6-1967

The Effect of Glucose on Neutrophilic Phagocytosis

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THE EFFECT OF GLUCOSE
ON
NEUTROPHILIC PHAGOCYTOSIS

by

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A Thesis in Partial Fulfillment of the
Requirements for the Degree of Master of Science
in the Field of Periodontics

June 1967

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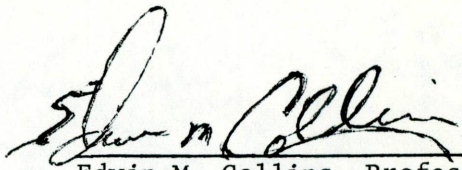

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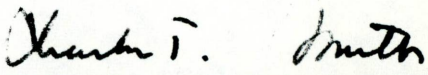

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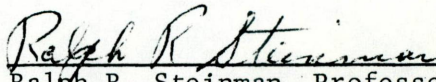

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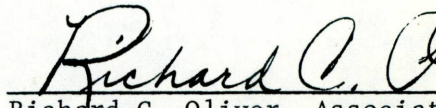

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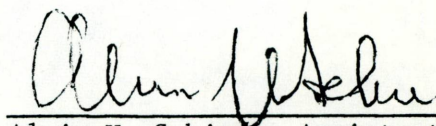

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ACKNOWLEDGMENTS

To Doctor Richard Oliver for his encouragement and patience and for providing the opportunity to make this work possible.

To Doctor Edwin Collins and Doctor Alvin Schiess for their assistance and guidance in the preparation of this thesis.

To Doctor Charles Smith and Doctor Ralph Steinman as members of my thesis committee.

To Doctor Al Egge and Doctor Irvin Kuhn for their early guidance and timely observations.

To Doctor William Thompson and the staff of the Loma Linda University Medical Laboratory for their able assistance in the collection of data.

To my typist, Thela Gray, to whom I owe my sincerest gratitude for her unselfish performance.

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I. INTRODUCTION

This study was designed to determine the effects of varying amounts of glucose on the activity of neutrophilic leukocytes in normal human blood. An *in vitro* system was used to isolate the reaction in an effort to control as many factors as possible. A review of the literature dealing with the role of the neutrophil in the defense mechanism of the human body and the phagocytosis of foreign material is presented along with a study of the effects of glucose on this phenomena.

Phagocytosis (Greek, *phagein*, to eat; *kytos*, cell), is described as the incorporation or introduction of particulate matter into a cell. Actually a specialization of a basic physiologic function in lower animal forms, the process has long been recognized as a major factor in the defense mechanism of higher animals. [13,73]

The violation of tissue integrity by a foreign substance elicits a multifaceted response by the body in an attempt to isolate and destroy the invader. The passive barriers of the outer skin and the internal tissue filters of conncetive tissue fibers and organs, such as the spleen and lymph nodes, are supported by a multitude of cells capable of being mobilized upon the stimulus of invasion. In normal individuals this mechanism includes an inflammatory response in which defense cells migrate through the blood vascular walls to the site of invasion and begin the process of destruction or inactivation of the foreign substances. Usually these cells overwhelm the invaders, repair occurs and tissue function returns to normal. [104]

The study of the phenomena of neutrophilic phagocytosis in certain diseases often provides useful information concerning the normal. Diabetes mellitus has proved useful in the study of the human defense mechanism. Diabetes mellitus, a disease of carbohydrate metabolism, is characterized by a rise in blood sugar levels and other changes which may inhibit the ability of the body defense mechanisms to function normally.[35,51,71,120] In the absence of diabetic acidosis, antibody activity, inflammatory responses, and other defense mechanisms are unaffected.[8] It is therefore assumed that other factors are responsible, such as the hyperglycemic state of the diabetic.

Since the purpose of this study was to determine differences in phagocytic capacities under different glucose concentrations, means and standard deviations of means were computed and 95% confidence intervals established for the differences of the means of each variable.

II. REVIEW OF THE LITERATURE

THE NEUTROPHILIC LEUKOCYTE

Ham states that there are two families of leukocytes, granular and non-granular, classified according to the contents of the cytoplasm.[39] Three granular cell types are described:

1) Neutrophilic
2) Acidophilic (acid staining) or eosinophilic
3) Basophilic (basic staining)

Two non-granular types are seen:

1) Lymphocytes
2) Monocytes

In a normal blood count of 5-10,000 leukocytes, 60 - 70 percent are neutrophils. Normally 1 - 2 percent of these are immature neutrophils (metamyelocytes or stabs). These counts can vary according to the physical activity and condition of the individual.[37,39] Neutrophils (also called polymorphonuclear leukocytes), are first seen in the liver in the hepatic hemopoietic period during the second embryonic month.[103] In the adult, the myeloid tissue is responsible for neutrophilic development and in a healthy individual only mature neutrophils are released into the blood. Neutrophilic maturity is determined principally by nuclear shape, and while the hematologist recognizes differences in white cells he cannot yet present an adequate scheme of the transformations from embryonic to mature states.[19]

Estimates of neutrophilic life spans vary from a few hours to over fourteen days.[16,90,119,128] In vivo this is nearly impossible to determine accurately due to the free flow of cells between tissue and vascular channels.[4,119] The first, the hematopoietic stage, represents

the period from beginning cell differentiation through maturation until it is released into the circulating blood. According to Wright [128] this takes approximately 14 days. The second stage represents the circulatory or intravascular period. The third, or tissue-migratory stage, is when the neutrophil is extravascular in location.

The neutrophils usually migrate through venule rather than capillary walls, passing between the end plates of the dilated endothelial cells. [39,74,117] In severe infections chemical messages are sent to the bone marrow where leukocytes, mostly neutrophils, are released into the blood. The factors which regulate formation, maturation and release of these cells, as well as their mobilization and subsequent destruction, are still little understood. [121]

The adult neutrophil is round with well defined cytoplasm and has a diameter of 12 - 18 microns, approximately twice that of an erythrocyte. The nucleus is deep-staining and usually contains 2 - 5 joined lobes with coarse, densely packed chromatin flakes. No nucleolus can be demonstrated. Few mitochondria, little endoplasmic reticulum and few free ribosomes are seen, but the Golgi apparatus is more prominent. [13,100] The neutrophil has a specific gravity of 1.065 and exhibits migration toward the positive pole. Since the mature cell has no capability of reproduction it is considered a differentiated end cell. [6,13] Glycogen and various enzymes increase as the cell matures. [17,114,115]

The cytoplasm occupies more space than the nucleus and contains very fine granules which are the most numerous of the organelles. These cytoplasmic granules oscillate in a Brownian movement. Some of the more

important granular constituents are: [12]

1) Phagocytin, a bacterial agent acting against gram positive and gram negative microorganisms,
2) Alkaline phophatase, which is increased in the presence of infection,
3) Lysozyme, a polysaccharidease,
4) Diphosphopyridine nucleotide (DPNH) oxidase, which upon release, may account for increased non-cytochrome linked oxygen consumption during particle ingestion.

Lieberkun, [63] in 1870, was evidently the first to observe leukocytic movement. He coined the term "ameboid motion". The speed of ameboid motion is variable but approximates 30.8 to 53.9 microns per minute.[116] Cell expansion during ameboid motion is not completely understood but may occur in one of several ways. Goldacre[38] theorizes that a new cell membrane is formed from soluble protein which is propelled forward by a contraction of the posterior cell membrane. Allen[1] feels that the endoplasmic contraction causes a streaming effect. In cell movement, a pseudopod is extended forward adhering to material in the cell environment. If the material consists of small particles, ingestion occurs. If the particle size is large, the cell will move over it.[5]

Chemotaxis is a directional response of an organism to a substance in its environment. Movement toward a given substance constitutes a positive chemotaxis; whereas, movement away from a substance is an example of negative chemotaxis.

McCutcheon[68] observed that leukocytes from inflammatory exudates immediately responded positively in the presence of bacteria. Sieracki[103] contends chemotaxis can be exhibited by attraction or repulsion up to

a distance of 1 millimeter.

Moon et al[77], in 1955, tested chemotaxis using neutrophils and various combinations of fragmented rat, fowl and rabbit tissue. The tissue fragments were placed in a capillary tube and the tube was placed in the leukocyte suspension. They observed positive chemotaxis in all the tissue combinations and concluded that chemotactic substances contained in normal tissues are released upon tissue injury and initiate an acute inflammatory response. However, Harris[43] showed that dead granulocytes, enzymatic products and altered tissues were not chemotactic to neutrophils. He questioned the validity of capillary tube methods in chemotaxis studies. He reasoned that convection currents between fluids inside and surrounding the tube may cause mechanical accumulation of leukocytes. Further, he suggested that any residual material used to produce the exudates and collect leukocytes may be very toxic to the leukocytes, thus destroying them. This finding seriously challenged the chemical basis of chemotaxis.

McCutcheon raised doubts about the chemotactic substances being given off by bacteria.[68] He found that repeated washings of microbes does not diminish the chemotactic response, nor the size of the chemotactic field. Chemotaxis is therefore most likely not chemical in nature. He suggests the existence of a concentration gradient present in the plasma.

The surface tension and electrical surface charge present on the granulocytes are believed to affect migration by the cell into areas of inflammation. These forces can also affect ameboid motion, phagocytosis and aggregation tendencies in adult cells.[103]

In 1905, during discussions summarizing his previous experiments

on the immune mechanism, Elie Metchnikoff described the evolution of phagocytic cells through various animal species to the highly specialized forms where they became a part of the resistance mechanism against infectious agents.[73,74] He observed that in simple multicellular animals, the phagocytes were present in the tissue between the skin and the intestine. In animals where a circulatory system has evolved, the phagocytes are found in the blood which thus enables them to become highly mobile. Metchnikoff was convinced that although some animals possessed entodermal digestive systems the mesodermal tissues retained a primitive digestive capacity. Recent studies on inflammation have reaffirmed the concepts of Metchnikoff. Current research techniques include human skin windows,[84,87] connective tissue spreads treated as blood smears,[22,108] and studies of individual leukocytes.[88]

Lewis,[60] in 1927, investigated a host of irritants and their effect on the mechanism of inflammation. Physical and chemical irritants such as histamine, burning, freezing, acids, bacteria, nettle stings and insect bites were used. Regardless of the type of irritant, a uniform reaction occurred. It consisted of local dilation and hyperemia of the minute vessels, and a reflex dilation of adjacent arterioles which caused a spreading flush and a pale circumscribed wheal. The latter reaction was caused by increased permeability of the capillary walls. Lewis found that the fluid of the wheal resembled blood plasma in protein content. He concluded that the reactions were not from the nature of the irritant but due to substances released by the injured cell in response to the stimulus.

Moon[76] stated that the cells of normal tissues contain histamine

in a non-diffusible form. Upon cell injury the histamine is released in a diffusible form causing dilation of adjacent capillaries and rendering them unresponsive to impulses or substances which may cause contraction. It is now felt that connective tissue mast cells contain cytoplasmic granules that are capable of releasing histamine and other substances.

Menkin[72] described a substance which he recovered from the pseudoglobulin of inflammatory exudate as being capable of eliciting leukocytic activity. He called this substance "leukocytosis-promoting factor" (LPF) speculating that LPF may be an inducer of leukocytosis following tissue injury.

Rebuck and Mellinger[85] in 1953, used a human skin window technique to observe leukocytic sequences in acute inflammation. The epithelium was scraped off the forearm by a sterile scalpel to the papillary layer of the corium. Glass cover slips were placed on the wound for 30 - 60 minutes, then removed and stained. The cells that had migrated to the underside of the slide were then studied. In the first 8 - 10 hours neutrophils increased in numbers. Small numbers of tissue histiocytes, monocytes and lymphocytes were also observed. Loss of size was observed in the neutrophils resulting from the continual disintegration of granule-containing cytoplasm and loss of these elements into extracellular tissues. Gradual pycnosis and shrinkage of nuclear lobes was also seen. Within ten hours, lymphocytic participation in the inflammation became evident and after 14 hours, the lymphocytes were the predominant cell type. Hour by hour, as the neutrophils shrank, the lymphocytes, which were engulfing the neutrophilic particles, increased in size.

PHAGOCYTOSIS

The unicellular animal is able to take on nourishment in the form of fluids or dissolved substances. Diffusion of fluids into the cell through the cell membrane is called absorption.[73] Lewis[61] in 1931, described a different process of taking on fluids whereby through ameboid motion an invagination is initiated in the outer cell membrane. The invagination closes around the nutrient and the fluid-filled vacuole is transported into the cytoplasm. This process is called pinocytosis or cell drinking (pinein: to drink). Particulate matter transported into the cell in a similar manner is said to be phagocytized, whereas pinocytosis refers to the ingestion of fluids only. Once within the cytoplasm the engulfed material, fluid or solid, is assimilated, destroyed or excreted into the extracellular environment.[13] The exact process of membrane transference is still unclear.

The two most important phagocytic cells in humans are:[4]

1) Polymorphonuclear leukocytes
2) Mononuclear elements of the reticulo-endothelial system (RES)

Phagocytic capabilities have also been described in other blood cells.[3,25,65,72,78,105,106,107,129]

The ability of a cell to phagocytize seems to be a function of the ratio of phagocytizable particles to the number of leukocytes. Fenn,[30,31,32] in 1921, mathematically formulated the interactions between cell and particle. In addition to finding that the amount of phagocytosis is proportional to the chances of collision between particle and cell, he also discovered that the rate of phagocytosis

was proportional to the number of particles remaining uningested. He determined that the probability of collision is related to the cell and particle size and their relative velocities. Jung[52] described a linear relationship between the concentration of leukocytes and the percentage of cells phagocytizing. As the number of leukocytes per unit volume increases the percentage of those phagocytizing decreases. The Fenn system included serum and since little phagocytosis will occur without serum[7,30,31,32] there are probably other factors involved in the interaction between leukocyte and particle. The nature of the particle being ingested has a highly variable effect on the rate of phagocytosis. For example, different strains of bacteria have different capacities for resisting phagocytosis.[14,53,95]

Rogers[92] injected large numbers of bacteria intravenously into animals and found certain characteristics of clearance from the blood were relatively constant. In the first phase, lasting up to five hours, a decrease of culturable bacteria of 90 - 99 percent was observed. This process is independent of the nature of the parasite, the animal used and the eventual outcome of the infectious process. The remaining bacteria are then slowly but continuously removed from the blood stream. During the rapid phase, the majority of clearance is accomplished by the reticulo-endothelial system. When staphylococci and pneumococci are injected, an immediate and profound leukopenia is seen, suggesting some alteration in the circulatory status of the leukocytes. This could be a transient stickiness in capillary areas allowing a phenomenon known as surface phagocytosis to occur. This is described by Wood, et al,[124,125] as an adherence of the leukocytes to the capillary endothelial wall with subsequent trapping of the

bacteria. Rogers[92] found the first phase of microbe removal nearly inviolate, unaffected by starvation, massive irradiation, shock, experimental diabetes, splenectomy, renal failure, overwhelming infection, corticotropins, adrenal steroids, endotoxin, heparin or platelet removal. He concluded that the outcome of a bacteremia seemed dependent upon the ability of the reticulo-endothelial and leukocytic systems to retain and destroy the bacteria. The disappearance of microorganisms from the circulating blood is not synonymous with destruction. Some bacteria may be trapped but resist engulfment, or engulfed and resist digestion. Some investigators feel that phagocytosis may actually protect the microbe from antibodies and antimicrobial drugs.[91,102,123]

Melly[70] found leukocytes containing viable bacteria were less vigorous than usual and in some cases bacterial toxins, such as streptolysin, caused actual degranulation leading to liquefaction of the cytoplasm, clumping of nuclear lobes and neutrophilic death.[48]

The subsequent course of infection usually depends upon whether or not the microorganism is easily ingested and, if so, its intracellular fate. The spread of an organism may be prevented by ingestion and inactivation regardless of whether the phagocyte destroys the invader.[123]

Brewer[5] studied human and guinea pig leukocytes by osmic fixation and electron microscopy and described three types of relationships between the phagocyte and bacteria:

1) A close association with the cell membrane curving around the cocci but not surrounding it,
2) Cocci inside the cell with the cell membrane very close

and surrounding them,

3) Cocci in a membrane-lined vacuole lying free in the cytoplasm. Rogers and Melly[94] also noted the presence of prominent vacuoles around ingested cocci.

Fenn[33] said the membrane was "unstable" and able to spread over the particle being ingested, while Palade[80] suggests that the cell membrane is in a dynamic and flowing state and substances bound to it are carried into the crevasses of the membrane. A segment of the membrane is then pinched off and passes into the cytoplasm.

Hirsch and Cohn[47] noticed that phagocytosis resulted in degranulation of leukocytes soon after ingestion with a subsequent release of the granular enzymes into the cytoplasm or the vacuole itself. Lockwood and Allison[66] theorize that the vacuole and granular membranes fuse and although the granules, per se, do not contact the bacteria[13] the granular enzymes are discharged into the phagocytic vacuole. Following particle ingestion granular lysis occurs as a result of a drop in intracellular pH to 6 or below due to an increase in lactic and organic acids produced during glycolysis.[12,13,45,54] Cohn, et al,[13] theorize that microbial leukocidin may also be responsible for the granular lysis. Cohn and Morse[15] found cells already engaged in phagocytosis are better phagocytes, possibly because of granular lysis already occurring. McKendrick[69] and Mudd[78] reported contrary findings in earlier work. They found no decrease in the ease of phagocytosis even after several particles were engulfed.

Cohn and Hirsch[12,13] and others[47] have noticed an explosive loss of granules in the immediate proximity of the bacteria 0.1 seconds after ingestion. They found a definite relationship between

the decrease in granules and the amount of phagocytosis and suggested that phagocytosis results in an intracellular redistribution of the enzymatic components either into the vacuoles or into the cytoplasm.

Little investigation related to leukocytic bacteriocides, with the exception of lysozyme, was done until 1956 when Hirsch[46] suggested two main mechanisms for bacterial destruction. First, by granular substances such as phagocytin or lysozymes and, secondly, by an increase in the acidic environment of the cytoplasm which immobilizes the bacteria. He discovered that an increase in the acid concentration enhanced phagocytin activity. Rous[98] had described this intracellular "inflammation" in 1925. By injecting litmus granules, that were subsequently ingested, the acidity surrounding the engulfed particle proved near pH 4.5. The tissues in general cannot develop a high degree of acidity without resulting in the death of the host. Leukocytes, being expendable, may develop this high acidity within the cytoplasm thus ridding the host of bacteria susceptable to phagocytosis.

Aside from the possibility of intracellular survival, the fate of a phagocytized organism depends upon its composition rather than the type or source of the phagocyte.[9] Degradation, digestion and oxidation of viable bacteria is more difficult than with heat-killed organisms.[55] Bacterial degradation includes depolymerization of lipids, nucleic acids, proteins and carbohydrates. Bacterial RNA is degraded faster than DNA. Some of the resultant components are utilized as leukocytic lipids but the majority are excreted into the medium.[9]

Mudd, et al,[78] and Berry and Spies[3] discuss many investigations

that deal with the effects of temperature on animal phagocytosis. Chickens, pigeons, guinea pigs and rabbits were studied with the general observation that phagocytosis increases with a rise in temperature up to about 40°C. Ellingson and Clark[26] reported that the optimum temperature zone for phagocytosis in humans is 38-40°C.

Changes in osmotic pressure can affect phagocytosis.[7,109] Hamburger[40] diluted horse serum with water and noted a slight depression of neutrophilic phagocytosis in hypotonic solutions. These changes were reversible. Hypertonic solutions were found to exert the same influence. Wright and Reid[127] disagree with Hamburger when observing phagocytosis in hypertonic solutions. They found that in the presence of serum, human leukocytes had a tendancy to phagocytize better, while in hypotonic solutions ingestion was suppressed. Neutrophils are best maintained in isotonic or slightly hypertonic solutions.[110]

The hormones of the adrenal cortex have been shown to have an inhibitory effect upon the phagocytic capacities of neutrophils.[18,22,85,86] Cortisone acts by inhibiting margination, diapedesis and migration of the leukocytes responding to irritational stimuli, and may also affect capillary permeability.[85]

Most early investigators found a decrease in phagocytosis with a change in hydrogen ion concentration. Hektoen and Ruediger[44] found that slight variations from neutrality suppressed cell activity. These observations were confirmed by Evans[28] and Fenn.[34] The pH of the solution seems to be most detrimental when it varies beyond a range of 6.3 to 7.3.[78]

Several authors[3,62,101,102,123] suggest that phagocytosis is

complicated by the presence of a bacterial capsule as seen in some virulent strains since encapsulation presents such an unusual surface to the leukocyte. Lactic acid[89] and oxylate[78] reduce phagocytosis while histamine aids the process.[105] Neutrophils from anemic blood show greater engulfment power because fewer erythrocytes exist as obstacles for neutrophil/particle collision.[3,49]

Fenn's studies[33] on the surface relationships between particle and cell showing a decrease in free surface energy when a particle is ingested indicated the importance of preparing the material to be ingested. Phagocytosis is enhanced by factors which decrease the surface energy of the cell or cell medium and increase surface energy on the particle. The most effective of the known phagocytosis-promoting factors are found in normal and immune serum.[82] Until the middle 30's these substances were known as opsonins, or materials present in normal sera, and bacteriotropins, which are serum antibodies present as a result of immunization.[19,78] The name opsonin (Greek, to prepare food), has since been suggested to denote <u>any</u> heat-labile antibody which prepares bacteria for phagocytosis. Opsonic action is due to a deposition of serum proteins upon the surface of a particle rendering it more easily phagocytized.[65,78,122]

Ward and Enders[118] and others[20,93] have demonstrated an antibody in immune sera of humans that induces phagocytosis of pneumococci. This organism, among others, can produce protein toxins that inhibit leukocytic chemotaxis. Cohn[10] noted that opsonins which normally enhance phagocytosis of bacteria actually delay intracellular degradation, for the opsonins by combining with the bacterial surface temporarily protect it from the digestive enzymes of the phagocyte.

Tullis and Surgenor[111] emphasize the importance of extracellular protein factors such as opsonins and complement in phagocytosis. Complement is a portion of blood serum or plasma that is considered to be a chemical system of several components aiding in the destruction of certain microorganisms. It is felt that the thermolabile complement group may act in conjunction with the antibody defenses to lyse certain cells or bacteria.[81]

Hanks[41] found that high numbers of particles increased the demands on the sensitizing opsonins in the serum resulting in poorer preparation of the particle surface. The effectiveness of the phagocytic system was consequently inhibited.

EFFECTS OF GLUCOSE

The literature is replete with studies of glucose effects on phagocytosis in vivo and in vitro on diabetic humans and alloxan-diabetic animals. Alloxan destroys insulin-producing beta cells in the pancreas and simulates most of the symptoms of diabetes except ketoacidosis and some of the wound healing impairment seen in human diabetics.[96] Tunnicliff[112] injected 0.2cc of a 50 percent dextrose solution intravenously in rabbits and found an increase in neutrophilic phagocytosis.

Bybee and Rogers[8] compared the phagocytic activity of neutrophils obtained from 31 diabetics under normal medical control and from 7 diabetics in ketoacidosis with that from normal patients. Their studies showed that leukocytes from non-acidotic or medically controlled diabetics phagocytized in a normal manner, while leukocytes obtained from ketoacidotic patients revealed impaired phagocytosis even in

normal serum. This was a reversible phenomenon for when the keto-acidosis was corrected normal leukocytic function returned. Normal leukocytes placed in 10 percent ketoacidotic serum displayed normal activity suggesting the possibility of a change in the acidotic leukocyte. Richardson[89] also found that phagocytosis did not vary significantly in normal and controlled diabetics but that acidotic conditions markedly suppressed phagocytic power.

Kijak, Foust, and Steinman[57] studied the phagocytic response of leukocytes obtained from known diabetics and from artifically produced diabetics ("pseudo-diabetics") who had been given varying amounts of orally administered glucose. The results of this study suggest that an inverse relationship exists between the level of blood glucose and percentage of phagocytosis by neutrophils.

Bybee[7] studied phagocytic variations of neutrophils in 10 percent and 50 percent serum concentrations. He used glucose concentrations ranging from 100-1000 milligrams percent and was unable to demonstrate any significant effect of the glucose on his phagocytic system. Drachman[23] observed a sharp drop in phagocytosis in hyperglycemic rats innoculated with pneumococci. Phagocytosis was depressed not only in diabetic states but in hyperglycemic conditions in normal serum. The various levels of sugar were not given. Interestingly, exudate leukocytes preincubated in normal plasma were not affected by hypertonic glucose solutions. Drachman concluded that impaired phagocytosis appeared to be due to the osmotic effects of the hyperglycemia in the extravascular tissues.

Cohn and Morse[15] found that, in the absence of serum, the presence or absence of glucose made little difference in the bacteriocidal effect

of the system. Leukocyte enzyme inhibitors blocked oxygen consumption, glucose utilization and phagocytosis. The conclusion was that in fresh serum adequate supplies of glucose are required for effective phagocytosis.

CELLULAR CARBOHYDRATE METABOLISM

Early observers in enzymology and analytical chemistry found that blood on standing showed a decrease in sugar content.[2] This was attributed to the decomposition of blood protein, amino acids and sugar. The reliance of the leukocyte on oxygen for metabolism was demonstrated in 1911 by Grafe.[113] Levene and Meyer,[59] noting that lactic acid production increased when sugar concentration decreased, determined that sugar was the source of oxygen for the leukocyte.

Glucose utilization by a resting cell occurs in the mitochondria and follows the tricarboxylic acid (TCA) cycle. Glycolysis is the process of glucose or glycogen breakdown which occurs primarily in the cytoplasmic matrix. It results in the formation of pyruvate which enters either the aerobic TCA cycle or is shunted to the anaerobic cycle with the formation of lactic acid. In the TCA reaction a series of hydrogen ion and electron transfers terminate in the formation of water and CO_2. Energy created by this oxidative phosphorylation results in ATP formation, the energy source for the cell.[24,64] Immature leukocytes, both myeloid and lymphocytic in origin, have a purely oxidative metabolism without lactate formation. Mature granulocytes, on the other hand, have shown strong lactic acid formation[56] and since no cell younger than a metamyelocyte is capable of phagocytosis[50] the ability to shunt to anaerobic glycolytic

metabolism may be related to phagocytic capabilities.

The contact between cell and particle triggers a mechanism producing rapid metabolic excitement.[130] The source of the immediate energy demanded during phagocytosis is glycolysis or a breakdown of glucose.[15,55,99] Leukocytes phagocytizing heat-killed bacteria increased their oxygen consumption, glucose utilization and lactic acid synthesis. Efficiency of the phagocytic system and the continued ingestion of bacteria relies on the availability of glucose. Since glycolysis is essential for phagocytosis and the initiation of ingestion also stimulates glycolysis, it was assumed that this is the process that supplies the necessary energy.[79]

In short term experiments, aerobic or anaerobic phagocytosis causes a transient increase in lactate production which last from thirty minutes to one hour.[15,29] Sbarra and Karnovsky[99] likened this phenomenon to oxygen debt in muscle. Under great demand for immediate energy, glycolysis occurs and lactate builds up. As phagocytosis is completed, energy demands lessen and oxidative phosphorylation catches up through operation of the TCA cycle. They noticed that neutrophilic phagocytosis in guinea pigs under aerobic conditions was accompanied by a doubling of oxygen uptake and a seven-fold increase in the conversion of glucose carbon-1 to CO_2. Rossi and Zatti[97] noted that the oxygen uptake or respiration of the neutrophil increased within 2 - 3 minutes after addition of particles and dropped to normal again after ingestion. The oxygen uptake is independent of lactic acid production, does not produce energy for particle ingestion, and thus is apparently non-essential for phagocytosis.

DPNH-oxidase stimulates the conversion of glucose carbon-1

to CO_2 triggering the large oxygen uptake. The DPNH-oxidase coupled with a TPNH-linked lactic dehydrogenase, stimulates the formation of lactate from pyruvate as well as the direct oxidative pathway for glucose-6-PO_4.[29,55] Both of these enzymes have pH optimims of 5 which is the point of lysis of the granules noted by Cohn and Hirsch.[11]

Other materials in the extracellular environment, when ingested along with the bacteria, may block glycolysis.[55,83] However, blockage of respiration or glycolysis by experimental inhibitors had no influence on intracellular degradation indicating a lack of dependence upon the major pathways of energy metabolism.[10,13]

SUMMARY OF THE LITERATURE

According to the literature, the neutrophilic leukocyte is an important element in the defense mechanism of higher animals. There are many factors which can affect the normal function of these cells, among them is glucose. Glucose is an essential element in the normal metabolism of the neutrophil but in excessive amounts, as seen in diabetes mellitus, it will cause suppression of such critical functions as phagocytosis. The exact mechanism of this suppression is not clearly understood.

The literature presents a large quantity of conflicting data. Several factors contribute to the difficulties of comparing experimental results of various workers:

1) Variation of cells studied: Such variabilities as species origin; anatomical sources, i.e., peripheral blood leukocytes vs exudative leukocytes; cell types; stage of maturity and mode of preparation.

2) Variation in analytical methods and types of incubation media used.

3) Variation in methods of reporting metabolic data such as dry weight, cell numbers, and cell nitrogen.[2]

III. MATERIALS AND METHODS

MATERIALS

Staphylococcus epidermidis was employed as the microorganism to be phagocytized. It is a relatively harmless, coagulase-negative, gram-positive, slightly oval organism, 0.8 - 1.0 micron in size. It is thermolabile, non-motile, non-flagellated, a non-spore former and grows best at 37°C. Staphylococcus epidermidis is considered a non-toxin producer, which renders it ideal where minimum interference with phagocytosis is desired. Bacterial colonies were obtained from the Department of Microbiology at Loma Linda University School of Medicine and grown on tryticase soy agar plates*.

Pure glucose in the form of blood sugar[36,42] was used for this study. Constant osmotic pressure was maintained for the glucose solutions by the addition of mannitol, a hexatomic, non-fermentable plant sugar ($C_6H_{14}O_6$) with a molecular weight nearly equal to that of glucose.[21]

The problem of varying osmotic pressure was controlled by the addition of compensating concentrations of mannitol to the glucose. The first few subjects supplied blood which was handled in the identical manner as the test procedure with the exception that 250 milligrams percent mannitol was added to isotonic blood instead of glucose. No significant variation was noted in phagocytic indicies between the mannitol alone and the mannitol with 50 milligram percent glucose as

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* Formula for the agar plates:

Bacto Tryptone (pancreatic digest of casein USP)	15 g.
Bacto Soytone (soy bean peptone)	5 g.
Sodium Chloride	5 g.
Bacto Agar	15 g.
Distilled Water	qs 1 liter

in tube No. 2. Since the hypertonicity was constant for all tubes and the mannitol takes no active part in phagocytosis suppression, it seems logical to conclude that the observed suppression in the remainder of the experiment was due to the glucose.

The sugar concentrations were mixed in advance, using Gey's balanced salt solution as a medium*. Prior to the drawing of blood samples a set of six test tubes was prepared for each donor, one of the six containing no sugar, the remaining five receiving by pipette 0.3 milligram of the graduated sugar concentrations as shown in the following listing:

Test Tube No.

1 -- no sugar								
2 -- 167 mg glucose	per	100	ml	Gey's	solution	(	50	mg% glucose)
663 mg mannitol	"	"	"	"	"			
3 -- 330 mg glucose	"	"	"	"	"		100	mg% glucose)
500 mg mannitol	"	"	"	"	"			
4 -- 500 mg glucose	"	"	"	"	"		150	mg% glucose)
330 mg mannitol	"	"	"	"	"			
5 -- 667 mg glucose	"	"	"	"	"		200	mg% glucose)
163 mg mannitol	"	"	"	"	"			
6 -- 830 mg glucose	"	"	"	"	"		250	mg% glucose)

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* Formula for 1 liter of normal Gey's balanced salt solution

NaCl	8.0 gm	Na_2HPO_4	0.112 gm
KCl	0.375 gm	KH_2PO_4	0.025 gm
$CaCl_2 \cdot 2H_2O$	0.365 gm	Glucose	2.0 gm
$MgCl_2 \cdot 6H_2O$	0.21 gm	$NaHCO_3$	0.25 gm

(Glucose was omitted for this study.)

(All glucose solutions and bacterial transplants were prepared by the investigator.

METHODS

Fresh bacterial colonies on agar plates were provided for the study. Using a sterile wire loop, a final transplantation of bacteria was made eighteen hours before the data were to be taken. The colonies were incubated at 37°C at the end of which time the bacteria were transfered with a sterile cotton swab to a tube of Gey's solution, and the bacterial count was determined by visual turbidity to a #6 density on the McFarland Nephelometer scale.[75] This method yielded a bacterial count of approximately 1.8 millions per milliliter.

The test group was comprised of thirty young adult male junior dental students from Loma Linda University. After the members of the test group had fasted for eight to twelve hours, 5 milliliters of blood was collected from each person, using venapuncture and a heparinized vacutainer tube. This sample was used for phagocytic studies and leukocyte counts. A second 5 milliliters was collected and set aside for blood sugar level determinations. All blood samples were taken within a three hour period of time. The first 5 milliliters of heparinized blood was centrifuged for ten minutes at 850 rpm using a model UV International Centrifuge, head number 279, in order to separate the serum from the solid elements. One milliliter of the separated serum was pipetted and discarded. It was replaced by one milliliter of the bacterial suspension in Gey's Solution. The blood/bacteria solution was gently mixed by hand rotation after which 0.7 milliliter was pipetted into each of the five sterile test tubes containing 0.3 milliliter of the varying sugar solutions and the one sterile test tube containing no sugar. Each of these six tubes was gently mixed by hand rotation and then placed in a Clay-Adams dry heat

incubator at 37°C and rotated for 30 minutes on an Eberbach Rotator.

At the end of the 30 minutes microscopic slides were made from samples of blood in each tube and then stained with Wright's stain. On each slide a random sample of one hundred neutrophils was counted and the number of those containing bacteria was noted. At the same time the total number of ingested bacteria was noted and the total was divided by one hundred. Each method resulted in a phagocytic index. All slides were read by the investigator to avoid variances in interpretation. If the intercellular identification of a coccus was in doubt it was not included in the study.

Recommendations of Martin and Green,[67] regarding physical and chemical handling of leukocytes, were heeded throughout the experiment such as a clean rapid venapuncture which eliminates tissue fluids and which would affect glycolysis and cell respiration. Bubbles and violent shaking were avoided in drawing blood, pipetting and centrifuging. High speed centrifugation was not used as it will completely disrupt normal function.

The fasting blood sugar levels were recorded by the Technicon Auto Analyzer method (normal fasting blood sugar range = 70 - 100 milligrams percent)[27] and the leukocyte counts were determined by a Coulter Counter. (Table I)

Data from the thirty subjects were grouped together by tube numbers and means were established for both the percentage of phagocytizing neutrophils and the number of bacteria per phagocyte. (Table II)

Since the purpose of this study was to determine differences in phagocytic capacities under different glucose concentrations, t-tests for the difference of the various means were run. Means and standard

deviations of the means were computed and 95 percent confidence intervals established for the differences of the means of each variable.

IV. RESULTS

When the means were established and plotted for each of the tubes, a constant decrease in phagocytosis occurred as glucose levels rose. Table IV illustrates a drop in the phagocytic index from tube #1 to #2 as the 50 milligrams percent glucose was added. This changed the original isotonic blood/bacteria solution to a hypertonic state. A nearly identical drop in phagocytosis was seen when pure mannitol was added during pilot studies, suggesting the hypertonicity, not the glucose was responsible at this point. The drop in the 'percent phagocytes' index from tube #1 to #2 was approximately 17 percent, 89.5 to 74.5. The 'bacteria per phagocyte' index dropped over 50 percent, from 10.7 to 5.1. From tube #2 to #6 the changes were slight but the phagocytic index for each tube was lower than the preceding index.

The only statistically significant change in the 50 milligrams percent concentration range was the change that occurred between 100 - 150 milligrams percent. (Table III) When 100 milligrams percent concentrations were compared, statistically significant suppression was observed in all tubes except those above 200 milligrams percent concentration. A drop of 10 percent in the 'percent phagocytes' index was seen from 50 milligrams percent to 250 milligrams percent glucose while a drop of 23 percent was observed in the 'bacteria per phagocyte' index. (Table IV)

At the same glucose concentrations neutrophils often displayed a variation in activity. One would be engorged with bacteria while another would contain few even though the bacterial concentration around

each appeared the same. Occasional neutrophils were capable of extensive phagocytosis regardless of the glucose concentrations. In higher glucose concentrations many actively phagocytizing and non-phagocytizing neutrophils were observed with disrupted cell membranes, as well as some with more condensed cytoplasm. Although not quantitatively evaluated, a general impression of increased erythrocyte crenation seemed related to these higher concentrations as well. (Figure I)

Engulfed bacteria were nearly always found in pairs although Staphylococcus epidermidis is not considered a diplococcus. (Figure I)

All cross correlations between fasting blood sugar levels, leukocyte counts and phagocytic indices were negative. Both the 'percent phagocytes' and the 'bacteria per phagocyte' methods of phagocytic index determinations were statistically equal.

The final leukocyte counts ranged from 4,200 to 12,400 cells per cubic millimeter. Fasting blood sugar levels ranged from 56 to 114 milligrams per 100 milliliters.

V. DISCUSSION

In a healthy individual, a microbial invasion triggers a mechanism in the body which supplies phagocytic cells to isolate and destroy the invader. In an infection the balance between the invading organism and the defense cells may be altered. Disturbed carbohydrate metabolism, which is characteristic of diabetes mellitus, has been shown to adversely affect the manner in which leukocytes respond to bacterial insult. This malfunction may result from a deficiency in the blood cell itself, from the ketoacidotic state of the diabetic or from the presence of excess glucose. The hyperglycemic state generally suppresses the phagocytic capabilities of leukocytes but whether this is due to an overwhelmed glucose metabolism in the cell or is contributory to another facet of the diseased condition is not known. Although glucose is necessary for cell respiration, it is possible that excessive amounts cause back-up in one or more of the metabolic shunts which overloads the remaining pathways. With ample oxygen available, respiration proceeds along the tricarboxylic acid cycle. In anaerobic conditions the lactic acid shunt is utilized until more oxygen is available at which time the condition is reversed. In studying closed systems neither oxygen or glycogen is replaced. This causes a "debt" in at least these elements that could adversely affect normal cell metabolism.

Few in vitro studies are found concerning the effects of glucose on the phagocytic system for a single individual. The study reported by Kijak, et al,[57] showed phagocytic variations with oral glucose ingestion in different individuals but only one level of glucose was

used for each of nine persons. With such a small test group, such factors as variations in gastrointestinal uptake of glucose, insulin or insulin-inhibitor activity, and other physical disorders cannot be ignored. Ideally, the fluctuation of only one variable should be studied in a given individual.

There are also risks in comparing data from animal and human studies, particularly where alloxan has been used. As mentioned previously, alloxan does not completely reproduce the human diabetic state, especially ketoacidosis.

A pilot study was undertaken by this investigator to observe phagocytosis in normal and diabetic individuals. Samples of blood to which bacteria were added were taken from 44 randomly selected hospital patients during each of the regular test periods of a three hour glucose tolerance test. The mixture was incubated for 30 minutes. Slides were prepared, stained and phagocytic indices determined on the first few cases. A wide index variation led to the conclusion that our "normals" were probably sick too, but not necessarily with diabetes, and that there were many metabolic disorders beyond our control that could affect phagocytosis. Therefore, this method was abandoned. Leukocytes were next isolated from the serum of normal healthy subjects and suspended in Hank's balanced salt solution. When no neutrophils were seen on the microscopic slides, it was discovered that the acidity of the solution which was below pH 6 had caused lysis of the neutrophils. It was determined that the distilled water used to mix the Hank's solution was slightly acidic. Gey's solution was eventually employed and buffered against this problem of lysis. In addition, the leukocytes were too difficult to maintain in balanced salt solutions without

clumping. From these initial attempts it was decided that the total isolation of leukocytes was not practical. The best method seemed to be to leave the cells in their normal environment. This was difficult, however, because adding the glucose would alter the isotonicity of the blood. The addition of mannitol solved this problem.

The comparatively large difference in phagocytosis observed between the means of tube #1 and #2 is due to differences in isotonic and hypertonic solutions and not merely the addition of glucose to tube #2. (Table IV) Not only are the numbers of bacteria per cell less but also the total percentage of phagocytes. This supports the findings of Drachman[23] and Kijak.[57] Upon comparing the percentages of phagocytic index suppression between tube #1 and #2 it is interesting to note that the percentage of phagocytes was not affected nearly so much as was the number of bacteria a neutrophil could engulf. While the effect of hypertonicity on the system caused a 17 percent drop in the number of cells phagocytizing a 50 percent drop was seen in the mean number of bacteria per phagocyte. The over-all drop in phagocytosis from 50 milligrams percent glucose concentration to 250 milligrams percent differed as well. A 10 percent change was seen for cells phagocytizing and 23 percent for bacteria per phagocytic cell. Therefore, we can see that the neutrophil is inhibited in its phagocytic ability by the presence of glucose as well as a hypertonic environment.

Data for single individuals, when analyzed separately, exhibited variations from tube to tube which made it impossible to predict the effect of glucose on phagocytosis in a single case. Some individual indicies dropped upon the addition of 50 milligrams percent glucose but rose as much as 15 percent when 100 milligrams percent glucose was

added, then dropped again. Usually the rise and fall of indices followed no specific pattern. Thus, it would be impossible to predict that a certain amount of glucose would cause a predictable degree of suppression or rise in phagocytosis. However, when the means were established for the total group for each glucose concentration, the over-all effect was phagocytic suppression inversely proportional to the glucose levels. (Table IV)

The group of young dental students was selected because they were considered normal healthy individuals. There were two variations from "normal" ranges of glucose and leukocyte counts. One subject had a fasting blood sugar level of 114, slightly above normal. His data were included with the rest when no relationship was found upon comparison with data from the other individuals or with the means of each glucose level. The same was true of another subject who had a leukocyte count of 12,400. One could assume that these variations from normal would ordinarily be balanced by the remainder of the "normals", but in these cases the individual indices were not significantly different than the means of the group. This points out the inherent dangers of specifically predicting phagocytic suppression in hyperglycemia. The significance of this finding is that glucose may have an unpredictably variable effect, even in clinically normal persons, and is probably not the only factor causing the leukocyte to malfunction in a hyperglycemic state.

The frequent observation of engorged neutrophils adjacent to relatively inactive cells refutes the statement by McKendrick that an actively phagocytizing cell is no better than a non-phagocyte. This study tends to support the observations by Cohn and Morse[15] that the

initiation of ingestion by a neutrophil triggers metabolic changes that favor further phagocytic activity. The question of this trigger mechanism in phagocytosis remains unanswered.

Quantitation of phagocytosis is accomplished by one of several methods. Leishman[58] and later Wright and Douglas[126] counted the average number of bacteria per leukocyte in a given time period. Hamburger[40] suggested determining the number of leukocytes phagocytizing in a given time period. These two methods are most commonly used. Fenn[30,31,32] also arrived at an index by computing the number of uningested particles per given time. However, the number of active neutrophils has been cited as slightly more accurate because although equal distribution of leukocytes and bacteria is theoretically desirable it is impossible to achieve. Therefore, by counting the phagocytes, the clumps become less significant.[3] Further, the Hamburger method has a tendency to equalize the effect of variations in individual cellular phagocytic activity. In this study, the methods of Leishman and Hamburger were both used. Statistical analysis subsequently indicated both methods were equally valid in serving as a phagocytic index. From a practical standpoint counting the numbers of phagocytes is less time-consuming and could reasonably be expected to yield adequate data.

Further related studies would help to clarify the role of other factors in phagocytosis. The presence of insulin is somehow related to the ability of glucose to cross the cell membrane.[36] Perhaps the lack of insulin, insulin-inhibitors or some little understood feature of diabetes combine to prevent normal entry of glucose during phagocytosis. Increased glucose intake <u>in vivo</u> would immediately create a demand for insulin. One disadvantage of <u>in vitro</u> studies is that in a closed

system the insulin is exhausted, as is the buffering capacity of blood, the immune system and possibly other unknown factors. Varying levels of glucose could be ingested by a single individual at different times under identical fasting conditions and the effect on phagocytosis observed. A study standardizing glucose levels and varying the insulin levels in single individuals would be informative.

This study concerned itself principally with artificially-induced blood glucose levels, but tissue concentrations of sugar may be of critical importance in the function of reticulo-endothelial phagocytic power. Skin window techniques could be used to study the relationship of blood glucose levels to tissue glucose levels in humans or animals.

VI. SUMMARY AND CONCLUSIONS

Thirty members of the Junior Class, Loma Linda University School of Dentistry, supplied blood to which bacteria and glucose were added in order to study the effect of increasing glucose on neutrophilic phagocytosis. Two methods of phagocytic index determinations were used.

In normal health young adult males, increasing concentrations of glucose significantly suppressed *in vitro* neutrophilic phagocytosis of coagulase-negative Staphylococcus epidermidis. As the glucose concentrations increased the suppression of phagocytosis increased. However, the percentage suppression for any glucose level was not found to be predictable nor was it established from these results that glucose alone will suppress phagocytosis in any one individual.

No statistically significant difference was seen in phagocytic index determinations between the bacteria per phagocyte method and the percentage of phagocytes method.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Allen, R. D. "A New Theory of Ameboid Movement and Protoplasmic Streaming," Exp. Cell Res. (Supplement) 8:17, 1961.

2. Beck, W. S. and W. N. Valentine. "The Carbohydrate Metabolism of Leukocytes. A review," Cancer Research, 13:309-317, 1953.

3. Berry, L. J. and T. D. Spies. "Phagocytosis," Medicine 28:239,1949.

4. Bierman, A. R., K. H. Kelly, and F. L. Cordes. "The Sequestration and Visceral Circulation of Leukocytes in Man," Ann.N.Y.Acad.Sc., 59:850, 1955.

5. Brewer, D. B. "Electron Microscopy of Phagocytosis of Staphylococci," J. Path. Bact., 86:299-303, October, 1963.

6. Bunting, C. H. Handbook of Hematology. New York: Hoeber, 1938, Vol. 1.

7. Bybee, J. D. Personal communication. 1966.

8. Bybee, J. D., and D. E. Rogers. "The Phagocytic Activity of Polymorphonuclear Leukocytes Obtained from Patients with Diabetes Mellitus," J. Lab. Clin. Med., 64:1-13, July, 1964.

9. Cohn, Z. A. "The Fate of Bacteria within Phagocytic Cells. I. The degradation of isotopically labeled bacteria by polymorphonuclear leukocytes and macrophages," J. Exp. Med.,117:27-42, January, 1963.

10. Cohn, Z. A. "The Fate of Bacteria within Phagocytic Cells. II. The modification of intracellular degradation." J. Exp. Med., 117:43-53, January, 1963.

11. Cohn, Z. A. and J. G. Hirsch. "The Isolation and Properties of the Specific Cytoplasmic Granules of Rabbit Polymorphonuclear Leukocytes," J. Exp. Med., 112:983, 1960.

12. Cohn, Z. A. and J. G. Hirsch. "The Influence of Phagocytosis on the Intracellular Distribution of Granule-associated Components of Polymorphonuclear Leukocytes," J. Exp. Med., 112:1015-1022, December, 1960.

13. Cohn, Z. A., J. G. Hirsch, and E. Wiener. "The Cytoplasmic Granules of Phagocytic Cells and the Degradation of Bacteria," Lysosomes, CIBA Foundation Symp., London: Churchill, 1963, p. 126.

14. Cohn, Z. A., and S. I. Morse. "Interaction Between Rabbit Polymorphonuclear Leukocytes and Staphylococci," J. Exp. Med.,110:419-443, 1959.

15. Cohn, Z. A., and S. I. Morse. "Functional and Metabolic Properties of Polymorphonuclear Leukocytes. I. Observations on the requirements and consequences of particle ingestion," J. Exp. Med., 111:667-687, May, 1960.

16. Cowdry, E. V. Problems of Aging. Baltimore: Williams & Wilkins, 1942, p. 629.

17. Cram, D. M., and R. J. Rossiter. "Phosphatase of Rabbit Polymorphonuclear Leukocytes," Can. J. Res., 27:290, 1949.

18. Crepea, S. B., G. E. Magnin, and C. V. Seastone. "Effect of ACTH and Cortisone on Phagocytosis," Proc. Soc. Exp. Biol. Med., 77:704, 1951.

19. Cushing, J. E., and D. H. Campbell. Principles of Immunology. New York: McGraw-Hill Co., 1957, pp. 162-163.

20. Delaunay, A. "Sur l'influence exercee par diverses toxines microbiennes sur le chimiotactisme leucocytaire," Compt. Rend. Soc. Biol., 137:265.

21. Die Redasksie, V. "Mannitol," So. Afr. Med. J.,38:792, Oct.,1964.

22. Dougherty, T. F., and G. L. Schneebeli. "Role of Cortisone in Regulation of Inflammation," Proc. Soc. Exp. Biol. Med., 75:854, 1950.

23. Drachman, R. H. "Phagocytic Defense Against Acute Bacterial Infection in Experimental Diabetes Mellitus," J. Clin. Invest., 44:1041, 1965. Abstract.

24. Egge, A. S. Personal communication. 1967.

25. Elberg, S. S. "Cellular Immunity," Bact. Rev., 24:67, 1960.

26. Ellingson, H. V., and P. F. Clark. "The Influence of Artificial Fever on Mechanisms of Resistance," J. Immunol., 43:65, 1942.

27. Escamilla, R. F. Laboratory Tests in Diagnosis and Investigation of Endocrine Functions. Philadelphia: F. A. Davis Co., 1962, pp. 209-224.

28. Evans, A. "The Toxicity of Acids for Leukocytes as Indicated by the Tropin Reaction," J. Immunol., 7:271, 1922.

29. Evans, W. H., and M. L. Karnovsky. "A Possible Mechanism for the Stimulation of Some Metabolic Functions During Phagocytosis," J. Biol. Chem., 236:PC30, 1961.

30. Fenn, W. O. "Phagocytosis of Solid Particles: Quartz," J. Gen. Physiol., 3:439, 1921.

31. Fenn, W. O. "Phagocytosis of Solid Particles: Carbon," J. Gen. Physiol., 3:465, 1921.

32. Fenn, W. O "Phagocytosis of Solid Particles: Carbon and Quartz," J. Gen. Physiol., 3:575, 1921.

33. Fenn, W. O. "The Theoretical Responses of Living Cells to Contact with Solid Bodies," J. Gen. Physiol.,4:373, 1921-1922.

34. Fenn, W. O. "Effect of the Hydrogen Ion Concentration on the Phagocytosis and Adhesiveness of Leukocytes," J. Gen. Physiol., 5:169, 1923.

35. Forscher, B. K., and H. C. Cecil. "Some Effects of Alloxan Diabetes on Acute Inflammation," J. Applied Physiol., 13:278, 1958.

36. Ganong, W. F. Review of Medical Physiology. Los Altos: Lange Medical Publications, 1965, 2nd Ed., pp. 378-380.

37. Garrey, W. E., and W. R. Bryan. "Variations in White Blood Cell Counts," Physiol. Rev., 15:597, 1935.

38. Goldacre, R. J. Int. Rev. Cytol., 1:135, 1952.

39. Ham, A. W., and T. S. Leeson. Histology. Philadelphia: J. B. Lippincott & Company, 1961, 4th Ed.

40. Hamburger, H. J. Physikalisch-chemische Untersuchungen uber Phagozyten. Wiesbaden, 1912.

41. Hanks, J. H. "Quantitative Aspects of Phagocytosis as Influenced by the Number of Bacteria and Leukocytes," J. Immunol., 38:159-176, 1940.

42. Harper, H. A. Review of Physiological Chemistry. 9th Ed., Palo Alto: Lange Medical Publications, 1963.

43. Harris, H. "Chemotaxis of Granulotyces," J. Path. Bact., 66:135, 1953.

44. Hektoen, L., and Ruediger. "Studies in Phagocytosis," J. Infect. Dis., 2:128, 1950.

45. Hirsch, J. G. "Biochemical Studies on the Bacteriocidal Power of Phagocytic Cells," Ann. N.Y. Acad. Sc., 66:382, 1956.

46. Hirsch, J. G. "Phagocytin: A bacterial substance from polymorphonuclear leukocytes," J. Exp. Med., 103:589, 1956.

47. Hirsch, J. G. and Z. A. Cohn. "Degranulation of Polymorphonuclear Leukocytes Following Phagocytosis of Microorganisms," J. Exp. Med., 112:1005-1014, December, 1960.

48. Hirsch, J. G., and Z. A. Cohn. "Digestive and Autolytic Functions of Lysosomes in Phagocytic Cells," Fed. Proc. 23:1023-1025, 1964.

49. Jacobs, A. "Phagocytosis in Normal and Anaemic Blood," J. Clin. Path. 17:185-187, March, 1964.

50. Jersild, M. "Phagocytic Activities of Various Types of Leukocytes," Acta Med. Scand. (Supplement) 213:238, 1948.

51. Joslin, E. Treatment of Diabetes Mellitus. Philadelphia: Lea & Febiger, 1952, 9th Ed.

52. Jung, R. W. "Correlation Between Number of Leukocytes and Percentage of Phagocytosis," Proc. Soc. Exp. Biol. & Med., 29:981, 1932.

53. Kaprol, F. A. "The Phagocytosis and Intracellular Fate of Staphylococci," Ann. N.Y. Acad Sc., 128:285-300, July 1, 1965.

54. Karnovsky, M. L. "Metabolic Basis of Phagocytic Activity," Physiol. Rev., 42:143-168, January, 1962.

55. Karnovsky, M. L., and A. J. Sbarra. "Metabolic Changes Accompanying the Ingestion of Particulate Matter by Cells," Am. J. Clin. Nutr., 8:147-155, March-April, 1960.

56. Kempner, W. "The Nature of Leukemic Blood Cells as Determined by their Metabolism," J. Clin. Invest. 18:291, 1939.

57. Kijak, E.,G. Foust, and R. R. Steinman. "Relationship of Blood Sugar Level and Leukocytic Phagocytosis," J. So. Calif. Dent. Assoc., 32:349, September, 1964.

58. Leishman, W. B. "Note on a Method of Quantitatively Estimating the Phagocytic Power of the Leukocytes of the Blood," Brit. Med. J. 1:73, January, 1902.

59. Levene, P. and G. Meyer. "On the Action of Leukocytes on Glucose," J. Biol. Chem., 12:265, 1912.

60. Lewis, T. Blood Vessels of the Human Skin and Their Responses. London: Shaw & Sons, 1927.

61. Lewis, W. H. "Pinocytosis," Bull. Johns Hopkins Hosp. 49:17, 1931.

62. Li, I. W., S. Mudd, and F. A. Kaprol. "Dissociation of Phagocytosis and Intracellular Killing of Staphylococcus Aureus by Human Blood Leukocytes," J. Immunol.,90:804, 1963.

63. Lieberkuhn, N. (Cited by Rebuck and Crowley). "Ueber Bewegungserscheinungen der Zellen," N. G. Elwert III Ed. Die farblosen Blutkorper. Marburg and Leipzig, Germany: pp. 357-365, 1870.

64. Lindberg, O., and L. Ernster. "Chemistry and Physiology of Mitochondria and Microsomes," Protoplasmatologie Bd. 3:1, 1954.

65. Litt, M. "Studies in Experimental Eosiniophilia VI Uptake of Immune Complexes by Eosinophile," J. Cell Biol. 23:355, 1964.

66. Lockwood, W. R., and F. Allison. "Electron Micrographic Observations of Changes Induced in Rabbit Exudate Cells by Phagocytosis of Pneumococcus," Clin. Res. 11:34, 1963.

67. Martin, S. P., and R. Green. "Methods for the Study of Surviving Leukocytes," Methods in Medical Research. Chicago: Year Book Publishers, Inc., Vol. 7, 1958, pp. 136-137.

68. McCutcheon, M. "Chemotoxis and Locomotion of Leukocytes," Ann. N.Y. Acad. Sc., 59:941, 1955.

69. McKendrick, A. G. Protoplasma, 3:611, 1927-1928. (Reviewed by E. Ponder.)

70. Melly, M. A., J. B. Thomison, and D. E. Rogers. "Fate of Staphylococci within Human Leukocytes," J. Exp. Med., 112:1121-1130, December, 1960.

71. Menkin, V. "Biochemical Factors in Inflammation and Diabetes Mellitus," Arch. Pathology, 34:182, 1942.

72. Menkin, V. "Factors Concerned in the Mobilization of Leukocytes in Inflammation," Ann. N.Y. Acad. Sc. 59:956, 1955.

73. Metchnikoff, E. Lectures on the Comparative Pathology of Inflammation. London: Kegan, Paul, Trench, Trubner & Co., 1893.

74. Metchnikoff, E. Immunity in Infectious Disease. Cambridge, England: Cambridge University Press, 1907, 2nd Ed.

75. Miller, S. E. Textbook of Clinical Pathology. Baltimore: Williams & Wilkins Co., 1955, 5th Ed., pp. 603-608.

76. Moon, V. H. "Mechanism of Acute Inflammation," Arch. Pathology, 20:561, 1935.

77. Moon, J. H., C. G. Grand, and G. A. Terschakovec. "Dynamics of Inflammation and of Repair IV Chemotactic Substances in Normal Tissues," Arch. Pathology, 57:44, 1954.

78. Mudd, S. M., M. McCutcheon, and B. Lucke. "Phagocytosis," Physiol. Rev., 14:210, 1934.

79. Oren, R., A. E. Farnham, K. Saito, E. Milofsky, and M. L. Karnovsky. "Metabolic Patterns in Three Types of Phagocytizing Cells," J. Cell Biol., 17:487-501, June, 1963.

80. Palade, G. E. Subcellular Particles. (Edited by T. Hayashi) New York: Ronald Press Company, 1959, p. 64.

81. Pillemer, L. "Recent Advances in the Chemistry of Complement," Chem. Revs., 33:1, 1943.

82. Ponder, E. "The Influence of Surface Charge and of Cytoplasmic Viscosity on the Phagocytosis of a Particle," J. Gen. Physiol., 11:757-777, 1928.

83. Racker, E., V. Klybas, and M. Schramm. "Tetrose Diphosphate, a Specific Inhibitor of Glyceraldehyde 3-Phosphate Dehydrogenase," J. Biol. Chem., 234:2510, 1959.

84. Rebuck, J. W. "Functions of Neutrophils and Lymphocytes in Acute Inflammation in Man," Anat. Record, 103:497, 1949.

85. Rebuck, J. W., and R. C. Mellinger. "Interruption by Topical Cortisone of Leukocytic Cycles in Acute Inflammation in Man," Ann. N.Y. Acad. Sc., 56:715, 1953.

86. Rebuck, J. W., R. W. Smith, and R. R. Margulis. Proc. 2nd ACTH Clinical Conference. Philadelphia: Blakiston, 1951, pp. 460-467.

87. Rebuck, J. W., R. W. Smith, Jr., and R. R. Margulis. "The Modification of Leukocytic Function in Human Windows by ACTH," Gastroenterology, 19:644, 1951.

88. Rebuck, J. W., and H. Woods. "Electron Microscope Studies of Blood Cells," Blood, 3:175, 1948.

89. Richardson, R. "Measurements of Phagocytic Activity in Diabetes Mellitus," Am. J. Med. Sc., 204:29, 1942.

90. Roberts, S. R., and R. R. Kracke. "Agranulocytosis; Report of a case," J. Am. Med. Assoc., 95:780, 1930.

91. Rogers, D. E. "Studies on Bacteriemia, I. Mechanisms Relating to the Persistence of Bacteriemia in Rabbits Following the Intravenous Injection of Staphylococci," J. Exp. Med., 103:713-742, 1956.

92. Rogers, D. E. "Host Mechanisms Which Act to Remove Bacteria from the Blood Stream," Bact. Rev., 24:50-66, March, 1960.

93. Rogers, D. E., and M. A. Melly. "Further Observations on the Behavior of Staphylococci within Human Leukocytes," J. Exp. Med., 111:533-558, April, 1960.

94. Rogers, D. E., and M. A. Melly. "The Dynamics and Immunology of the Phagocytosis of Staphylococci," Trans. Assoc. Amer. Physicians, 74:216-224, 1961.

95. Rogers, D. E., and R. Thompsett. "The Survival of Staphylococci within Human Leukocytes," J. Exp. Med., 95:209, 1952.

96. Rosen, R., and I. F. Enquist. "The Healing Wound in Experimental Diabetes," Surgery, 50:525, 1961.

97. Rossi, F., and M. Zatti. "Changes in the Metabolic Pattern of Polymorphonuclear Leukocytes During Phagocytosis," Brit. J. Exp. Path., 45:548-559, October, 1964.

98. Rous, P. "The Relative Reaction within Living Mammalian Tissues," J. Exp. Med., 41:379, 399, 451, 1925.

99. Sbarra, A. J., and M. L. Karnovsky. "The Biochemical Basis of Phagocytosis. I. Metabolic Changes During the Ingestion of Particles by Polymorphonuclear Leukocytes," J. Biol. Chem., 234:1355, 1959.

100. Schwind, J. L. "The Supravital Method in the Study of the Cytology of Blood and Marrow Cells," Blood, 5:597, 1950.

101. Shayegani, M. G., and F. A. Kaprol. "The Immediate Fate of Staphylococci after Phagocytosis," J. Gen. Microbiol., 29:625-636, December, 1962.

102. Shayegani, M. G., and F. A. Kaprol. "The Eventual Intracellular Destruction of Staphylococci by Mononuclear Cells," J. Gen. Microbiol., 29:637-644, December, 1962.

103. Sieracki, J. C. "The Neutrophilic Leukocyte," Ann. N.Y. Acad. Sc., 59:690, 1955.

104. Speirs, R. S. "How Cells Attack Antigens," Sc. Amer., 210:58-64, February, 1964.

105. Suter, E. "Interaction Between Phagocytes and Pathogenic Micro-organisms," Bact. Revs., 20:94-132, 1956.

106. Taliaferro, W. A. "The Cellular Basis of Immunity," Ann. Rev. Microbiol., 3:159, 1949.

107. Tompkins, E. H. "The Monocyte," Ann. N.Y. Acad. Sc., 59:732, 1955.

108. Townsend, W. A., and B. Campbell. "The Effects of Roentgen Rays on the Inflammatory Cells of the Mouse and Rabbit," Blood, 4:1346, 1949.

109. Tullis, J. L. "The Leukocytosis of Diabetic Acidosis," Am. J. Med. Sc., 215:424, 1948.

110. Tullis, J. L. Blood Cells and Plasma Proteins. New York: Academic Press, 1953, p. 265.

111. Tullis, J. L., and D. M. Surgenor. "Phagocytosis-promoting Factor of Plasma and Serum," Ann. N.Y. Acad. Sc., 66:386, 1956.

112. Tunnicliff, R. "Promotion of Phagocytosis by Calcium Gluconate, Sodium Iodide, Dextrose, and Other Substances," J. Inf. Dis., 48:161-166, 1931.

113. Valentine, W. N. "Quantitative Biochemical Studies on Leukocytes in Man: A Review," Blood, 6:845, 1951.

114. Vercauteren, R. "Leukocyte Metabolism in Health and Disease," Proc. of the 2nd Int'l. Symp. on Enzymes in Clin. Chem., Ghent. 1961.

115. Wachstein, M. "Alkaline Phosphatase Activity in Normal and Abnormal Human Blood and Bone Marrow Cells," J. Lab. Clin. Med., 31:1, 1946.

116. Wagner, R. "Studies on the Physiology of the White Blood Cell," Blood, 2:235, 1947.

117. Waller, A. "Microscopic Observations on the Perforation of the Capillaries by the Corpuscles of the Blood, and on the Origin of Mucous and Pus Globules," London, Edinburgh, and Dublin: Philosoph. Mag. and J. Sc., 29:397, 1846.

118. Ward, H. K., and J. F. Enders. "An Analysis of the Opsonic and Tropic Action of Normal and Immune Sera Based on Experiments with the Pneumococcus," J. Exp. Med., 57:527-547, 1933.

119. White, L. P. "The Intravascular Life Span of Transfused Leukocytes Tagged with Atabrine," Blood, 9:73, 1954.

120. Williams, R. H. Diabetes. New York: Paul B. Hoeber, Inc., 1965, Third printing.

121. Wintrobe, M. M. Clinical Hematology. Philadelphia: Lea & Febiger, 1951, p. 43.

122. Wood, W. B., Jr. "White Blood Cells v. Bacteria," Sc. Amer., February, 1951.

123. Wood, W. B., Jr. "Phagocytosis, with Particular Reference to Encapsulated Bacteria," Bact. Rev., 24:41-49, March, 1960.

124. Wood, W. B., Jr., M. R. Smith, J. W. Berry, and W. D. Perry. "Studies on the Cellular Immunology of Acute Bacteremia," Trans. Assoc. Am. Physicians, 64:155-159, 1951.

125. Wood, W. B., Jr., M. R. Smith, W. D. Perry, and J. W. Berry. "Studies on the Cellular Immunology of Acute Bacteriemia. I. Intravascular Leukocytic Reaction and Surface Phagocytosis," J. Exp. Med., 94:521-534, 1951.

126. Wright, A. E., and S. R. Douglas. "An Experimental Investigation of the Role of the Blood Fluids in Connection with Phagocytosis," Proc. Roy. Soc., (London), 72:357-370, 1903.

127. Wright, A. E., and S. T. Reid. "On the Possibility of Determining the Presence or Absence of Tubercular Infection by the Examination of a Patient's Blood and Tissue Fluids," Proc. Roy. Soc., 77:211, 1906.

128. Wright, C. S. "Leukemia Polycythemia and Related Diseases," Am. J. Roentgenol. Radium Therapy, 64:907, 1950.

129. Wright, C. S., and M. C. Dodd. "Phagocytosis," Ann. N.Y. Acad. Sc., 59:945, 1955.

130. Zatti, M., F. Rossi, and V. Meneghelli. "Metabolic and Morphological Changes of Polymorphonuclear Leukocytes During Phagocytosis," Brit. J. Exp. Path., 46:227-233, April, 1965.

APPENDIX

TABLE I

FASTING AND BLOOD SUGAR AND LEUKOCYTE COUNTS

Subject Number	Fasting Blood Sugar	Leukocyte Count	Subject Number	Fasting Blood Sugar	Leukocyte Count
1	70	4,500	16	63	8,300
2	56	7,700	17	72	9,200
3	97	7,600	18	59	4,400
4	67	7,300	19	72	5,600
5	114	7,600	20	65	4,200
6	80	6,900	21	71	7,800
7	68	8,300	22	70	7,500
8	73	7,200	23	68	5,100
9	76	6,500	24	73	6,000
10	77	9,100	25	64	6,500
11	66	7,900	26	60	8,100
12	63	6,400	27	72	5,100
13	69	7,100	28	72	12,400
14	57	9,100	29	73	8,100
15	62	7,300	30	66	8,400

TABLE II

MEANS OF PHAGOCYTOSIS

Tube No.	Milligrams Percent Glucose	Percent Leukocytes Phagocytizing	Bacteria per Phagocyte
1	0	89.5	10.7
2	50	74.5	5.1
3	100	72.2	4.9
4	150	69.6	4.1
5	200	67.6	4.0
6	250	67.0	3.9

TABLE III

DIFFERENCE OF THE MEANS*
(Percent Phagocytes)

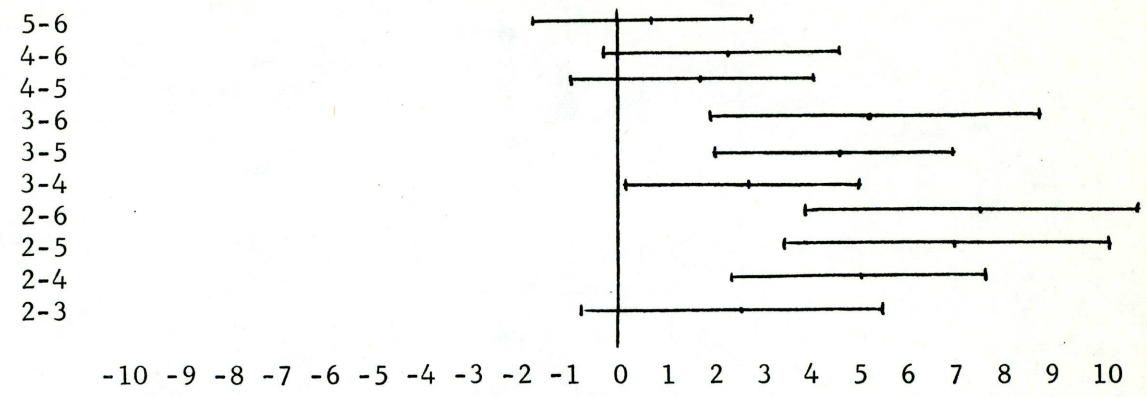
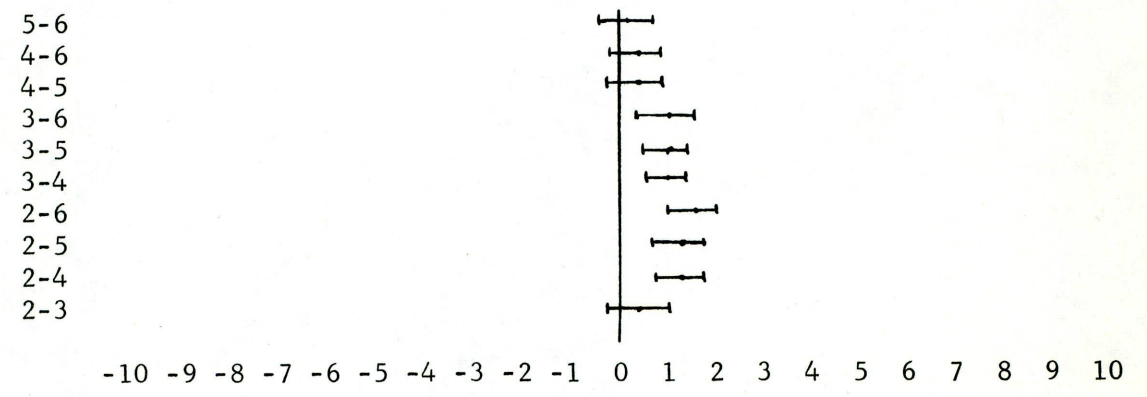


TABLE IV

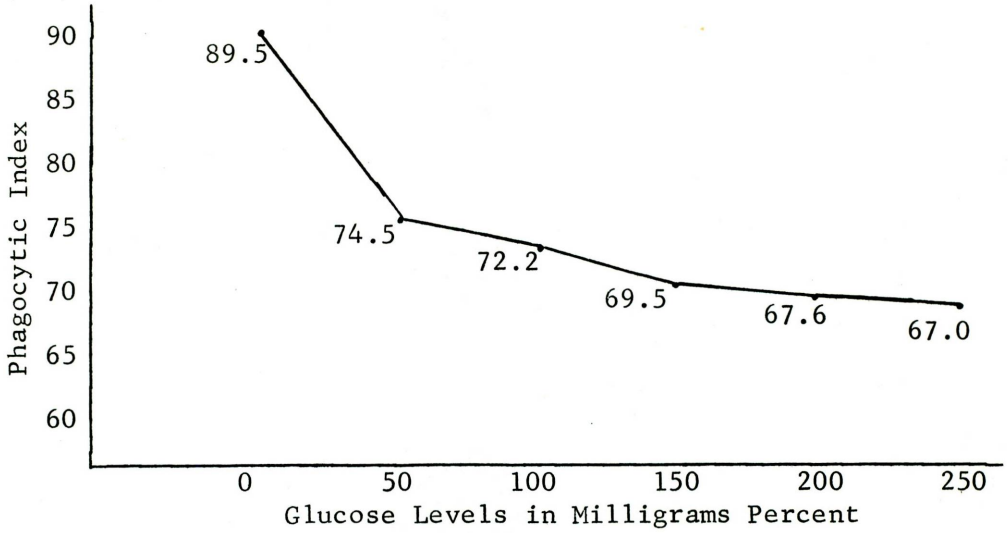
DIFFERENCE OF THE MEANS*
(Bacteria per Phagocyte)



* Confidence intervals crossing 0 line indicate differences of means that are not significant ($\alpha = .05$)

TABLE V

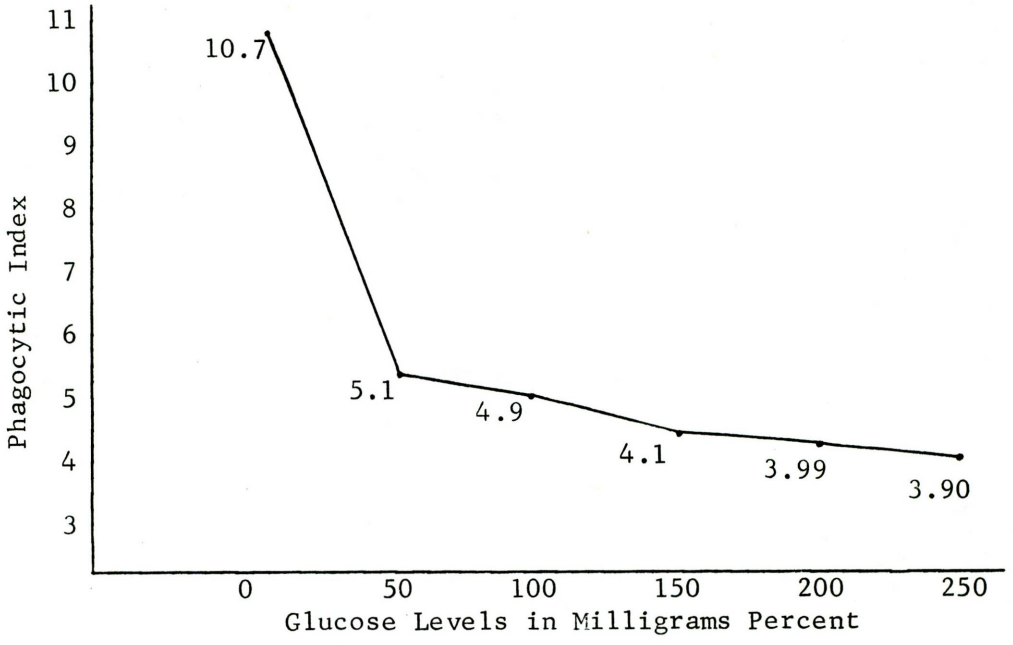
PHAGOCYTIC INDEX CURVE (Percent Phagocytes)



Tube No.	1	2	3	4	5	6

TABLE VI

PHAGOCYTIC INDEX CURVE (Bacteria per Phagocyte)



Tube No.	1	2	3	4	5	6

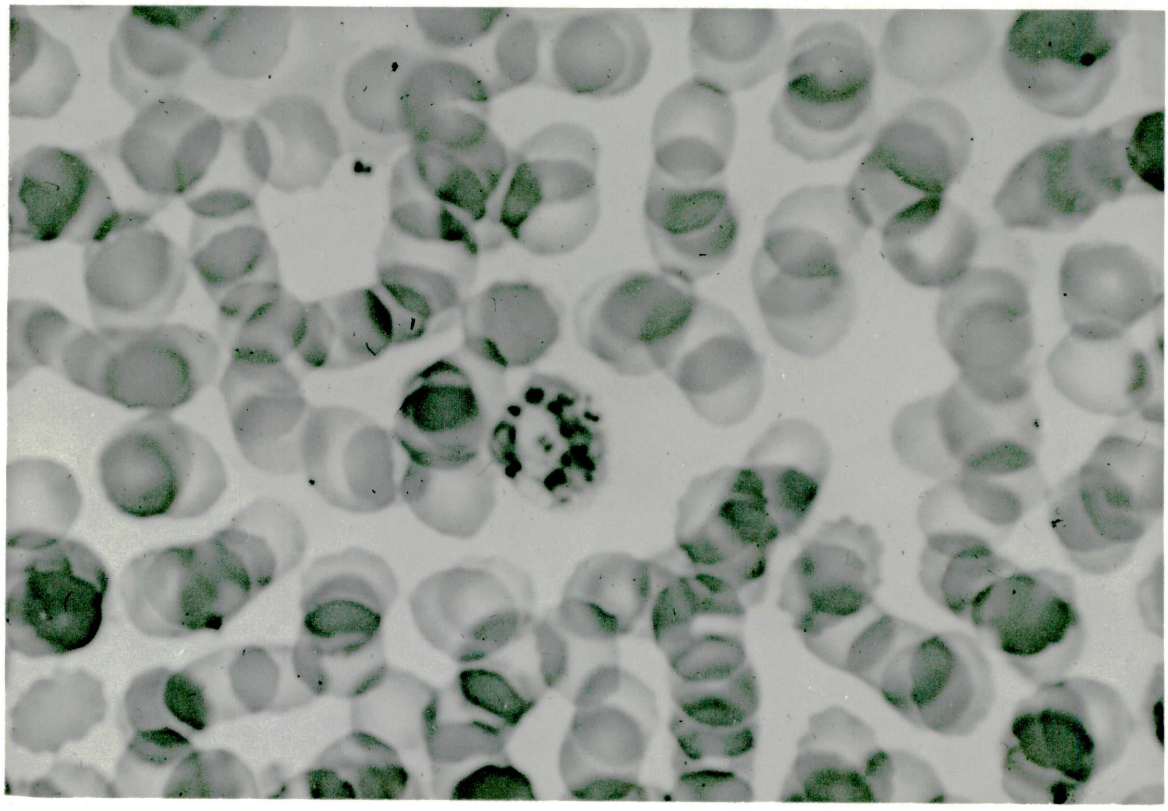
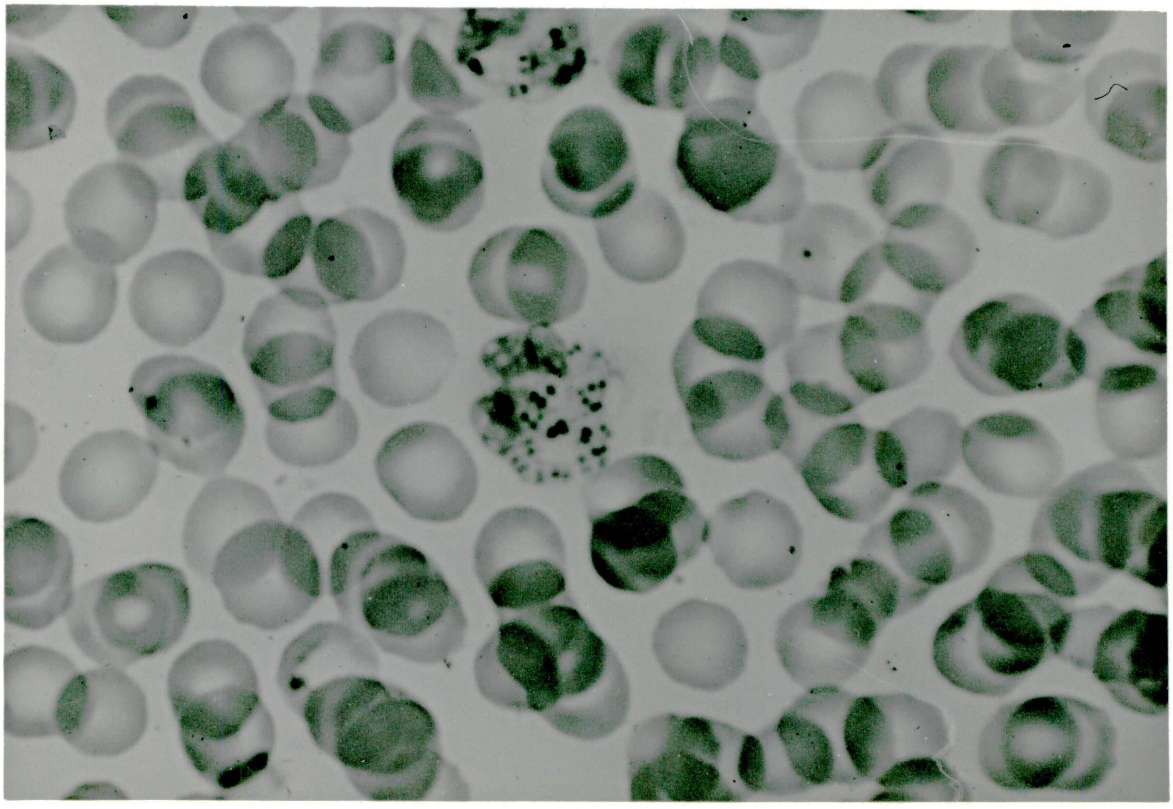


Figure I. PHAGOCYTIZING NEUTROPHILS



LOMA LINDA UNIVERSITY

Graduate School

THE EFFECT OF GLUCOSE ON NEUTROPHILIC PHAGOCYTOSIS

by

Donald F. Adams

An Abstract of a Thesis in

Partial Fulfillment of the Requirements for the

Degree of Master of Science in the Field of Periodontology

June 1967

ABSTRACT

Samples of blood were obtained from thirty healthy young adult males (Loma Linda University School of Dentistry), and contaminated with bacteria. Varying concentrations of pure glucose were added to this mixture to observe the effect of the glucose on in vitro neutrophilic phagocytosis. As the glucose levels increased a statistically significant suppression was noted in the ability of the neutrophilic leukocytes to engulf Staphylococcus epidermidis.

It was not possible, however, to predict the degree of suppression for any particular level of glucose added nor is it possible to state that suppression will occur in any one individual.

Two methods of phagocytic index determinations were used. The first recorded the percentage of neutrophils that had phagocytized and the second counted the number of bacteria per phagocyte. Statistically no difference was noted between the two methods.