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The Effect of Diet on Neutrophilic Phagocytosis

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THE EFFECT OF DIET ON NEUTROPHILIC PHAGOCYTOSIS

by

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Each person whose signature appears below certifies that this thesis in his opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

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CHAPTER I

INTRODUCTION

The ability of polymorphonuclear leukocytes (PMNL) to engulf bacteria and particulate matter by phagocytosis is a major defense mechanism of higher organisms (Metchnikoff, 1893; Menkin, 1940). Although various circulating leukocytes engulf bacteria, neutrophils and monocytes are the most active phagocytes (Anthony, 1967).

The study of diabetes mellitus has provided information about human defense mechanisms. This is a disease characterized by a severe disturbance in carbohydrate metabolism, including elevated blood sugar levels. Diabetes has also been associated with subnormal resistance to bacterial infection (Menkin, 1950). A study of the mean number of bacteria engulfed by leukocytes from diabetics suggests that the higher the blood sugar level, the lower the phagocytic index (Kijak, Foust, and Steinman, 1964).

Data from this laboratory showed that the ingestion of 100 grams each of various sugars (glucose, fructose, sucrose, honey, and concentrated orange juice) significantly decreased the phagocytic index for more than five hours. Similar results were not obtained with the ingestion of starch (Sanchez et al., 1973). Other researchers gave graded levels of oral glucose to 1 to 4 subjects per group (Kijak et al., 1964), which is too few subjects to provide statistically meaningful conclusions. One of the purposes of this study is to determine the effects of varying levels of oral glucose on phagocytosis by use of a statistically valid number of subjects.

In a normal diet sugars are usually found in combination with other nutrients such as proteins, fats, and starch. An additional purpose of this study is to test the effects of glucose when ingested in combination with protein, fat, and other carbohydrates.

CHAPTER II

REVIEW OF LITERATURE

All organisms resist internal invasion by foreign substances, and more complex animals are protected against such invasion by passive and active defenses. Skin is the first of the passive defenses. Then connective tissue fibers throughout the body and especially in organs of the reticulo-endothelial system, such as the spleen and lymph nodes, act as filters to trap foreign materials and prevent it from being spread into other tissues by body fluids. These passive defenses are incomplete and rather easily breached (Hektoen and Ruediger, 1950). Active defenses include phagocytosis of invading organisms by reticulo-endothelial cells and by mononuclear and polymorphonuclear leukocytes (PMNL), which constitutes one of the important agencies of host defense against infectious disease (Hirsch, 1956; Selvaraj, McRipley, and Sbarra, 1967).

The vital role played by phagocytic cells in protecting animals from certain infectious diseases was first emphasized by Metchnikoff (1893) over 80 years ago and is now a well accepted concept (Hirsch, 1956). Inadequate host resistance, rather than exposure to the parasite, is the prime determinant of disease. As an example, staphylococci, which are widely spread in the human population, produce disease only in the rare individual in whom the mechanisms of natural resistance have failed (Hirsch, 1960). The phagocytic system has a reserve capacity which is rarely exceeded by the invading organism. Living bacteria which enter the blood stream of animals or man usually disappear rapidly from the

circulation (Rogers, 1960; Wright and Dodd, 1955).

The leukocytic reaction to an invading organism is a complex biologic event involving many stages: migration of the PMNL from the blood vessels, locomotion, response to chemotaxis, engulfment, and finally intracellular killing and digestion of the parasite (Hirsch and Strauss, 1963; Selvaraj, McRipley, and Sbarra, 1967). These stages will be discussed in the following sections.

Bacterial clearance

The cells of the reticulo-endothelial system, and those to which they give rise, are especially phagocytic. Though widespread throughout the body, they are concentrated in lymph nodes, spleen, bone marrow, and liver. Though scattered, these cells are uniform and are spoken of collectively as the reticulo-endothelial system (RES). The two main cell types found in the RES are (1) stationary or fixed cells, and (2) the free or circulating cells, known as macrophages in tissues, and monocytes in the bloodstream (Smith, 1969).

The liver and spleen are principally responsible for clearing bacteria from the blood, but they remove different organisms with different degrees of efficiency (Carpenter, 1965; Rogers, 1960). Rogers (1960) states that the saturation of or exhaustion of the splanchnic trapping mechanism does not play an essential role in the course of natural infections. Instead, the ability of the fixed and circulating phagocytic cells to retain and destroy bacteria determines the outcome of the system's fight against bacteremia.

The PMNL, produced in the bone marrow, is one of the most important of the derivative cells of the RES (Smith, 1969). The PMNL are the first

cells to arrive at the site of a developing inflammatory reaction. These cells are followed by the mononuclear phagocytes (monocytes). This sequential order seems to be due to the localized lactic acidosis that develops with the progress of acute inflammation. At a pH between 6.0 and 5.5 the leukocytes, including macrophages, are to a large extent incapable of survival, and frank pus results (Menkin, 1955).

The initial rapid phase of bacterial clearance by PMNL is not affected by such factors as starvation, massive irradiation, the production of agranulocytosis, shock, experimental diabetes, splenectomy, renal failure, or the presence of overwhelming infection. In contrast, the ability of reticulo-endothelial tissues to contain and destroy entrapped bacteria may be considerably influenced by a wide variety of stresses applied to the host (Rogers, 1960).

Motility

Motility of PMNL and monocytes is essential for the cellular response to inflammation. The speed of migration is approximately 29 to 34 micrometers per minute, determined either as random movement on a coverslip or as emigration from a buffy coat, the layer of white blood cells formed when blood is centrifuged (Suter, 1956).

Amebae and leukocytes are much alike in appearance. Although the handmirror shape of leukocytes in motion is similar to the shape of moving amebae, the constriction rings noted in moving leukocytes in a culture medium do not play an essential role in movement (DeBruyn, 1946).

Granulocytes normally move intravascularly with the current of blood. With the entrance of bacteria into the blood stream, the granulocytes stick to the endothelium of capillaries and assume ameboid motility,

becoming potentially phagocytic (Wright and Dodd, 1955).

Anesthetics and metabolic blocking agents inhibit migration. Of the latter those inhibitors which also reduce aerobic respiration of PMNL are most effective, suggesting that aerobic oxidative metabolism supports migration. These inhibitors frequently have no influence on phagocytosis (Suter, 1956).

Chemotaxis and opsonization

Chemotaxis of leukocytes, a directional response of the organism to chemical substances in its environment, is termed positive or negative depending on whether there is attraction or repulsion. Positive chemotaxis does not affect the rate of PMNL motility, but directs the movement of the phagocyte toward the bacteria and increases the number of the leukocytes emigrating into the area, thereby increasing contact between phagocytes and microorganisms in the tissues (McCutcheon, 1946, 1955; Suter, 1956). Chemotaxis, displayed especially by the PMNL, has been observed in mammals, birds, and amphibia. Monocytes (macrophages) react only weakly to chemotactic stimuli under most conditions (McCutcheon, 1946, 1955).

Among the various substances that attract or repel leukocytes, the best known are products of tissue injury and particles such as bacteria, silicates, and collodion. Certain aluminum silicates are powerful repelling agents for leukocytes *in vitro*, whereas collodion and certain bacteria attract them (McCutcheon, 1955; Suter, 1956). Certain microorganisms produce surface-coating antiphagocytic factors which inhibit their ingestion (Wright and Dodd, 1955). Still other particles such as carbon, have no chemotactic effect (McCutcheon, 1955).

Certain factors in serum, which promote the uptake of bacteria by leukocytes, are known as opsonins and tropins. These components of immune and normal sera, respectively, are the most effective and most important of all known phagocytosis-promoting substances (Mudd, McCutcheon, and Lucke, 1934). There are 3 classes of opsonins: (a) heat-labile serum factors, which increase phagocytosis by adsorbing onto and presumably changing the nature of the bacterial surface (Hirsh and Strauss, 1963; Menkin, 1955; Mudd, McCutcheon, and Lucke, 1934; Suter, 1956); (b) specific antibodies directed against antiphagocytic surface components of microorganisms; and (c) certain basic proteins which apparently exert a nonspecific phagocytosis-promoting effect under some conditions (Mudd, McCutcheon and Lucke, 1934).

Chemotaxis, phagocytosis, and intracellular digestion are separate activities of the leukocyte which depend on different mechanisms (McCutcheon, 1946). Phagocytosis is not necessarily preceded by chemotaxis, nor is chemotaxis always followed by phagocytosis.

PMNL - Phagocytic activity

Of the mature leukocytes, only the granulocytes and monocytes are potential phagocytes. The neutrophilic granulocytes (PMNL) are more active phagocytes than eosinophils and basophils. The younger mature PMNL with only 1 or 2 nuclear lobes are the most effective phagocytes (Anthony, 1967; Wright and Dodd, 1955).

Phagocytic activity may be affected by a wide variety of agents which influence the effectiveness of the phagocytic defense mechanism, and thus alter nonspecific resistance to infection. Various researchers (Crepea, Magnin, and Seastone, 1951; Kass and Finland, 1953; Menkin, 1955;

Rebuck and Mellinger, 1953; Suter, 1956; Wood, 1960; Wright and Dodd, 1955) have observed reduced phagocytosis in human subjects following treatment with ACTH, cortisone, and other steroids, and general anesthesia. Various substances such as formalin, $CaCl_2$, $BaCl_2$, $MgCl_2$, K_2SO_4, and $NaHCO_3$, in high concentrations, may decrease phagocytosis by binding or neutralizing opsonins (Hektoen and Ruediger, 1950). This decreased phagocytosis results in the establishment and progress of various infections, especially those caused by streptococci, pneumococci, and other microbes. Tunnicliff (1931) found that a few suitably diluted substances have stimulated phagocytosis. Among these are $CaCl_2$, neoarsphenamine, neosalvarsan, sodium salicylate, and quinine salts.

Vaccination of animals with bacteria increases the engulfment of these bacteria and causes several alterations of their surface properties, such as increased cohesiveness, decrease in surface electrical potential differences (Suter, 1956), and decrease in wettability by oil (Lucke et al., 1929). Roger's findings (1960) indicate that bacteremia can be profoundly modified by immunity, although initial host mechanisms acting to clear the blood stream are probably not dependent on prior experience with the invading microorganism.

In testing the influence of temperature on phagocytic activity, Ellingson and Clark (1942) found that phagocytosis was greatest from 38 to 40 degrees Celsius, a range somewhat above the normal body temperature.

PMNL - metabolism during phagocytosis

Phagocytosis was once thought to be a passive process, with no net expenditure of energy on the part of the engulfing cell (Mudd et al.,

1934). The act of engulfment is now understood to be an active process requiring metabolic energy, specifically glycolytic energy (Karnovsky, 1968; Sbarra et al., 1971).

Numerous biochemical activities occur immediately after foreign material strikes a phagocytic cell. There is an increase in enzymatic activity, respiratory activity, glycolysis, and oxidation of glucose through the hexose monophosphate shunt (Cline, 1956; Karnovsky, 1968; Karnovsky and Sbarra, 1960; Patriarca et al., 1970; Paul et al., 1972; Rossi and Zatti, 1964; Sbarra et al., 1971; Selvaraj et al., 1967; Wachstein and Middletown, 1946; West, 1968). The citric acid cycle (Krebs) is less important in PMNL, as is expected from examination of their structure--which reveals rather few mitochondria in mature cells (Karnovsky, 1968).

Bactericidal activities

Many of the oxidative metabolic stimulations that occur when a phagocytic cell comes in contact with bacteria or foreign material are closely associated with the antimicrobial activities of the cell. The increased hexose monophosphate shunt may serve to supply hydrogen peroxide to the phagocyte by reoxidation of NADPH by its oxidase in the presence of molecular oxygen. Subsequently this H_2O_2 would act as a bactericidal agent in the cell. Also, certain heat-labile components of blood, which aid in phagocytosis, are necessary for the subsequent intracellular destruction (Sbarra et al., 1971).

Nutrition and phagocytic activity

Nutrition of both humans and laboratory animals has been shown to

affect phagocytic activity. Cottingham and Mills (1943, 1945) found that a single deficiency in thiamine, riboflavin, pyridoxine, pantothenic acid, choline, or ascorbic acid severe enough to retard growth in laboratory mice resulted in reduced phagocytic activity.

Nungestar and Ames (1948) found a direct relationship between the ascorbic acid content of exudative PMNL from guinea pigs and the phagocytic activity. An increased ascorbic acid content also resulted in decreased fragility of the leukocytes.

The bactericidal activity of the PMNL is often reduced by nutritional deficiencies with no reduction in phagocytic activity. Moderate nutritional deficiencies of thiamine, riboflavin, or vitamin A have been shown to decrease the bactericidal activities of the phagocyte, but have no effect on phagocytic activity of the peritoneal fluid of rats (Guggenheim and Buechler, 1946). One study of undernourished infants and children (Seth and Chandra, 1972) revealed a significant decrease in bactericidal killing as compared with normal healthy controls, although phagocytic activity was comparable in the two groups. In another study of children and adults Arbeter (1971) suggests that iron utilization and deficiency may affect an iron-dependent enzyme, myeloperoxidase, essential for intracellular killing of bacteria.

A prolonged moderate or sudden severe restriction of food intake in rats has been shown to increase phagocytosis, but decreases the bactericidal action of the PMNL from peritoneal fluid (Guggenheim and Buechler, 1946). Sanchez et al., (1973) found a significant increase in the phagocytic index of 7 subjects following a fast of 36 or 60 hours.

Diabetes and phagocytosis

Patients with diabetes mellitus are characterized by elevated blood glucose levels and decreased resistance to infection. Several researchers (Bybee and Rogers, 1964; DaCosta and Beardsley, 1908; Richardson, 1942; Schauble and Baker, 1957) have also observed impaired neutrophilic phagocytosis in diabetics as compared with normal subjects. The higher the blood sugar level of 45 diabetic patients, the lower the phagocytic index (Kijak et al., 1964). Humans with uncontrolled diabetes and depancreatized cats with acidosis showed a significantly decreased phagocytic activity when compared with controlled states (Richardson, 1942).

Bybee and Rogers (1964) investigated the phagocytic capacity of PMNL from nonacidotic and acidotic diabetic patients. Leukocytes with normal activity phagocytized normally when added to serum from ketoacidotic diabetic patients. These results indicate that this reduced phagocytosis is due to a defect in the leukocyte itself when studied in this altered metabolic state.

The mechanism of reduced phagocytosis is yet unknown. Esmann (1961) found a low glycogen content and decreased glycogen synthesis in leukocytes obtained from patients with poorly controlled diabetes. When the observations were repeated with leukocytes obtained after return to controlled state, glycogen content and synthesis were normal. Martin and his associates (1953) demonstrated diminished glucose utilization and lactate production in leukocytes from diabetic subjects. This abnormality was corrected in vitro by addition of insulin.

Since insulin regulates the functions of several rate limiting enzymes in the glycolytic pathway, the functional abnormalities may

result from a disturbance in energy production (Weber, Stamm, and Fisher, 1965). As an energy-requiring process, phagocytosis depends on continued metabolism of glucose (Bagdade, Nielson, and Bulger, 1972). Since insulin does not appear to be required for the transport of glucose across the granulocyte membrane (Esmann, 1965), the abnormality of phagocytosis may be related to the intracellular actions of insulin (Bagdade et al., 1972).

Phagocytosis and sugar ingestion

In studies conducted to determine the effects of carbohydrate ingestion on neutrophilic phagocytosis and the duration of the effects, Sanchez et al. (1973) found that the ingestion of 100 grams of each of 5 sugars (glucose, fructose, sucrose, honey, and concentrated orange juice) significantly reduced the phagocytic index for up to 5 hours postprandial. Starch ingestion did not produce the same results. The greatest effects occurred between 1 and 2 hours postprandial. The decreased phagocytic index was not significantly associated with the number of neutrophils. These data suggest that the function and not the number of phagocytes was altered by ingestion of sugars.

Kijak et al. (1964) found that, in a limited number of subjects, the action of the leukocyte varied with different levels of blood glucose. The subjects were given varying amounts of glucose orally; the blood glucose was increased at 45 minutes postprandial. The more glucose consumed up to 100 grams, the higher the blood glucose level was raised, and the lower the phagocytic index dropped. Leukocytes from a blood sample of normal glucose concentration (60 to 100 mg. per cent) were very active, in contrast to leukocytes from blood with higher than

normal blood sugar concentration. The sluggish cells showed an increased amount of fat vacuoles (Kijak et al., 1964).

METHODOLOGY

Experimental design

The first series of tests were designed to determine the effects of varying amounts of oral glucose by measuring the phagocytic response of subjects after an overnight fast and at 1/2, 2, 3, and 4 hours post-prandial. The amounts of glucose administered were 12.5, 25, 50, and 100 grams in 10 ounces of water. The same 7 to 10 subjects participated for each of the different amounts of glucose tested.

The second series of experiments were designed to test the effects of glucose ingestion on phagocytosis when combined with a meal of known amounts of protein, fat and starch. The amount of glucose selected to use in the experiments, as determined by the previous tests, was 75 grams, an amount which produced maximal results. A total of 11 subjects participated in each of 4 separate test runs. In each test the subjects were divided into 4 groups, each following a different pattern of glucose and meal ingestion. All of the test diets were liquid in order to control for liquid vs. solid variables of digestion.

Group I received the "meal" which consisted of Isopro, a soy protein isolate, cornstarch, and corn oil, providing 20 grams of protein, 75 grams of carbohydrate, and 10 grams of fat. Group II received the "meal with glucose," which consisted of Isopro, glucose, and corn oil, providing 20 grams of protein, 88 grams of carbohydrate, and 10 grams of fat. Group III received 75 grams of glucose. Group IV received the "meal" 30 minutes following ingestion of 75 grams of glucose. Fasting, 2, and 4 hour blood

samples were drawn from subjects in groups I, II, and III. In group IV, samples were drawn at fasting, 2 hours 15 minutes, and 4 hours 15 minutes following ingestion of the glucose.

Most of the subjects were students, ranging in age from 20 to 40. In each series of experiments, most of the subjects participated in all the tests performed.

Experimental procedure

All blood samples were drawn from the arm of the subject into heparinized tubes. Fasting samples were taken in the morning following a minimum of 12 hours fast. For the 1/2, 2, 3, and 4-hour samples, time was computed from the point when the subject finished drinking the glucose or test meal. Because leukocytes are fragile, disappearing several hours after blood is drawn (Green, 1968), the phagocytic index was determined within 1/2 hour after each blood sample was drawn.

The slide technique was used to determine the phagocytic index. Staphylococcus epidermidis, a coagulase negative, gram positive, spherical organism, about 0.8 to 1.0 micrometer in diameter, was obtained from the Loma Linda University Department of Microbiology for use in this study. The organism is non-pathogenic and does not interfere with phagocytosis.

For the first series of experiments the culture was prepared as follows: 9.5 ml. of Todd-Hewitt broth was inoculated with 0.5 ml. bacterial stock culture and incubated at 38 to 40 degrees centigrade for 36 hours. Then the cells were washed by centrifuging the broth culture at 2000 rpm for 10 minutes, discarding the supernatant, suspending in physiological saline, and re-centrifuging. After discarding the saline

supernatant approximately 14 ml. of saline was added to the cells to provide a turbidity measurement of 20 per cent transmittance in a Bausch-Lombe spectrophotometer set at a wavelength of 420 nm. At this measurement the average phagocytic index at fasting was computed to be 18, using the researcher's own blood as the standard. At an average of 18, enough bacteria were present to demonstrate the leukocyte's phagocytic activity under the specific conditions imposed on it, and yet there were not so many bacteria to cause cells to burst from engulfment of them.

To 0.9 ml. of heparinized blood was added 0.1 ml. of the bacterial suspension. This mixture was rotated in a mechanical rotator at 10 rpm end over end at 38 to 40 degrees Celsius for 30 minutes. All pipettes, test tubes, and stoppers used for the blood were siliconized to prevent destruction of leukocytes by surface contacts. Immediately following the 30-minute incubation period blood slides were made for the phagocytic index determination.

In the second series of experiments, a 24-hour culture was used instead of 36 hours. The 0.1 ml. of bacterial suspension was added to 1.0 ml. of blood instead of 0.9 ml. as in the first series of experiments.

Duplicate slides were prepared for each blood sample and stained with Wright's stain. The bacteria were counted in 50 cells from one of the 2 slides to determine the phagocytic index, the average number of bacteria per leukocyte. Slides from 4 subjects in the first series of experiments were chosen with an average phagocytic index of 12 to 13. The duplicate slides were also read to compare reading 50 vs. 100 cells.

Table 1 shows no significant difference between reading 50 and 100 cells. Also there was no significant difference in the readings from the duplicate set of slides from the first set. To determine the consistency of phagocytic index readings from different areas on the same slide, 3 other slides were re-read. On each slide the bacteria were counted in 50 cells from 3 different areas of the slide. Table 2 shows no significant difference between reading center, upper, or lower margins of each slide.

Paired T-tests were used to compare fasting phagocytic indices with determinations at subsequent time intervals. Also general linear models were used to make comparisons between the different amounts of glucose tested in the first series of experiments and between the different diets of the second series. The model included subject effects as a design variable and the fasting phagocytic indices as a covariate. Diet comparisons were made between diets I and II, I and III, II and IV, III and IV. The null hypothesis was tested for an identical response to all 4 test diets.

Table 1 - Phagocytic index calculated from 50 vs. 100 cells per slide.

Slide #	1 - 50 cells	51 - 100 cells	100 cells
1	12.0	12.4	12.2
2	13.1	14.0	13.6
3	13.7	13.6	13.7
4	12.5	12.1	12.3
5	12.6	11.4	12.0
6	12.8	13.1	13.0
7	13.7	13.2	13.5
8	12.7	13.2	12.9
9	12.5	12.4	13.0
10	13.2	12.0	12.6
11	12.3	12.7	12.5
12	12.5	11.6	12.1
mean	12.9	12.7	12.8
S.E.	0.2	0.2	0.2
S.D.	0.6	0.8	0.6

Table 2 - Comparison of phagocytic index readings from 3 different areas on the same slide.

	Area Read		
Subject	Top	Center	Bottom
C-fasting	22.8	18.9	21.4
F-fasting	10.7	9.4	10.2
J-3 hour	13.5	12.3	15.4
mean	15.7	13.5	15.7
S.E.	3.6	2.8	3.2
S.D.	6.3	4.9	5.6

RESULTS AND DISCUSSION

The original experimental design included only the fasting, 1/2, 2, and 3 hour postprandial samples. However, a preview of the results after the first 2 runs indicated a need for the 4 hour sample. Therefore, for each different amount of glucose tested several subjects missed the 4 hour sample. Also there was a wide variation in individual response to each amount of glucose. For these reasons data are included from only the 6 to 8 subjects who participated in all 5 experimental time periods, even though data from 11 subjects were collected for various of the time periods.

From determinations on the researcher's own blood it was decided to use 20 per cent transmittance for the bacterial suspension, which provided an average fasting phagocytic index of 18. An average of about 15 at fasting is desirable to provide possible differences due to the ingestion of glucose. However, several of the 6 to 8 subjects had fasting phagocytic indices between 6 and 13. This would tend to decrease the potential for per cent decrease of phagocytic index following glucose ingestion.

Tables 3 to 6 show the phagocytic index and per cent decrease in phagocytic index following ingestion of the 5 different amounts of glucose. The fasting values show a high degree of individual variation even though the mean phagocytic index was similar for all fasting groups (see tables 3 to 7). This wide variation was not only between subjects, but subjects varied widely in phagocytic index from one time

Table 3 - Phagocytic index following ingestion of 12.5 gms glucose.[a]

Subject	fasting P.I.[b]	1/2 hour P.I.	1/2 hour % decrease[c]	2 hour P.I.	2 hour % decrease	3 hour P.I.	3 hour % decrease	4 hour P.I.	4 hour % decrease
C	26.3	21.0	20.2	20.4	22.6	18.9	28.4	21.1	20.1
D	9.9	14.6	-47.6[d]	12.0	-21.0	12.2	-22.6	12.3	-23.8
E	12.0	12.1	13.0	11.4	18.6	12.4	11.5	10.9	22.2
F	10.0	11.2	-12.0	11.6	-15.3	10.9	-8.2	15.9	58.6
H	18.5	16.4	11.1	21.4	15.68	14.2	23.5	13.2	28.5
J	16.8	16.5	1.6	13.4	20.2	14.3	14.9	10.9	34.7
K	8.7	7.9	9.2	8.2	6.2	7.5	13.3	6.9	20.2
mean	15.0	14.3	-0.6	14.0	2.2	12.9	8.7	13.0	22.9[e]
S.E.	2.4	1.6	8.7	1.9	7.2	1.3	6.8	1.7	9.3
S.D.	6.3	4.3	23.1	5.0	19.1	3.5	18.0	4.5	24.6

[a]Data only from subjects who participated in all 5 blood-drawings.

[b]Phagocytic index.

[c]Per cent decrease in phagocytic index from fasting levels.

[d]Negative figures indicate an increase in phagocytic index.

[e]Statistically significant at the alpha = .05 level.

Table 4 - Phagocytic index following ingestion of 25 grams glucose.

	fasting	1/2 hour		2 hour		3 hour		4 hour	
Subject	P.I.	P.I.	% decrease	P.I.	% decrease	P.I.	% decrease	P.I.	% decrease
A	6.9	8.9	-29.7	9.4	-37.2	7.1	-3.2	4.6	33.7
B	14.2	13.7	3.1	11.9	17.6	10.8	23.8	10.5	26.1
D	12.6	10.5	16.8	11.0	12.6	10.6	15.9	11.0	12.3
E	15.3	13.3	13.2	15.4	0.7	12.2	20.5	11.3	26.0
H	13.8	14.3	-3.5	11.8	14.5	10.5	24.3	12.9	6.8
J	16.7	15.8	5.6	15.5	7.2	17.2	-2.6	15.0	10.2
K	9.1	8.1	11.2	8.9	2.0	8.9	2.0	6.7	26.7
mean	12.7	12.1	2.4	12.0	2.3	11.0	11.5	10.3	20.2[a]
S.E.	1.3	1.1	5.9	1.0	7.0	1.2	4.7	1.4	3.9
S.D.	3.5	2.9	15.7	2.6	18.6	3.2	12.4	3.6	10.3

[a]Statistically significant at the alpha = .01 level.

Table 5 - Phagocytic index following ingestion of 50 grams glucose.

	fasting	1/2 hour		2 hour		3 hour		4 hour	
Subject	P.I.	P.I.	% decrease	P.I.	% decrease	P.I.	% decrease	P.I.	% decrease
D	20.3	18.1	10.6	20.4	-0.5	15.3	24.5	13.4	33.8
G	14.8	12.4	15.9	10.9	26.0	11.2	24.1	9.9	33.1
H	13.4	10.8	18.9	13.1	1.8	12.6	5.9	10.0	25.0
I	8.7	7.7	10.9	6.2	28.6	6.4	25.9	9.0	-4.2
J	10.4	9.8	5.4	10.8	-4.4	12.0	-15.8	10.3	1.0
K	7.9	9.1	-15.7	8.5	-7.9	10.5	-32.4	6.4	19.7
mean	12.6	11.3	7.6	11.7	7.3	11.3	5.3	9.3	17.9[a]
S.E.	1.9	1.5	5.0	2.0	6.5	1.2	10.0	0.9	6.6
S.D.	4.6	3.7	12.3	4.9	15.9	2.9	24.5	2.3	16.2

[a]Statistically significant at the alpha = .05 level.

Table 6 - Phagocytic index following ingestion of 75 grams glucose.

	fasting	1/2 hour		2 hour		3 hour		4 hour	
Subject	P.I.	P.I.	% decrease	P.I.	% decrease	P.I.	% decrease	P.I.	% decrease
A	6.6	8.7	-31.9	10.2	-55.0	7.46	-13.4	4.9	25.5
B	14.0	11.9	15.2	12.4	11.3	11.3	18.9	10.5	24.9
D	19.4	14.2	27.0	21.3	-9.9	16.4	15.6	13.0	22.9
F	19.6	19.7	-0.2	15.0	23.3	13.5	31.2	14.1	28.0
H	22.0	22.7	-3.2	16.0	27.1	16.3	25.7	16.9	23.2
I	10.1	10.9	-7.7	8.8	13.6	8.4	16.8	9.6	5.7
J	12.0	12.8	-7.0	12.5	-4.4	12.3	-3.0	11.2	6.5
K	10.9	8.4	23.2	7.6	30.7	5.4	50.5	7.4	32.5
mean	14.3	13.6	1.9	13.0	4.6	11.4	17.8[a]	11.2	21.2[b]
S.E.	1.9	1.8	6.8	1.6	9.9	1.4	7.0	1.4	3.5
S.D.	5.4	5.1	19.3	4.5	28.0	4.0	19.7	4.0	9.8

[a]Statistically significant at the alpha = .05 level.

[b]Statistically significant at the alpha = .001 level.

Table 7 - Phagocytic index following ingestion of 100 grams glucose.

	fasting	1/2 hour		2 hour		3 hour		4 hour	
Subject	P.I.	P.I.	% decrease	P.I.	% decrease	P.I.	% decrease	P.I.	% decrease
A	12.6	7.9	37.3	7.9	37.3	8.5	32.0	10.0	20.7
B	8.5	10.6	-24.7	8.1	5.2	8.1	4.7	10.5	-23.3
C	23.9	22.5	5.7	20.5	14.3	18.0	25.5	15.9	33.2
D	19.3	16.5	16.7	20.2	-4.7	16.5	14.3	11.8	39.1
F	17.8	15.6	12.2	13.9	21.6	12.3	30.7	15.6	12.3
H	17.5	14.0	20.1	17.2	1.5	14.9	14.5	22.8	-30.4
J	20.7	22.1	-6.9	16.8	18.9	16.3	21.5	14.6	29.5
mean	17.2	15.6	8.3	14.9	13.4[a]	13.5	20.3[b]	14.5	11.6
S.E.	2.0	2.1	7.5	2.0	5.4	1.5	3.7	1.7	10.5
S.D.	5.1	5.5	19.8	5.2	14.2	4.0	9.8	4.4	27.7

[a]Statistically significant at the alpha = .05 level.

[b]Statistically significant at the alpha = .001 level.

to another. For example in table 3, subject D had a low index of 9.9 and subject J had a high index of 16.7. The reverse of this is seen in table 5, where subject D had a high index of 20.3, and subject J, a low index of 10.3. For this reason the per cent decrease for each subject was included along with the actual decrease in number of bacteria per PMNL.

While certain subjects maintained consistently high phagocytic indices, and others were consistently low, some subjects showed an increased phagocytic index following ingestion of the glucose. Table 8 shows that the average phagocytic index decreased over 20 per cent for the 50, 75, and 100 grams doses of glucose. These findings were statistically significant ($P < .05$) at 2 hours for 100 grams glucose, at 3 hours for 75 and 100 grams glucose, and at 4 hours for 25, 50, and 75 grams glucose.

Tables 9 to 12 show the phagocytic index and per cent decrease in phagocytic index after ingestion of the following 4 test diets:

Diet	gms. CHO[1]	gms. Pro.[2]	gms. Fat	Source of CHO
I. Meal	75	20	10	Cornstarch
II. Meal with glucose	88	20	10	Glucose
III. Glucose	75			Glucose
IV. Glucose, meal[3]	75	20	10	Glucose Cornstarch

[1]Carbohydrate
[2]Protein
[3]The meal was taken 30 minutes following glucose ingestion.

Following ingestion of the meal alone, there was a 9.4 per cent decrease in phagocytic index at 2 hours and 12.2 per cent at 4 hours (see table

Table 8 - Average phagocytic index and per cent decrease in phagocytic index following ingestion of glucose.

	Fasting	1/2 hour		2 hour		3 hour		4 hour	
Gms Glucose	P.I.	P.I.	% decrease	P.I.	% decrease	P.I.	% decrease	P.I.	% decrease
12.5[a]	15.0	14.3	-0.6	14.0	2.2	12.9	8.7	13.0	22.9[d]
25[a]	12.7	12.1	2.4	12.0	2.3	11.0	11.5	10.3	20.2[d]
50[b]	12.6	11.3	7.6	11.7	7.3	11.3	5.3	9.3	17.9[d]
75[c]	14.3	13.6	1.9	13.0	4.6	11.4	17.8[d]	11.18	21.2[d]
100[a]	17.2	15.6	8.3	14.9	13.4[d]	13.5	20.3[d]	14.5	11.6

[a] 7 subjects

[b] 6 subjects

[c] 8 subjects

[d] Statistically significant at the alpha = .05 level.

Table 9 - Phagocytic index and per cent decrease in phagocytic index following ingestion of the meal.[a]

Subject	fasting	2 hour		4 hour	
	P.I.	P.I.	% decrease	P.I.	% decrease
1	10.4	8.4	19.3	5.5	47.2
2	8.7	8.2	5.8	5.6	35.8
3	8.0	8.4	-4.5	10.3	-28.3
4	8.3	8.1	2.4	8.0	3.4
5	9.1	6.6	27.6	10.7	-17.3
6	13.2	10.6	19.8	11.3	14.0
7	11.7	14.6	-25.6	14.4	-23.3
8	7.9	7.7	2.5	5.6	28.6
9	13.7	9.3	32.2	7.8	42.7
10	12.7	10.8	15.1	10.2	19.5
mean	10.4	9.3	9.5	8.9	12.2
S.E.	7.2	7.2	5.4	0.9	8.7
S.D.	2.3	2.3	17.0	3.0	27.7

[a]20 grams protein, 75 grams carbohydrate (from starch), and 10 grams fat.

Table 10 - Phagocytic index and per cent decrease in phagocytic index following ingestion of the meal with glucose.[a]

Subject	fasting P.I.	2 hour P.I.	2 hour % decrease	4 hour P.I.	4 hour % decrease
1	7.5	6.2	17.1	5.8	21.9
2	8.5	8.4	1.6	6.6	22.1
3	12.5	8.7	30.6	10.1	19.4
4	9.7	9.4	2.9	7.3	24.4
5	- -	- -	- -	- -	- -
6	13.1	8.8	32.8	10.2	22.3
7	12.1	15.3	-26.3	14.0	-16.0
8	8.7	6.2	28.3	5.2	39.6
9	11.2	11.3	-7.1	- -	- -
10	12.7	11.4	10.1	12.1	4.7
mean	10.7	9.5	10.0	8.9	17.3[b]
S.E.	0.7	0.9	6.5	1.1	5.8
S.D.	2.1	2.8	19.6	3.2	16.4

[a]20 grams protein, 88 grams carbohydrate (75 grams from glucose), and 10 grams fat.

[b]Statistically significant at the alpha = .01 level.

Table 11 - Phagocytic index and per cent decrease in phagocytic index following ingestion of 75 grams glucose.

Subject	fasting P.I.	2 hour P.I.	2 hour % decrease	4 hour P.I.	4 hour % decrease
1	9.1	6.1	33.7	6.8	25.6
2	6.3	8.1	-28.7	5.9	6.7
3	11.8	9.8	17.2	8.7	26.5
4	9.2	11.1	-20.6	6.6	28.4
5	11.6	10.3	11.2	11.2	3.8
6	14.1	12.8	9.1	11.3	19.8
7	18.0	11.6	35.2	16.3	9.0
8	9.8	9.1	7.0	7.9	19.5
9	13.5	10.9	19.4	9.8	27.5
10	12.3	12.0	2.8	10.4	14.0
mean	11.6	10.2	8.61	9.5	18.1[a]
S.E.	1.0	0.6	6.5	1.0	2.9
S.D.	3.2	2.0	20.58	3.1	9.2

[a]Statistically significant at the alpha = .001 level.

Table 12 - Phagocytic index and per cent decrease in phagocytic index following ingestion of 75 grams glucose 30 minutes before the meal.

	fasting	2 hours 15 min.		4 hours 15 min.	
Subject	P.I.	P.I.	% decrease	P.I.	% decrease
1	6.4	5.8	9.1	6.4	-0.3
2	8.5	5.2	39.1	7.7	9.2
3	12.5	9.3	25.6	10.2	18.6
4	8.7	7.3	16.3	10.2	-17.4
5	12.3	7.7	37.6	10.4	15.2
6	11.7	12.1	-3.2	11.7	-0.2
7	19.6	17.3	11.8	14.5	26.2
8	9.4	8.4	10.6	9.1	3.6
9	13.7	9.7	29.1	8.6	37.3
10	8.6	9.3	-7.7	11.6	-34.8
mean	11.2	9.2	16.8[a]	10.2	5.7
S.E.	1.2	1.1	5.0	0.7	6.6
S.D.	3.8	3.5	15.9	2.2	20.9

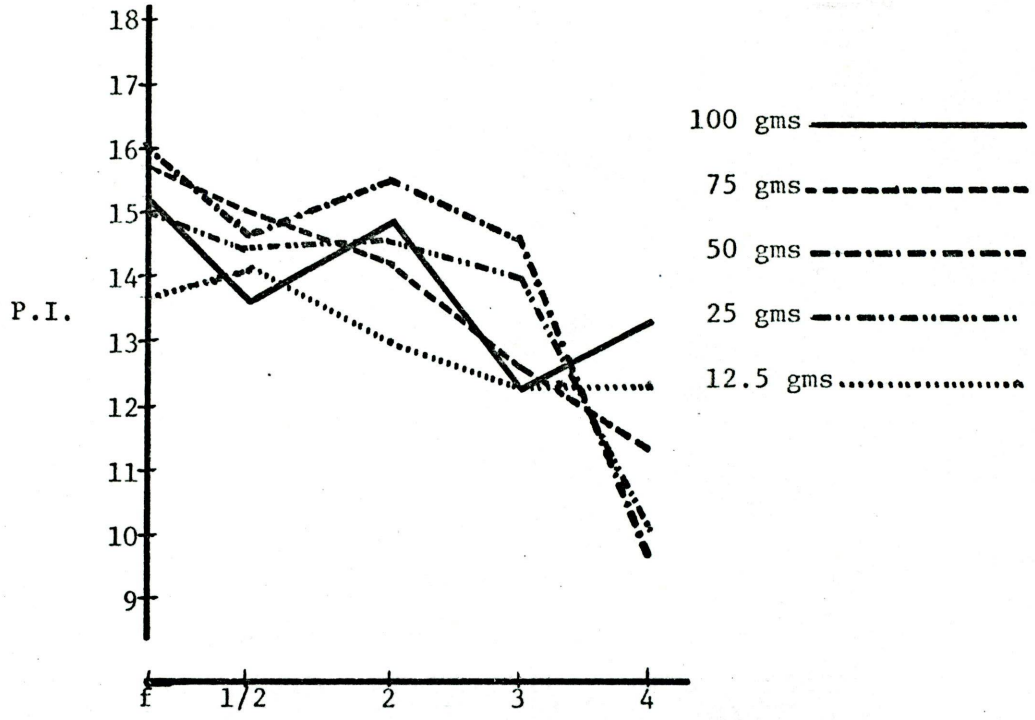
[a]Statistically significant at the alpha = .01 level.

8). For the meal with glucose, the phagocytic index decreased 10.0 per cent at 2 hours and 17.3 per cent at 4 hours (see table 10). Following ingestion of 75 grams of glucose alone, there was a decrease in phagocytic index of 8.6 per cent at 2 hours and a statistically significant decrease ($p < .001$) of 18.1 per cent at 4 hours (see table 11). The ingestion of the meal 30 minutes after ingestion of the glucose reduced the phagocytic index by 16.8 per cent at 2 hours 15 minutes, and 5.7 per cent at 4 hours 15 minutes (see table 12). This was statistically significant ($P < .01$) at 2 hours 15 minutes. As in the first series of experiments, there was a wide variation in the individual subject's response at fasting and 2 and 4 hour samples.

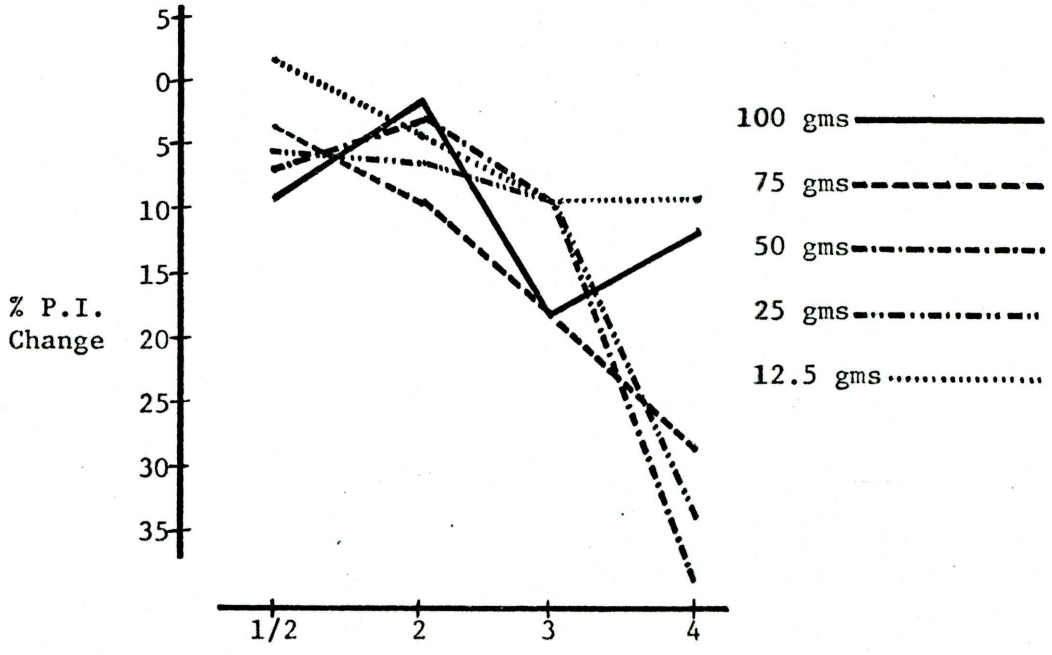
A general decrease in phagocytic index from fasting levels is observed in both the first and second series of experiments (see graphs 1 through 8), although this decrease is not strongly related to the amount of glucose or the diet ingested. The responses to the various diets and levels of glucose were not significantly different from each other.

The variations found in the present study are due to some factors yet unknown. Duffy, Phillips, and Pellegrin (1973) have observed large intraindividual variations in blood glucose levels from time to time in normal subjects. Variables shown to affect the blood glucose levels include posture, nausea, anxiety, and exercise. Also, arterial blood glucose values may be 20 to 70 milligrams per cent higher postchallenge (Duffy et al., 1973). Previous studies have found a significant decrease in phagocytosis at 2 hours following ingestion of various sugars even in the presence of these possible variations (Sanchez et al., 1973), using

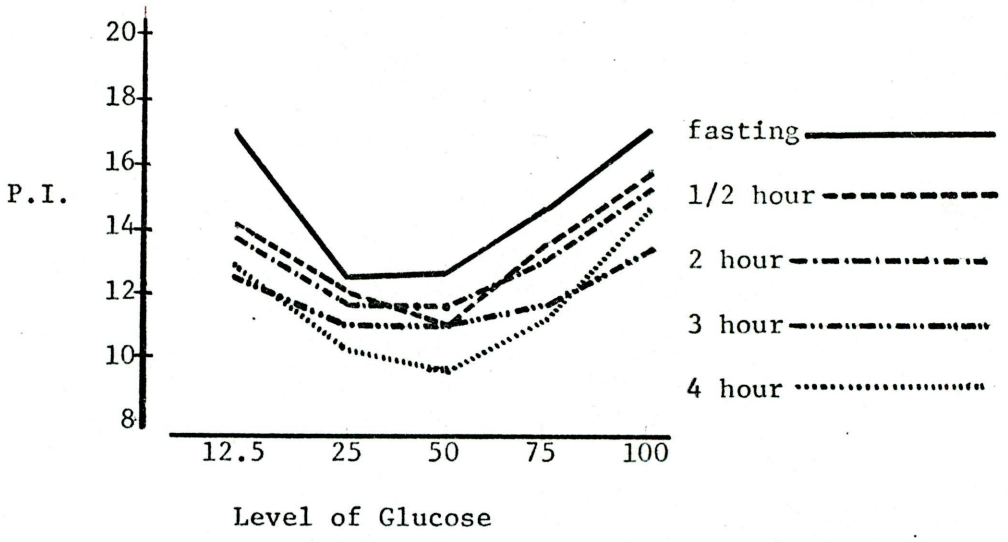
Graph 1 - Phagocytic index following ingestion of 5 levels of glucose.



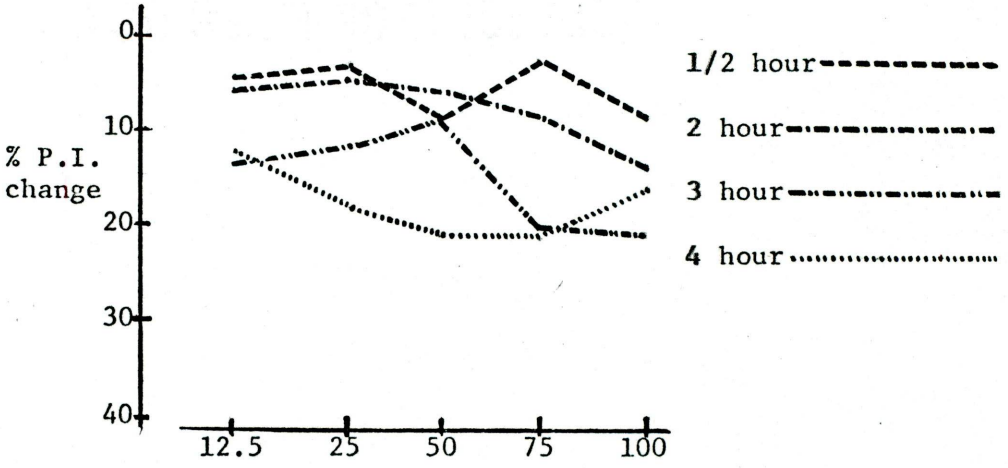
Graph 2 - Per cent change in phagocytic index from fasting levels following ingestion of 5 levels of glucose.



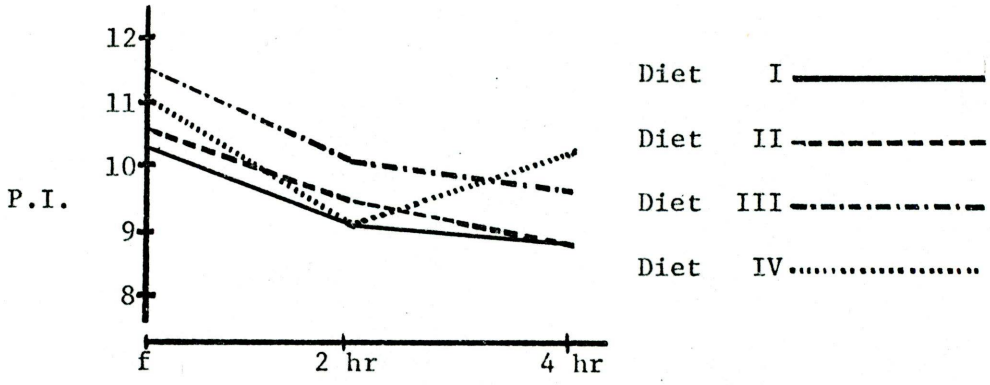
Graph 3 - Phagocytic index at different time intervals following ingestion of glucose.



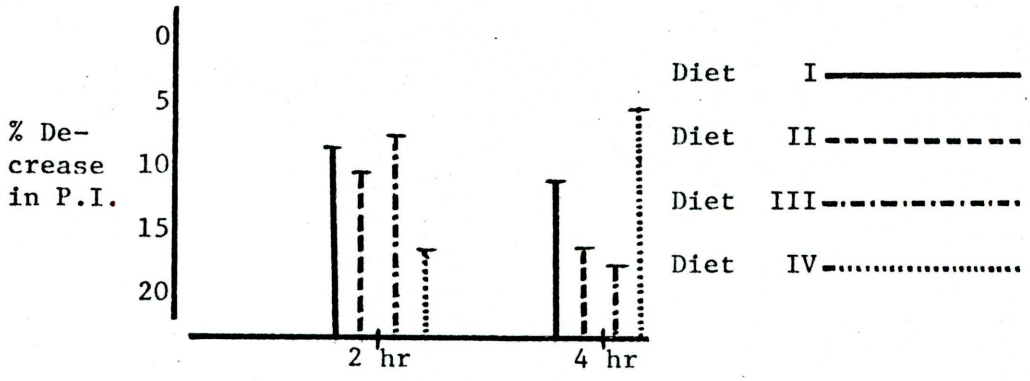
Graph 4 - Per cent change in phagocytic index at different time intervals following ingestion of glucose.



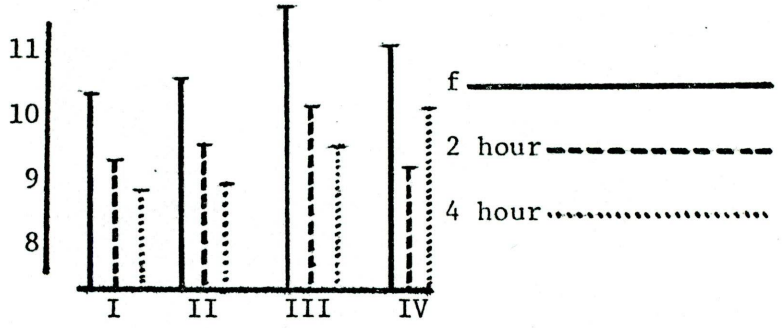
Graph 5 - Phagocytic index following ingestion of 4 different test diets.



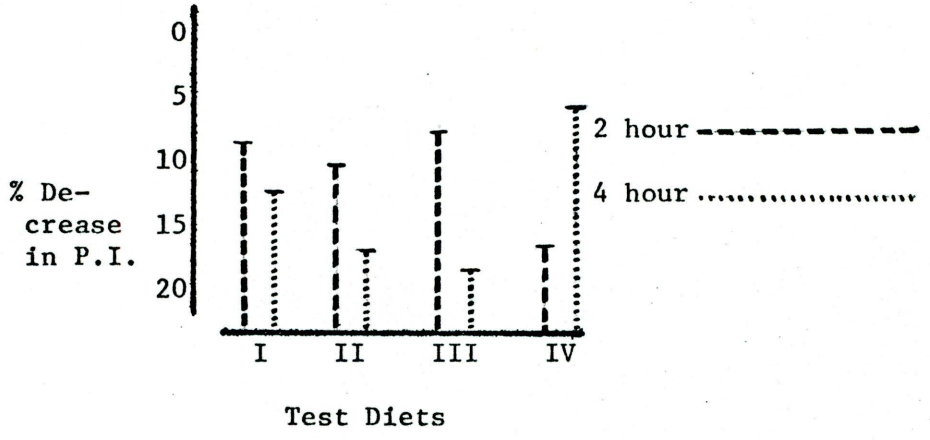
Graph 6 - Per cent decrease in phagocytic index following ingestion of 4 different test diets.



Graph 7 - Phagocytic index at fasting, and 2 and 4 hours following ingestion of 4 test diets.



Graph 8 - Per cent decrease in phagocytic index at fasting, and 2 and 4 hours following ingestion of test diets.



the same slide technique.

After the first series of experiments were run, it was discovered that subject A had taken a depressant drug the night before one of the test runs, and reported that she had not been feeling well. Also, subject I was found to be type IV hyperlipoproteinemia. Although these subjects showed low fasting phagocytic indices, this did not differ widely from results for some of the other normal subjects.

Since a number of subjects exhibited a low fasting phagocytic index subsequent experiments were designed to increase the ratio of bacteria to leukocyte, thereby increasing the fasting values for all subjects and hopefully decreasing the wide variations in fasting values.

Table 13 shows the fasting and 2 hour phagocytic index of 12 outpatients taking a 100 gram dose of glucose. Using a bacterial suspension of 8 per cent transmittance, the fasting phagocytic indices were higher, averaging 16.3, and showed less variation than in all the previous data. However, the 2-hour sample showed no significant decrease in phagocytic index compared to fasting levels. Normal healthy subjects were likewise tested. Table 14 shows the fasting and 2 hour phagocytic index of 6 subjects, again using 8 per cent transmittance for the bacterial suspension. The 2-hour postprandial phagocytic index showed no significant decrease from the fasting index of 27.6. The effect of glucose at 1 hour postprandial was also tested. Table 15 shows no significant difference between the fasting, 1, or 2 hour samples.

Reports (Sanchez et al., 1973; Kijak et al., 1964) indicate that glucose ingestion decreases phagocytosis. The present study shows no correlation between glucose ingestion and phagocytosis. The first two

Table 13 - Phagocytic index of 12 hospital out-patients following ingestion of 100 grams glucose.

Subject	Fasting	2 Hour
A	13.6	12.2
B	13.9	12.9
C	18.6	18.0
D	13.0	- -
E	12.0	10.9
F	13.9	11.0
G	19.3	16.4
H	18.5	16.0
I	16.1	15.5
J	21.0	18.6
K	17.4	16.7
L	18.6	15.6
Mean	16.3	15.2
S.E.	0.8	0.8
S.D.	2.9	2.7

Table 14 - Phagocytic index of 6 healthy subjects following ingestion of 100 grams glucose.

Subject	Fasting	2 Hour
A	24.8	22.6
B	32.4	28.7
C	30.9	25.7
D	27.8	27.1
E	30.0	27.1
F	21.0	23.6
Mean $\pm$ S.D.	27.6	25.8
S.E.	1.8	0.9
S.D.	4.3	2.3

Table 15 - Phagocytic index of 5 hospital out-patients following ingestion of 100 grams glucose.

Subject	Fasting	1 Hour	2 Hour
A	22.7	19.1	19.0
B	14.3	15.5	13.0
C	16.3	17.6	20.0
D	15.6	14.2	16.9
E	12.4	12.2	14.6
Mean	16.3	15.7	16.7
S.E.	1.7	1.2	1.2
S.D.	3.9	2.7	2.9

of the present experiments showed no significant decrease in phagocytic index following glucose ingestion. However, the average fasting phagocytic index was probably too low to provide an adequate test of phagocytosis. The third group of experiments did demonstrate a consistently high fasting phagocytic index of about 16, but again no decrease in the phagocytic index following ingestion of glucose. Methodology or some other unknown factors have caused the lack of correlation between this study and previous research.

Subsequent experiments were designed to determine the sensitivity of the methods used in the present study. Calcium, known to stimulate phagocytosis at relatively low concentrations (Tunnicliff, 1931; Stossel, 1973), was added to various samples of blood from 9 healthy subjects in 3 test runs. Two tests involved fasting subjects, and one involved non-fasting subjects. The phagocytic index was measured using an 8 per cent transmittance for the bacterial suspension. Table 16 shows a decreased phagocytic index with addition of EDTA, but the decrease was not statistically significant with the number of subjects used in each group tested. With the addition of EDTA plus calcium, the phagocytic index averaged the same as for the control samples, as was expected. The average fasting phagocytic index for group 2 was much lower than anticipated, probably not providing an adequate test of phagocytosis. Each group contained too few subjects to make any conclusions concerning the sensitivity of the technique used in this study.

Five fasting and 5 2-hour postprandial slides from the previous study (Sanchez et al., 1973) were read in order to compare present data with previously recorded data. Table 17 shows no significant decrease

Table 16 - Phagocytic index of blood with in vitro additions of EDTA, $CaCl_2$, and concanavalin A (Con A).

Subject	Control	0.5 mg. EDTA	0.5 mg. EDTA + .055 mg. $CaCl_2$
		Group 1	
A	23.9	16.3	3.6
B	36.3	30.1	30.1
C	24.7	5.3	22.3
Mean	28.3	17.2	18.7
S.E.	4.0	7.2	7.9
S.D.	7.0	12.5	13.6
		Group 2	
D	11.5	4.6	10.5
E	9.4	7.9	9.2
F	12.0	10.8	14.0
Mean	11.0	7.8	11.3
S.E.	0.8	1.8	1.4
S.D.	1.4	3.1	2.5
		Group 3	
G	20.4	1.4	16.7
H	20.1	23.0	24.3
I	17.5	19.3	18.6
Mean	19.3	14.6	19.9
S.E.	0.9	6.7	2.3
S.D.	1.6	11.6	4.0

Table 17 - Phagocytic index on 5 subjects as determined from reading slides of the previous study.

Sugar	Subject	Fasting	2 Hour
Glucose	B	17.2	10.5
Fructose	C	17.6	16.8
Honey	D	16.8	14.4
Surose	E	18.6	14.4
O.J.	F	18.8	19.2
Mean		17.8	15.1
S.E.		0.4	1.4
S.D.		0.9	3.2

in phagocytic index 2 hours following ingestion of various sugars. However, the previous data on these same slides did show a significant decrease at 2 hours (see table 18). There was no significant difference between the fasting phagocytic indices of present and previous observations, but the 2-hour phagocytic index was significantly lower in the previous study as compared to present readings. This difference in the 2-hour readings could account for the significant decrease in phagocytic index noted in the previous study which has not been found in the present study.

The technique of slide reading used in the previous study appears to differ from the technique used in the present study. In the former study, it appears that slides were read with the reader's knowledge of the subject, the carbohydrate ingested, and the time the sample was taken (fasting, 1/2 hour, 1 hour, etc.). All slides in the present study, and in this investigation of the previous study, were read without the reader's knowledge of the subject, the amount or kind of diet ingested, or the time the sample was taken. The researcher believes the more objective reading technique to be preferable, especially with the particular difficulties encountered in counting Staphylococcus epidermidis, which commonly occur in pairs. The total of only 10 slides read in this investigation provides insufficient evidence for a final conclusion concerning the accuracy of previous data.

Since both Kijak et al. (1964) and Sanchez et al. (1973) have reported a significant decrease in phagocytosis following ingestion of sugar, then it is possible that the technique used in the present

Table 18 - Phagocytic index on 5 subjects as reported in the previous study.

Sugar	Subject	Fasting	2 Hour
Glucose	B	20.6	9.5
Fructose	C	15.4	9.1
Honey	D	16.0	11.7
Surose	E	14.5	8.3
O.J.	F	19.6	10.7
Mean		17.2	9.9
S.E.		1.2	0.6
S.D.		2.7	1.4

study was inadequate to provide correlating results. The sensitivity of the methods used have not been adequately tested. It is well recognized that variations can occur in bacteria (Browning and Mackie, 1949; Smith, 1969). If in the present study a strain of Staphylococcus epidermidis was used which differs from that used in previous studies, this could be a possible reason for the lack of similar response to phagocytosis which is observed in this study. However, if the strain is the same, there is need for further research into the lack of correlation between present and previous studies.

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LOMA LINDA UNIVERSITY

Graduate School

THE EFFECT OF DIET ON NEUTROPHILIC PHAGOCYTOSIS

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ABSTRACT

Two series of experiments were conducted in order to determine the effects of diet on neutrophilic phagocytosis. The first series was designed to determine the effects of varying amounts of oral glucose on phagocytosis. Blood samples from 6 to 8 subjects were drawn at fasting, and at various time periods following ingestion of 12.5, 25, 50, 75, and 100 grams of glucose. In the second series of experiments, blood samples were taken from 11 subjects after an overnight fast and 2 hours following ingestion of one of 4 different liquid diets, which included the following: the meal (I), consisting of protein, fat, and carbohydrate from a starch source; the meal with glucose (II), consisting of protein, fat, and carbohydrate mainly from glucose; 75 grams of glucose only (III); 75 grams of glucose followed 30 minutes later with the meal (I). Following a 30-minute incubation and rotation of all blood samples with bacteria, slides were prepared with Wright's stain to determine the phagocytic index, the average number of bacteria engulfed per leukocyte.

For the first series of experiments, statistical analysis revealed a significant decrease in phagocytic index 2 hours following ingestion of 100 grams glucose, 3 hours following ingestion of 75 and 100 grams glucose, and 4 hours following the 25, 50, and 75 grams glucose. In the second series of experiments significant decreases in phagocytic index were observed 2 hours 15 minutes following ingestion of the glucose and meal of diet IV, and 4 hours 15 minutes following ingestion of diet III, 75 grams glucose. Although 4 of the tests showed a statistically

significant decrease in phagocytic index from fasting, the responses to the various diets and levels of glucose were not significantly different from each other. Also, there were wide variations in phagocytic index from one subject to another. In these experiments, the average fasting phagocytic index was low, possibly not providing an adequate test of phagocytosis.

Additional experiments were run in order to decrease the wide variations in the previously determined phagocytic indices. This was done by increasing the fasting phagocytic index as a result of increasing the bacteria to leukocyte ratio. Although the variations between samples were decreased, there was no significant decrease in phagocytosis 2 hours following ingestion of 100 grams of glucose.

Since previous work (Sanchez et al., 1973; Kijak et al., 1964) has reported a significant decrease in phagocytic index following ingestion of glucose, two approaches were taken. First, the sensitivity of the present technique was tested by using compounds that decrease phagocytosis (EDTA) and increase phagocytosis ($CaCl_2$), using 9 subjects. There was a decreased phagocytic index with the addition of EDTA, although it was not statistically significant with the few subjects involved. The control and the EDTA plus calcium samples showed the same average phagocytic index, as expected. Too few subjects were involved to make a final conclusion concerning the sensitivity of the present technique.

The second approach was an investigation of data from previously reported studies (Sanchez et al., 1973). Several slides from these studies were re-read and were compared with the phagocytic indices previously reported. A significantly lower 2-hour postprandial phagocytic index was reported in the previous study than was observed in

the present reading of the same slides. Too few slides were read to make a final conclusion concerning the accuracy of these data.

As it is recognized that variations can occur in bacteria (Browning and Mackie, 1949; Smith, 1969), possibly the Staphylococcus epidermidis used in these experiments differs from that used in previous work, causing the observed difference in response to phagocytosis. However, if the strains are the same, then there is a need for further research into the lack of correlation between the present and previous studies.