of erythrocytes, whereas those which enhance tuberculous infection remove lecithin from red cells. It is speculated that the active agents remove hydrophobic lipids from the surface of the tubercle bacillus and render it more susceptible to enzymatic attack within the phagocyte (129). Triton WR 1339 is completely inactive as an inhibitor *in vitro*. Its action must be a specific one on the surface of mycobacteria resulting in an alteration of the interaction between the bacilli and the phagocyte. These experiments show that phagocytes can not only protect against antibacterial agents but can also act as the site for chemotherapeutic action, by accumulating the active agent and thus providing an environment detrimental to the survival and multiplication of the pathogen.

V. FACTORS ALTERING PHAGOCYTIC ACTIVITY

The pharmacology of the phagocytic system is poorly understood, and only fragments of information on the effect of chemical and physical agents are available.

1. Histamine. Histamine is one of the naturally occurring amines which has a well known pharmacological effect. It stimulates the RES as measured by the removal of India ink or of radioactive chromium phosphate from the circulation (101, 149). The antagonistic effect of antihistaminics causes depression of the activity of the RES. These antihistaminics also increase the occurrence of bacteremia in local infections by inhibiting the mobilization of phagocytes and other factors of an inflammatory exudate (121). Histamine also stimulates phagocytosis by blood leukocytes *in vitro* (181). Choline increases the rate of removal of radioactive chromium phosphate (133). It has also a direct effect on the transformation of fibroblast-like cells into macrophages in tissue culture (49). These findings suggest that choline increases the regenerative capacity of the RES, thus augmenting its efficiency.

2. Mucin. Mucin has been found to reduce the minimal number of some pathogenic microorganisms required to cause a fatal infection in animals by the intraperitoneal route. Most experiments were done with hog gastric mucin, but homologous mucins and mucin from other sources than the stomach were equally effective (103, 303, 332). The activity of mucin, as discussed in an earlier review (241), depends on decreasing phagocytosis by coating the microorganisms and damaging the leukocytes, and on diminishing the extracellular bacteriolysis (240, 302).

Similarly, intravenous injection of levan increases susceptibility to intraperitoneal injection with some organisms (138). It was found that mice treated with mucin 24–26 hours before injection with meningococci suspended in mucin were more resistant than untreated animals or those injected only 2 hours prior to infection (223). This was explained as local stimulation of defense mechanisms, or as increased capillary permeability allowing adequate dispersal of the pathogens and local mobilization of plasma and cellular elements (103, 284). These findings agree with results reported recently according to which pretreatment of mice with cell-wall preparation of *Escherichia coli* alters the susceptibility of these animals to a subsequent challenge infection with *E. coli*. If the cell-wall injection is given 30 minutes before challenging, the animals are more susceptible than controls, but if it is given 48 hours earlier, then they are more resistant. An explanation was found when the serum level of properdin in the pretreated mice was determined: 2 hours after the injection of cell-wall fractions the properdin level fell to about 20 per cent of its normal value, whereas it reached a titer four times higher than normal 48 hours after injection (277). Evidence has been presented for the importance of the properdin-system for natural resistance to infection (251, 252). Mucin, some levans, and dextrans combine with properdin in serum. This indicates that a number of substances have in common the property to inactivate properdin and to reduce resistance to infection.

3. *Chemotherapy.* The effect of chemotherapy on the interaction between pathogens and phagocytes can be mentioned only briefly. Results with sulfonamides are contradictory and no uniform conclusion could be reached (23). Antibiotics were found in some instances to reduce phagocytic activity, whereas in others no influence was observed (89). Organisms grown in suboptimal bacteriostatic concentration of various antibacterial agents are frequently phagocytized more efficiently than organisms grown in normal media. It may be assumed that under the former conditions the organisms are unable to synthesize surface components which counteract phagocytosis (167). For example, it has been shown that suboptimal concentrations of thiosemicarbazone inhibit the production by tubercle bacilli of components essential for their pathogenicity, although the organisms grow as abundantly as in normal media (31).

4. *Pathological conditions.* Several pathological conditions induced by chemical or physical means are known to alter resistance to infection due in part to impairment of phagocytic function.

a. In traumatic shock, especially shock following massive hemorrhage, it is suggested that a bacterial factor contributes to the irreversibility of the condition. The evidence for the importance of this factor follows:

(i) Dogs in irreversible shock remove incompletely intravenously injected bacteria.

(ii) Homogenates prepared from tissues taken

from dogs in irreversible shock induce the irreversible state when injected into dogs in the reversible state of shock, and penicillin treatment neutralizes this effect (289).

(iii) The phagocytosis enhancing effect of serum taken from dogs during shock is reduced (290).

(iv) The tolerance of shocked rabbits for *E. coli* endotoxins is greatly reduced (288). It has been suggested that some clostridia harbored normally in tissues are responsible.

(v) The properdin level falls progressively during hemorrhagic shock, indicating that the lower resistance of the animal is not due to reduced phagocytic activity alone.

b. Hormones of the adrenal cortex and ACTH lower resistance to acute bacterial infections in man (88) and to experimental infections in animals (128, 154, 186), and also activate normally harmless commensales (172, 294). Because of the profound influence of these hormones on the economy of cells and tissues, it can be expected that the effect on infections is due to direct and indirect mechanisms. As a result of the inhibitory activity on the inflammatory response many manifestations of bacterial infections are depressed or abolished, especially those connected with inflammation induced either directly or through hypersensitivity, although the underlying infection is unchanged (186, 259, 272). Consequently, real improvement can be expected only in situations in which the reactivity of the host tissues, rather than direct bacterial activity, causes the pathological state. In most infections in which parasitic invasiveness is the dominant feature a detrimental effect results from administration of ACTH and adrenal cortical hormones (154). Analysis of the mode of action of these hormones on resistance is complicated by the many variables involved, some of which are species, physiologic state of the host, age and sex, infective agent and type of disease, type of hormone, dosage and mode of treatment. The literature on this subject has recently been admirably reviewed (152). Only some of the findings leading to an interpretation of the mode of action of cortisone and ACTH will be listed.

(i) The inflammatory response is partially suppressed (359) due to alterations in the capillary bed, such as lowering of the arteriolar tone and capillary permeability (225, 368), lack of stickiness of the endothelium usually developing upon injury (84), and reduced rate of production of leukotaxin and LPF by PMN in the ensuing exudate (213). Thus, humoral and cellular defenses are mobilized only inefficiently, especially the MN in the cellular exudate (61, 106, 329).

(ii) The granulomatous response is greatly inhibited either by reduction of proliferation of fibroblasts (212, 323) or of mobilization of macrophages (258, 309).

(iii) Phagocytosis by PMN and MN is reduced (59, 60), whereas the uptake of bacteria by the RES has been found either decreased (237, 353), unaltered (106) or increased (186). It is interesting that the regenerative capacity of the RES after blockade is greatly reduced by cortisone, thus delaying the restoration of normal phagocytic function (20, 134).

(iv) Intracellular digestion of phagocytized material and possibly of bacterial endotoxins is reduced (153, 325). The latter effect results in an alteration of the generalized Shwartzman phenomenon (single i.v. injection of endotoxin causes generalized Shwartzman). The aggravating influence of cortisone on malaria may be due to decreased intracellular digestion of the parasite, to a lack of macrophage reaction or of reticuloendothelial regeneration (286).

(v) Antibody production is reduced considerably, a fact which influences both resistance and hypersensitivity (158).

c. Ionizing radiation is frequently followed by a decrease of resistance to microbial invasion (297) or to bacteremia developing from normal intestinal organisms (224). At least two distinct syndromes can be observed after irradiation: (i) The intestinal syndrome connected with functional failure of the bowel, in which death results from changes in the fluid and electrolyte balance and infection plays little part. (ii) The "bone marrow" syndrome due to bone marrow failure, in which infection is a frequent complication and cause of death (35). Besides infection, pancytopenia, thrombocytopenia and anemia are characteristic symptoms. The mechanisms by which the resistance is lowered are not clear yet, but a number of pertinent observations are as follows: (a) The number of available phagocytes is reduced as a result of the breakdown of the regenerative capacity of the marrow (35). (b) The bactericidal capacity and the properdin level of the serum are lowered (197), as illustrated by the fact that injections of properdin into irradiated animals prevent to some extent post-irradiation infection (272). (c) One to three days after irradiation the clearing capacity of the RES is

found to be incomplete (114, 291), especially regeneration after blockade (102). (d) PMN from the blood taken 3–5 days after irradiation show reduced migration and phagocytosis (298). (e) The phagocytes lose their ability to dispose of ingested material as measured by a decrease of the bactericidal activity of PMN against phagocytized bacteria (93), or increased susceptibility to toxin and somatic antigen (299). (f) Antibody production is impaired (132, 146). Infection after irradiation can be symptomatic or the direct cause of death. Only in the latter case will treatment with antibiotics decrease mortality (201).

In all these conditions, a number of deficiencies develop which can result in increased susceptibility to bacterial invasion or sensitivity to their products. A common underlying mechanism seems possible, such as a decrease of enzymatic function as measured by the reduction of antibody production and of intracellular digestion. The impairment of the phagocytic function is only one single component of a complex effect. Further exploration and identification of the many factors involved in the reduction of resistance to infection under these conditions will not only contribute knowledge about the effects of radiation, hormones and shock, but will also add to an understanding of the mechanisms of natural resistance to infection.

VI. CONCLUSIONS

The present review is far from complete, for the interaction between phagocytes and pathogens has its repercussions in every phase of host-parasite relationship. Many aspects of phagocytic function in relation to infection have by necessity been omitted. New advances toward an understanding of the initiation and maintenance of the inflammatory response (150) and the site of antibody formation (58) could not be discussed here.

In spite of the overwhelming number of observations on the various aspects of interaction between phagocyte and pathogen, our understanding of the mechanisms involved is fragmentary. It has been common practice to study either the host or the pathogen, whereas the actual interaction between the two has just begun to become the topic of many investigations. We therefore have a considerable knowledge on the intermediary metabolism of microorganisms and of cells and tissues of higher animals, but information on the biology of the pathogen in the host, or on the biochemical alterations occurring in the tissues of the host due to infection, is incomplete or missing. A beginning has been made by a number of investigators. Bacillary products isolated from the infected host (304), the metabolism of the pathogen either after growth in a host (293) or when ingested by phagocytes (311), and the influence of metabolic products of the host on the pathogen (78) have recently been or are being studied. It would be desirable to learn more about the mechanisms involved in the maintenance or destruction of function and life as a consequence of the relationship between phagocytes or host and pathogens (246), as the interaction between the two embraces the problem of virulence which "is the resultant of the opposed systems of forces exerted physiologically by both the parasite and its host, in the effort of each to maintain its life and health" (231).

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