THE TOTAL AND DIFFERENTIAL LEUCOCYTE COUNTS IN COLLEGE WOMEN

THE EFFECT OF HIGH FAT INTAKE ON THE LEUCOCYTE

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INTRODUCTION

The blood plays a dominate role in the living body as a fluid tissue consisting of corpuscles suspended in plasma. Its main function is to serve as a medium or carrier through which all the raw nutritive materials and eliminated cellular products may be transported to and from the individual cells. To fulfill this function, the cells and the serum may act independently or in collaboration, and the transported material is distributed according to its ability to diffuse. The corpuscles function like other tissue cells in preserving a constant lipid composition, characteristic of that particular group of cells, but not characteristic of only that species. The composition of the serum tends to be a variable and is characteristic of the species.

The lipids are present in the blood as colloidal aggregates, except possibly for phospholipids. The passage of the lipids in and out of the blood stream is a more complex process than is that of other food materials due to their insolubility.

The blood and lumph are believed to transport fatty substances about in the body. After fat hydrolysis in the small intestine and resynthesis in the walls of the intestine, it is assumed that about sixty per cent of the fat is absorbed via thoracic duct into blood stream. It is proposed that the rest must be absorbed directly into the portal circulation.

It has been found that in normal human blood the total lipid content of the white cells is four times greater than in the plasma; therefore, it is possible that they have an important role in fat transportation.

Studies show that there is a normal lipid level in the blood plasma

in man, and this level is variable, differing both with individuals and with nutritional factors, so that care must be taken in interpreting analytical results. The white cells tend to be more constant in composition than plasma, not only in individuals of the same species but in different species. The composition also varies with the amount of fat consumed by the individual. A normal individual may have values that differ widely from the average. Lipids in the blood of individuals from different geographic areas vary. For instance, the races of the tropics tend to have a lower lipid value than races of the temperate zone.

In view of the fact that the blood is an important means of fatty acid transportation, and the leucocytes are probably concerned in the process, this study was undertaken to observe the effect of high fat intake upon the total and differential white blood counts. A fat staining procedure was also used to observe the distribution of fat in the cells.

In order to notice any changes that may occur in the blood on an experimental diet, it was necessary first to determine what the average blood picture is for the group from which subjects were chosen. Furthermore, as total and differential white cell counts are considered of some diagnostic value, it is important to have additional information about the amount of variation that may occur in these values under normal conditions in women who are in average health.

The purposes of this study, then, are as follows:

(1) To determine average total and differential white blood counts in thirty randomly selected college women.

(2) Using subjects selected from the group in (1) to determine;

(a) The amount of variation that will occur in the values from one individual to another.

(b) The individual variation that may occur in these values as determined weekly over a period of six weeks.

(3) To observe any effect that a diet high in fat may have on;

(a) The total leucocyte count,

(b) The differential leucocyte distribution, and,

(c) The fat content of the cell.

(4) To develop a stain technic for the observation of fat distribution in the white blood cells.

REVIEW OF LITERATURE

A study of the effect of diet upon the leucocyte necessitates some knowledge of the morphology and physiology of this cell under normal conditions and a knowledge of the normal blood picture. A large portion of this literature review, therefore, will be concerned with a consideration of present knowledge of the nature and function of this particular type of cell. The material for this literature review has been obtained partly from text sources.

The discovery and early history of the white blood cells are associated with the earlier knowledge of the connective tissues, for very little study was made on the circulating cells. Although Jones (1846) distinguished the leucocytes as a finely granular cell, and Schultze (1865) noted the cells in fresh blood preparations, Ehrlich (1879) was the first to clearly point out the morphological characteristics of the leucocyte.

From these early beginnings, the study of the blood cells and the study of their roles in physiological processes have continued. At the present time, the average blood picture is quite well defined, and variations from this average are considered diagnostically significant in clinical examinations.

NORMAL BLOOD PICTURE

The plasma makes up 50 to 60 per cent of the blood by volume. Plasma consists of about 90 per cent water. Of the 9 per cent of solids which are present in the blood, some 7.5 per cent are due to proteins

(albumin, globulin, fibrinogen, prothrombin). Firbrinogen plays a specific role in blood coagulation, and the proteins of the blood maintain the water balance between the blood and the tissues. Carbohydrates in the form of glucose constitute about .07-.08 per cent of the blood. There are fat and fat-like substances constantly appearing in the circulating tissue in varying qualities. Salts, nitrogenous waste products, enzymes, hormones and antibodies are also found in the blood.

The solids of the blood make up 40 to 50 per cent of the blood. The erythrocyte, forming a large portion of the solids, is made up of protoplasmic material (stroma) which encloses the oxygen-carrying pigment, hemoglobin. This pigment accounts for more than three quarters of the total solids.

White cells or leucocytes, of which there are several varieties, are larger in size than the erythrocytes and possess a nucleus. They also possess the power of ameboid movement, whereby they can leave the cells and wander into surrounding tissues.

Blood platelets, or thrombocytes, are believed to be of importance in the blood coagulation. They are round oval disks, and have a diameter of about one third that of the erythrocytes.

Since part of this study was carried on using the albino rat as an experimental animal, it is also necessary to point out the differences between the blood picture in this animal and that found in man.

The neutrophilic granulocytes of the rat blood differ from those in the human blood in having two types of nuclei; the pycnotic ringed nucleus and the polymorphic nucleus. The eosinophiles differ in the rat by having

an annular or ring-like nucleus and in containing spherical acidophilic granules. The basophilic granulocytes also differ by having the ringed nuclei.

The agranulocytes are similar to those of the human blood, but there is an unknown type of cell. Pathologically, an increase in the number of lymphocytes is associated with an increase in the unknown cell.

Table 1. Comparison of Rat and Human Blood Cellular Counts per cu. mm.

CELL	HUMAN BLOOD		RAT BLOOD	
	Range	Average	Range	Average
Total Erythrocytes in (M)	4.5 - 5.0	4.75	6.1 - 11.0	7.5
" Platelets in (M)		0.50		1.2
" Leucocyte Count in (T)	5 - 10		9.0 - 13.0	11.0
Neutrophiles in (%)	62 - 75	68.0	16 - 25	28.0
Eosinophiles	2 - 4	3.0	1 - 3	1.5
Basophiles	0 - 1	0.5	0 - 1	0.5
Lymphocytes, Small	20 - 25	23.0	60 - 70	62.0
Lymphocytes, Large	3 - 5	4.0	5 - 10	7.0
Monocytes	1 - 2	1.5	0 - 2	1.0

Note: (M) - Millions; (T) - Thousands; (%) - Percentages

VARIATIONS IN THE BLOOD PICTURE

The importance of blood analysis and the significance of the variations from the normal have been realized in clinical diagnosis for a

long time. Normal variations of the blood occur in an individual each day. There may be a 200 to 500 leucocyte increase in an individual during the day. Although the normal individual has a variable blood picture, this variation is not so great as the variation between species.

There is a slight sex difference in the blood picture; the total erythrocyte count is 4.5 million in women and 5 million in men, but the total leucocyte count is expected to be only slightly lower for women than for men.

Seasonal and environmental influences cast some light on a varying blood picture, however, this has had little study.

The influence of fatigue has been studied by Goldberg and Lepskaja (1931), and they concluded that muscular and mental work affected the white blood count. They found that the neutrophile count increased to 82 per cent after heavy work, and that continuation of such work produced lymphopenia. Immature leucocytes appeared in the blood. This neutrophilic change was caused by waste products of the activity. They also concluded that mental activity had a greater influence.

Macy I. G. (1936) and others noted through their observations that the composition of the blood followed a pattern to some extent during different stages of lactation. During gestation there are definite changes in the blood concentration of calcium, amino acids, urea, and total non-protein nitrogen and some change in lipid content.

An adequately balanced diet has a profound influence on the maintenance of a normal blood picture.

WHITE BLOOD CELL

Granular Leucocytes

Granulocytes contain granules elaborated in their cytoplasm which are in the same form in any given cell, but are distinctly different in the various classes of granulocytes. Granular leucocytes have a characteristic nucleus, spherical and compact in the young cell, but lobated in the mature cell. The nucleus of the mature granulocytes, constricted into a varying number of lobes, is quite different from the spherical, slightly indented or kidney-shaped nucleus of other white cells. The most convenient classification of the granulocytes is based upon a combination of the morphology and staining reaction of the granules.

Neutrophiles. Approximately two thirds (60 to 70 per cent) of the circulating white blood cells in man are of the type designated as the polymorphonuclear neutrophiles. It may be described as a differentiated end-cell, without power of reproduction, characterized by an horseshoe shaped or S shaped nucleus, and by fine granulation of its cytoplasm.

Functionally, the salient feature of the polymorphonuclear neutrophile is its power of ameboid movement and of phagocytosis. Neutrophiles also furnish a proteolytic enzyme, opsonin, which is capable of destroying bacteria. Thus, neutrophiles become of great importance in the disposal of necrotic tissue and removal of inflammatory fibrin through the action of its proteolytic enzyme. Neutrophiles may play a role in blood coagulation, for it is possible that they may yield a thrombokinase.

The bone marrow throughout extrauterine life is the natural source of the erythrocytes, the granulocytes and the blood platelets. The agranulocytes utilize the vascular bed as an avenue of distribution, but rarely arise in the bone marrow. Longcope (1906) and Sabin (1928) showed that the lymph nodes, spleen and diffuse connective tissues are the normal sites of agranulocyte development.

Morphologically, the commonly accepted limits of variation in diameter of the polymorphonuclear neutrophiles are nine and twelve micra as given by Schultze (1865). The cytoplasm seems to be a fluid endoplasmic protoplasm enclosed by a somewhat denser outer layer. It contains specific granules centrosome and centrosphere mitochondria, vacuoles and Golgi apparatus. The nucleus of the normal neutrophile is distinct from that of any other body cell. It is termed polymorphous to describe the cell as polynuclear and also to indicate the great variability of the structure in different cells in the same drop of blood. The lobes range from one to five connected by bridges of loops. Arneth (1904) called attention to the nuclear formation in the neutrophiles. The relative age of the cell may be judged by the increasing subdivisions of the nucleus. Sabin (1923) states that in 95 per cent of the cases the total number of leucocytes in the peripheral circulation may vary between 5000 to 6000 per cubic millimeter.

A sharp rise in neutrophile counts, and an increase in the number of the cells (leucocytosis) occurs in man as a result of a great variety of infections. An extreme example, is the leucocytosis of gangrene in which the count rises to 60,000 or 80,000 leucocytes per cubic millimeter with 90 per cent neutrophiles.

Leucopenia, diminished number of circulating leucocytes, especially neutrophiles, may be found under a variety of conditions. Among the causes of a leucopenia may be noted the infections of virus diseases and the direct action of drugs such as benzol.

The characteristic variations in the leucocytes in certain conditions may be of distinct value in determining the type of disease, its severity and progress.

In the majority of acute inflammatory processes, those caused by pyogenic microorganisms, the leucocytes present characteristic variation, type and structure in different stages of the disease.

In mild infections, a bone marrow hyperplasia is adequate for meeting the demand of the neutrophilic leucocytes to maintain the circulating cells at levels above normal range.

Coincident with the increase in neutrophiles, in infections, there is a decrease in other cells, particularly eosinophiles which frequently disappear from the blood stream completely. As the infection subsides, the number of neutrophiles decreases with a corresponding increase of other cells.

Eosinophiles. Compared to the neutrophile, the second class of granulocytes, the eosinophile, is slightly larger and is found in much fewer numbers in the circulating blood, only 2 to 4 per cent. It has less motility and phagocytic ability. Sabin (1933) states they can move as rapidly but not for so long a period. The outstanding characteristic of this cell is the acidophilic nature of the few large granules. In unstained condition the granules are yellowish or greenish in color, but stained with Wright's stain the granules become brilliantly red which

accounts for their name.

The functions of the Eosinophile are concerned in: their intimate relationship with intestinal secretory activity; their hormonal importance upon proper cellular activity; and in their functioning in the metabolism of iron (Petry, 1908). It is generally believed that eosinophiles possess a detoxifying agent.

Morphologically the eosinophiles are spherical cells, and their nuclei present a simpler configuration then do those of the neutrophiles. Their nuclei are either bilobed or trilobed and the plump nuclear lobes are connected by broad or extremely delicate protoplasmic connections. Mitochondria (minute particles of protoplasm of living cells believed to be bearers of heredity qualities in the cell) are present in some eosinophiles.

In pernicious anema, a typical nucleus is encountered with four or five lobes, also in eosinophile hyperleucocytosis. Eosinophilia occurs in trichinosis more than in any other disease. It has been recorded as high as 80 per cent. Clinically, eosinophilia may be a postinfective phenomenon. Allergic conditions, many skin diseases, and scarlet fever all produce eosinophilia. During menstruation eosinophilia is a normal physiological response. Foreign proteins are the chief factors in the production of eosinophilia. Hypoeosinophilia and sometimes aneosinophilia may occur in acute cases of infection or intoxication.

Basophiles. These granulocytes average about 0.5 per cent of the white corpuscle count. The nucleus is elongated, usually bent in the form of an S, and provided with two or more constrictions. The basophile

leucocyte has a looser and paler chromatin network than the eosinophile and does not contain any nucleoli. Investigations have failed to observe phagocytosis in these cells. The basophiles are difficult to distinguish because of their small numbers and the properties of their granules.

Clinical and pathological observation may show under what conditions basophiles react, but no definite evidence as to what they accomplish when they do react can be given, therefore, we have little knowledge concerning basophiles.

Cytologically, it is generally believed, basophiles have specific forestages in the bone marrow. Morphologically, they exhibit variations in cytoplasm and nucleus, in staining reactions, and in the solubility of their granules. The granules are stained mostly by basic dyes.

Clinically, basophiles are like eosinophiles, they disappear from the peripheral blood stream during acute inflammation, and in recuperative stages younger forms appear. Elephantiasis favors the accumulation of mast cells as does any stagnation of the lymph stream. Anent skin diseases, there is a slight increase of mast leucocytes. In infectious diseases, they may disappear entirely, while in ameliorated influenza young nucleated forms occur. Basopenia may be noticed in the blood in leucocytosis, chlorosis, polycythemia vera, and secondary anemia, but in pernicious anemia, lymphatic leucemia, and myelogenous leucemia, the basophiles remain characteristically low.

Non-granular Leucocytes

The non-granular leucocytes of the blood are divided into two main

classes, the lymphocytes and the monocytes.

Lymphocytes. These cells are characterized by having a spherical nucleus which may partially fill the cell in large lymphocytes and completely fill the cells in the small lymphocytes; the cytoplasm appears as a thin border around the nucleus. Approximately 20 to 25 per cent of the total number of colorless corpuscles of the blood are lymphocytes.

The lymphocyte function in the blood is unknown. As large numbers of lymphocytes are always found in the epithelium of the gastro-intestinal tract, however, it is usually states that the lymphocytes leave the body through this passage.

Practically all investigators agree that the blood lymphocytes of adult mammals arise in the lymphatic tissue. Most of the lymphocytes arise homoplastically, that is, through mitosis of pre-existing lymphocytes. Small lymphocytes of the peripheral blood vary in size from 4 to 6 micra. The large lumphocytes, at 8 micra, are the size of the red corpuscle or slightly larger.

The cytoplasm contains one or several highly refractile droplets shown to be lipid in nature. In the large lymphocyte the amount of cytoplasm is greater and stains somewhat paler than in the small lymphocyte. The cytoplasm also contains a number of mitochondria, varying in size and usually adjacent to the nuclear membrane. Wiseman (1934) claims that the degree of basophilia of the cytoplasm is an accurate indication of the age of the lymphocyte. That is, the younger the cell the more basophilic its cytoplasm. Clinical hematologists consider the smaller lymphocytes older cells.

The nucleus shows an irregular structure; a nucleolus can occasionally be seen. The nuclear membrane is usually indented in one or more places.

It was once believed that the lymphocytes were not able to move. However, numerous investigators have disproved this theory. The number of small lymphocytes that appear in the blood is about 20 per cent and the number of large lymphocytes approximate 3 to 8 per cent of the leucocytes.

Monocytes. These cells are larger mononuclear and transitional leucocytes. They are larger than the erythrocytes, and are usually slightly larger than the neutrophile leucocytes, but they may be about the same size as the larger eosinophiles, 10 to 11 micra in diameter in the spherical form. Some of the monocytes appear with rounded or only slightly indented nuclei, and usually have relatively more cytoplasm than the larger lymphocytes. It is in this group that one has the greatest difficulty in classifying the cells, for they resemble lymphocytes. Sabin (1925), Simpson (1922) and others believe the monocyte nucles shows more finely distributed arrangement of the chromatin.

At present there is little question that the monocyte migrates into inflamed tissues and hypertrophies there becoming a large macrophage. They hypertrophy first into large phagocytes and eventually into fibroblast-like cells. They also play, according to Maximow and others (1925), a role in the formation of the epithelioid tissue of tuberculosis.

There are numerous theories as to the origin of the blood monocyte. Most of them point to the reticulo-endothelial system as the origin.

The monocyte cytoplasm, unstained, shows fine granules which are a distinguishing feature between monocytes and lymphocytes. The cytoplasm of the monocytes is usually pale blue when stained with eosin-azure. The wondering monocytes in the peripheral blood constitute the 'macrophages' and are regarded as specialized derivatives of the fixed cells. It is generally accepted that 3 to 5 per cent of the blood leucocytes of man are monocytes.

LIPIDS IN THE BLOOD

Manner in which Lipids enter the Blood

The hydolyzing lipase and the emulsifying bile change the fats into products that are absorbed through the villi of the small intestine. There are several theories regarding the real absorption path of fat. It may be mostly by the lymphaticovenous communications and to lesser extent by way of direct passage into capillaries of the villi in the form of finely emulsified substances. This has been a well established belief. However, later authorities tend to emphasize the role of the capillaries in fat absorption as do Hawley and Carden (1944).

Absorbed fat reaches the blood in the form of tiny globules called 'chylomicrons'. These increase during fat absorption. Chylomicrons are fat droplets of about one micron in diameter and their properties indicate that they are surrounded by a protective protein layer which behaves like a mixture of albumin and globulin. The protein acts thus as a

protective colloid.

The rate of fat absorption varies; however, the greatest over all absorption of fats into the blood stream has been found three to six hours after ingestion.

Plasma Lipids

The lipid content of the plasma shows considerable individual and species variation, but the variation in the same individual is not so great as that between different species. There appears to be a definite relation between plasma lipid levels and the amount of fat habitually taken in diet. In all animals whose food contains considerable fat, a higher content of plasma cholesterol and phospholipid is found. Bloor (1943) states that there is a reasonable inference that these two lipids are concerned with the transport of the fat in the plasma and probably with its metabolic changes in the tissues.

The nature of the fat in both plasma and corpuscles in normal animals is as follows; neutral fat in small amounts; and phospholipid, lecithin and cephalin in relatively large amounts. In the plasma, lecithin predominates; in the corpuscles, cephalin and sphingomyelin make up most of the phospholipid. Cholesterol in the corpuscles is almost entirely in the free form; in the plasma 60-70 per cent of it is found as an ester.

Lipemia, a milky appearance of plasma due to high lipid content, is believed to be a failure to remove fat from the blood. In persistent lipemia, the content of the corpuscles is not markedly higher than normal, (Bodansky, 1931).

Definite variations in the lipid content of plasma are found in infections in which there is certainly a fall in blood cholesterol and probably in phospholipid as well. In menstruation there is a fall in cholesterol esters. In diabetes the blood lipids are higher than the normal range. The plasma lipids are characteristically high in nephrosis. The fall in cholesterol in infections may be connected with the immunity processes, since the level comes back to normal again on recovery. The high values in cases of diabetes may probably be due to the large use of fat, which would bring about an increase both in the phospholipid and cholesterol content.

Cellular lipids

An examination of the literature upon the subject of lipids of the leucocyte reveals the fact that little information is available. In order to study the lipid content of the leucocyte, there must first be a knowledge of the lipid content of the plasma and its relationship to the cellular lipids.

There is general agreement that the corpuscles of warm blooded animals are of quite constant composition, not only in individuals of the same species but in different species. In other words, corpuscles seem to be like tissue cells in having a characteristic composition

The corpuscles tend to preserve their characteristic lipid composition (Bloor, 1943). New Corpuscles formed after result of severe hemorrhage may be an exception, for their compositions may vary. In these, the lipid content is high, as it is in young cells in general.

Boyd's (1933) study on the lipid content of the white blood cells

of normal women as compared with human plasma pointed out that the total lipid content of the white cell is four times greater than that of the plasma. His study also shows that the free cholesterol and the phospholipid content was four or more times greater in the leucocyte than in the plasma and twice as great as in the red cells.

It was found that the white blood cells resemble tissue cells in phospholipid content. Cholesterol ester and phospholipid content are reciprocal in their variation.

The participation of the corpuscles in the transportation of absorbed fat was found by Bloor (1915) and later confirmed by Bodansky (1931). They noted that the relative increase of total fatty acids in the corpuscles is much greater than the increase in the plasma. They pointed out also there was a greater increase of lecithin and cholesterol esters in the corpuscles than the plasma. Bergel (1921) ascribed lymphocytosis to the presence of excess lipids in the blood. Vahlquist (1931) presented findings contrary to those of Bloor and Bodansky in examination of separate corpuscles by a method different from that used by Bloor and Bodansky, found no changes in the corpuscles during fat absorption. It is quite possible, however, the difference in method may account for this observation.

The blood corpuscles as lipid transporters were studied by Artom (1933), who injected dogs intravenously with emulsions of iodized fats. At intervals of time, the amounts of iodized lipids found in the liver, plasma, and corpuscles were determined to find any increase of iodized lipids in the blood, notably, in the corpuscles. The acetone-insoluble

iodine was markly higher in the corpuscles than in plasma. Later Artom and Peretti (1933) in their experiments found definite amounts of iodine in the acetone precipitate of the corpuscles. These findings demonstrate that exogenous fatty acids are partly introduced in the phospholipid molecule, but show direct evidence that blood corpuscles are largely concerned in transportation of ingested or mobilized lipids, (especially phospholipids) as was first suggested by Bloor.

Chiwachi (1933) found an increase in the cholesterol esters of the corpuscles greater than the increase in the plasma. The ratio of the cholesterol esters to free cholesterol rose from the fasting value of 0.18 to that of 2.0 to 2.5 during cholesterol absorption.

Comparison of White Blood Cells and Plasma Lipids

Compared with human blood plasma, the white cells contain approximately three to four times as much total fatty acids. The white blood cells have a much higher percentage of the total fatty acid present as phospholipid fatty acid, and, conversely, a lower percentage as cholesterol ester fatty acids. In contrast to human plasma, the leucocytes contain 50 to 100 per cent more cholesterol, about the same mean percentage of combined cholesterol and about four times as much free cholesterol. As a result, cholesterol esters compose a much higher percentage of the total in plasma than in leucocytes.

Bloor (1923) and others have emphasized that there tends to be a constant relationship between the various constituent lipids of the plasma and the cells. It has been concluded that under controlled conditions the

variations in the lipid rations were identical with the variations in the lipids used in each control experiment.

Williams and Maynard (1934) found some indication that blood lipids were influenced by the amount of fat in the diet. In the plasma of lactating goats fed a diet of extremely low fat content, the total lipids, phospholipids and total and free cholesterol decreased gradually, and again increased when fat was added to the diet.

Since the role of the leucocyte in the absorption and transport of fat has not been clearly defined, this study was designed to point out certain blood pictures which might indicate, in part, the role of these cells in the use of fat.

EXPERIMENTAL PLAN

In this study the total and differential leucocyte counts of thirty college women were first taken to determine the average picture. Data such as age, weight, height, recent illness, stage in the menstrual cycle and remarks were kept. These records were made to observe any relationship that might exist between the blood total and differential leucocyte counts and these factors.

Four women were chosen from the group of thirty for further experimentation. After a regular, normally balanced meal, the total and differential leucocyte counts were made and micro-staining of lipid granules in the leucocytes was done. Using the same subjects, similar tests were made following a meal of high fat content.

Because it was possible to feed rats for longer experimental periods than could be used for human subjects, a similar study was made using six young adult albino rats. They were fed Steenbock V (the stock diet) for one week. At a given time after meals on the fifth and seventh days, total and differential leucocyte counts were made. From these counts, averages for these rats were obtained. The rats were then placed on a high carbohydrate diet for one week succeeded by one week of the Steenbock V ration. A high fat diet for one week supervened by one week of Steenbock V ration was then fed. Each week was followed with the same counting procedures as those used in the first week.

At the close of the week of high fat diet feedings, the total plasma lipids were determined and the micro-staining technic of lipid granules in the leucocyte was completed on three of the rats. The same

determinations were made on the last three rats following a final week of Steenbock V ration.

BIOLOGICAL METHOD

Human Subjects

Selection. The randomly chosen college women who served as experimental subjects for this study were living in the dormitories at the Virginia Polytechnic Institute during the winter and spring of 1945. The subjects used on the experiment had maintained average good health for considerable length of time.

Diet Fed. The thirty subjects used in the determination of the standard leucocyte counts were consuming the meals as prepared in the dormitory. The four subjects chosen from this group had the same diet, with the supplementary feedings of four tablespoons of butter.

The high fat diets fed to the subjects consisted of the following percentage compositions; first day, 12.5 per cent protein, 47.5 per cent carbohydrates and 40 per cent fat; second day, 11.5 per cent protein, 49.5 per cent carbohydrates and 39 per cent fat. The total calorie content of the first day's meal was 1,278 and that of the second day was 1,249.

Experimental Animals

Selection of Animals. In this study, female rats bred from the stock colony belonging to the Home Economics Nutrition laboratory were used. They were from 137 to 147 days of age and weighed from 167 to 230 grams. They were the second generation rats, born of parents raised on the Steenbock V stock diet.

Care of Rats. The rats were caged in pairs. Their weights were recorded daily and their food consumption was carefully checked. There was an average consumption of 11.8 grams per rat per day.

Diet Fed. A modification of the Steenbock V ration as used in the Nutritional Laboratory of Iowa State College was fed.

Steenbock V Rat Ration

Yellow cornmeal	64.0 grams
Crude Casein	5.0 "
Linseed Meal	16.0 "
Ground Alfalfa	2.0 "
Sodium Chloride	00.5 "
Calcium Carbonate	0.5 "
Brewers Yeast	1.5 "
Irradiated Yeast	0.5 "
Wheat Germ	10.0 "
	100.0 grams

The high fat and carbohydrate diets were figured on percentage bases, using as nearly as possible the individual pure foodstuffs. The diet was planned to include all of the known nutritional elements. In the high fat diet, lard was used, while dextrose was used in the high carbohydrate diet. The diets were prepared weekly and carefully stored in the refrigerator to prevent rancidity. The rat is very sensitive to even slight evidence of rancidity, so only one day's food supply was placed in the food cups at each feeding. Securely fastened cups permitting practically no loss of food were used; therefore, food consumption could be determined with considerable accuracy. The rats were fed each of the individual control diets for a period of one week.

Table 2. Composition of Special Diets Fed Rats

Constituents	High Carbohydrate			High Fat		
	grams	per cent	Calories per gram	grams	per cent	Calories per gram
Casein	100	20	.8	100	20	.8
Glucose	300	60	2.4	200	40	2.4
Lard	0	0	.0	100	20	1.8
Brewer yeast	50	10	.5	50	10	.5
Cod liver oil	5	1	.09	5	1	.05
Wheat germ oil	20	4	.36	20	4	.36
Wheat germ	25	5	.36	25	5	.35
Total	500	100	4.51	500	100	6.16

Adequate minerals and vitamins were added to the diets to meet the requirement of rats.

HISTOLOGICAL PROCEDURE

Sampling Procedure

Human Blood. The blood was collected in a paraffin cup from a freely flowing cut secured by a puncture of the finger tip with a spring lancet. The lancet and finger were cleaned with 70 per cent alcohol before making the puncture. The first drop of blood was discarded. These collections were made at regular times after meals on the four subjects, but always at least two hours after a meal on the

thirty women.

Rat Blood Samples. The animals were placed in a box stanchion with tail freely accessible. The blood was obtained from the tip of the tail by cutting with a razor blade after the tail had been rubbed gently with xylene to dilate the veins. The first drop was always discarded. When a generous flow was not obtained, the tail was stroked from base to tip, but was not squeezed. The collection was made in a paraffin cup. The tip of the tail was seared with a hot metal to check bleeding after the sample was obtained. These collections were made approximately three hours after feedings on the fifth and seventh days of a particular dietary.

Determination of Total and Differential Leucocyte Counts

Total Counts. Standardized blood-counting pipettes and doubled-counting chamber, a Spencer (Bright-Line) Haemacytometer, were used for the leucocyte cell determinations. Blood was drawn to a calibrated mark in the pipette and diluted 1:20 with diluting fluid. The pipettes were shaken uniformly, and air bubbles were carefully avoided when filling the pipette. This diluted sample was permitted to stand for one or two minutes. The first two or three drop from pipette were discarded before placing a drop on the surface of the counting chamber.

A one per cent solution of glacial acetic acid was used for the diluting fluid in making the leucocyte counts. The counts were made by counting sixteen square millimeters of the counting chamber each time. When 1:20 dilution was used, the estimate of leucocytes could be obtained by adding two ciphers to the number of cells counted.

The average of two leucocyte counts was recorded from each sample of blood taken in each of the cases.

Differential Counts. The differential counts were made with the use of commercially prepared Wright's stain. A drop of blood was placed on a slide. With the edge of another slide held at a 60 degree angle, the drop of blood was pulled slightly back and then pushed forward. Care was taken to spread evenly and gently, in this manner, a thin film of blood. This was permitted to air dry. The preparation was covered with a measured quantity (in drops) of Wright's stain one-half to one minute. Next, distilled water was added to the staining fluid in a quantity equal to that of the stain. This mixture was allowed to remain two or three minutes, according to the intensity of the stained that was desired. Eosinophilic granules were best brought out by briefer staining. The slide was gently washed in distilled water until a thin film of a pinkish tint was left. The slide was permitted to air dry and counts were made.

Micro-staining Technic of Lipid Granules of Leucocyte

A thin film of blood was spread on a carefully cleaned slide with a cover slip held at approximately an angle of twenty five degrees to the slide. The film was permitted to air dry at about thirty six degrees centigrade for one-half to one hour or longer. The film was covered with Sudan III stain and kept at 56 degrees centigrade for five minutes. The slide was washed with 40 per cent alcohol for one minute or less. It was then thoroughly washed in a gentle stream of distilled water approximately one to two minutes. The preparation was counter-

stained with Weigert's iron hematoxylin for thirty to sixty seconds, and rinsed with a gentle stream of distilled water. This was followed by differentiating in acid alcohol for two to three seconds, and was washed in a stream of gently flowing distilled water for about one minute. This preparation was permitted to air dry at approximately thirty six degrees centrigrade, and was then covered with glycerin to prevent the rapid fading of the stain, followed by immediate microscropic observation.

This is a short, simple and reliable micro-staining method for lipid granules in the leucocyte with Sudan III as developed by Basich (1937).

Microscopic Dark-field Observation

Although it was not part of the basic plan of the study, in order to obtain another blood picture, light and dark field examinations of fresh blood droplets were made. If a fresh drop of blood from an experimental animal or human subject was quickly observed with the ordinary microscope, using an oil immersion objective, erythrocytes, leucocytes and occasionally the thrombocytes were clearly seen. Faint glimpses here and there of the fibrin filaments, and minute dancing granules were observed.

When the same or a similar preparation was placed under the dark-field microscope with oil immersion objective, the picture was strikingly different. The field became blackened and the various bodies seemed to shine by their own light. The erythrocytes appeared as if they had white rims, the leucocytes looked like tiny pop-corn balls,

and many of them clearly showed amoeboid movement, if the preparation was made immediately. The fibrin, when seen, resembled a winding cob-web and on some slides was plainly visible.

The most distinguishing difference between the light-field and the dark-field microscopic pictures was the multitude of small, brilliant, dancing particles.

In a preparation made of blood from an individual who had fasted a twelve hour period, there were considerably fewer granules than after an ordinary meal. The blood seemed to be literally alive with these particles after the high fat meals, in which large portions of butter were fed. These differences were very marked.

OTHER PROCEDURES

Plasma Lipid Content Determination

This method employed an application of the volumetric principles described by Allen (1933). A special Allen fat test bottle was used in this test.

Three milliliters of well mixed plasma were pipetted into test bottles, while a very gentle suction was attached to the reading neck of the test bottles to draw the sample into the bulb of the bottle as rapidly as it flowed from the pipette. Five milliliters of alkaline reagent were added to the sample in the same manner by allowing air to be drawn gently through the mixture. Tightly stoppered filling necks prevented any sample from being forced back out of this opening during the digestion process at 85° to 90° C. The fat was liberated by

digestion of the blood plasma with the alkaline reagent which dispersed the protein and phospholipids into a molecular state.

After centrifuging for five minutes hot distilled water was added by hypodermic needle until the liquid was raised well into the reading neck. This was followed by two minutes of centrifuging to remove air bubbles that might have been present in the fat column. More hot liquid was added followed by two minutes of centrifuging if the fat column failed to enter in the reading neck adequately. The centrifugal force separated the fat to allow it to become visible in the reading neck of the U shaped test bottle.

A small wooden plug was inserted into top of the filling neck of the test bottles and the samples were then placed into the bath at 60° C for five minutes. The purpose of the wooden stopper was to prevent the fat column from shifting to the opposite neck of the test tube. The fat was measured volumetrically in the calibrated neck of the specially designed test tubes. Dividers were employed in measuring the length of the fat column. To insure accuracy in reading measurement was made from the top of the upper meniscus to the top of the lower meniscus of the fat column.

Securing Blood by Cardic Puncture

To secure sufficient blood plasma for the blood fat determination it was necessary to obtain all available volumn of blood from the rat by the heart puncture method.

The rat was injected with nembutal subcutaneously in the abdomen below the diaphragm. After ten to fifteen minutes the rat became

desensitized. Immediately a ventral incision was made through the abdominal and thoracic region. The heart was exposed and the twenty gauge needle was inserted in the left ventricle from which the blood was obtained. As the first drops of blood appeared at the hub of the needle, the piston of the syringe was drawn upward to obtain all available blood and to prevent the escape of the blood into the tissues. This whole operation was done very rapidly. A saturated solution of sodium oxlate was used as anticoagulant. After obtaining the blood it was centrifuged to secure the plasma.

RESULTS OF STUDY WITH HUMAN SUBJECTS

OBSERVATION OF THE NORMAL

Average Leucocyte Counts in Thirty Subjects

Total Leucocyte Counts. The mean value for the total leucocyte count of the group of thirty women, as presented in Table 4 is 6,785. This average obtained in the current study is comparable to that which Sabin (1923) found. He indicated that 95 per cent of the white cell counts clustered near 5000 to 6000 rather than near 10,000 as some investigators seem to generalize in their statements.

Differential Leucocyte Counts. The average differential leucocyte counts found by other investigators compared to those obtained in this study may be found in Table 3.

Table 3. A Comparison of the Differential Blood Counts Found by Different Workers

Investigators	Neutrophiles	Eosinophiles	Basophiles	Monocytes	Lymphocytes
Schilling (1929)	63	3	1	6	23
Bunting & Downey (1938)	62--75	2--4	0.5	4--6	20--25
Arneth (1930)	64	3.5	0.45	2.0	29.0
Lineberry (1946)	65.5	3.0	0.7	2.0	28.3

Table 4. Thirty Womens' Total and Differential Leucocyte Counts

Subject	Neutrophile	Eosinophile	Basophile	Lymphocytes	Monocytes	Total Count
I	60	3	0	37	0	6500
II	65	5	2	25	3	5900
III	60	4	1	31	3	6300
IV	68	2	2	27	0	8100
V	60	3	1	34	2	7100
VI	65	5	0	30	0	7800
VII	60	2	0	30	4	6200
VIII	58	6	1	34	1	8400
IX	72	3	1	23	2	6200
X	63	2	1	22	2	7100
XI	60	2	0	35	2	4800
XII	60	1	1	34	4	10000
XIII	64	4	1	31	0	6300
XIV	75	3	2	21	1	6700
XV	75	3	1	20	1	7900
XVI	69	10	2	19	1	5100
XVII	60	2	0	36	2	7400
XVIII	58	2	1	38	1	6800
XIX	59	3	0	33	3	3800
XX	66	4	1	26	3	8200
XXI	66	4	1	28	1	5100
XXII	60	1	1	34	4	4900
XXIII	62	4	0	33	1	7100
XXIV	74	2	0	25	0	6200
XXV	75	2	1	19	2	6200
XXVI	60	3	1	28	4	5000
XXVII	78	4	0	14	4	7400
XXVIII	75	2	0	21	1	6400
XXIX	75	1	1	22	1	7900
XXX	62	2	1	33	2	6600
Mean Value	65.5	2.7	1.2	28.4	1.7	6786
Standard Deviation	7	0.8	0.25	5	0.5	1184

Table 5. Date Recorded on Human Subjects

Subjects	Age	Weight in pounds	Height in inches	Percentages	
				Over-weight	Under-weight
I	27	107	62		11.5
II	24	114	66		15.5
III	19	132	65		
IV	20	170	68	18.8	
V	20	118	65		7.9
VI	24	128	62	4.8	
VII	28	132	64	3.1	
VIII	22	122	65		4.6
IX	19	108	65		15.6
X	20	107	60		20.0
XI	12	117	63		4.2
XII	20	134	63	9.1	
XIII	19	117	63		4.2
XIV	23	108	63		11.8
XV	21	147	64	15.2	
XVI	21	105	68		25.0
XVII	19	120	62	0.9	
XVIII	20	140	68		
XIX	19	122	63		
XX	20	140	66	5.8	
XXI	21	140	68		
XXII	28	128	66		5.1
XXIII	23	112	63		9.4
XXIV	20	135	63		9.6
XXV	20	120	63		1.6
XXVI	21	112	62		5.9
XXVII	21	126	65		1.5
XXVIII	22	138	64		10.0
XXIX	18	132	67		2.9
XXX	19	114	65		11.5

* Calculated from the tables published by the Life Extension Institute as given by Chaney and Ahlborn (1943)

It will be noticed that the results in this study are comparable to those established as normal by other workers.

A consideration of the figures for total and differential leucocyte counts of the thirty women brings out a few factors worthy of comment. The highest total leucocyte counts were found in persons who were overweight. The increase in total counts is not proportional, however, to increasing weight throughout the whole group. The two highest leucocyte counts were due to tooth extraction and a cold.

From Table 4 it will be noted that Subject XXIX, who is anemic, had no basophiles present. Three counts were made for this subject and no basophiles were recorded in any of these counts. Subject XXVII shows 14 per cent lymphocytes which is considered a low percentage.

Subject XVI revealed a high eosinophilia and the recorded remarks indicated that this person was extremely allergic to milk. Ringoen (1937) found foreign proteins in the blood stream to be the chief cause of eosinophilia. Other allergic conditions may also result in eosinophilia.

Variation and Average Leucocyte Counts in Four Subjects Over a Period of Six Weeks

In Subject I the average total and differential counts are cited in Table 6. It will be noted that the total count range is from 5,300 to 7,700. This increase was due to a cold which the subject developed. An increase was noted in the total count as the cold developed, and in the differential count a "shift to the left" was notices. Arneth (1904)

traced the "shift to the left" or polynuclear formation and showed that it was due to an increase in the number of the neutrophilic cells. Thus, the subject had a higher neutrophilic average as well as an increase in total count.

Subject II was without a cold during the study and seemed to have a slight increase in the total leucocytes count during the menstrual period. The same was true with Subject III. However, Subject IV failed to show this trend. The grouping of the neutrophiles according to the appearance of their nuclei was not significant in Subjects II, III, and IV. The age of the subject had no apparent effect upon the leucocytes in either phase of the study.

In an observation of all four cases, eosinophilia was noted during the menstrual period. In subject III, it will be noted from Table 6, the condition was quite pronounced. The average total leucocyte counts in all four subjects over the period is comparable to that of the average as has been established in the past. It will be noted that even without the influence of any special factor such as a cold the total count may vary. These variations or fluctuations may be pointed out when the subject remains in a normal state of health. In such a study a range or variation may be influenced by a number of factors such as the menstrual cycle, gestation, lactation, nutritional status and the ability of the individual to produce the cells.

Table 6. Total and Differential Counts Recorded on Four Subjects for Six Weeks Period

Subject	Weight in pounds	Height in inches	Age	Leucocytes: Polymorphonuclear Neut.	Eosin.	Baso.	Lympho-cytes	Mono-cytes	Total Leucocyte counts
I	114	66	24	74	4	0	24	0	5300
				75	2	0	20	3	5900
				81	3	1	12	3	7400
				76	3	0	23	2	7700
				60	2	0	36	2	6700
				66	4	2	25	3	6100
				72	2.6	0.5	23.9	2.1	6516 Ave.
II	107	62							
			27	67	6	1	29	2	6900
				73	3	0	23	1	6300
				59	3	1	32	2	5400
				56	5	0	36	3	5500
				63	3	0	32	1	5600
				55	2	2	37	2	6400
				62.1	4.3	0.66	30.4	1.9	6016 Ave.
III	128	62	24	50	11	2	36	1	6700
				53	6	0	39	2	6300
				57	4	1	37	3	5800
				71	3	0	23	2	5900
				81	3	1	14	3	8100
				60	6	1	31	3	6900
				62	5.5	0.9	31.2	2.5	6616
IV	132	64	28	65	4	1	29	1	6100
				68	6	0	25	2	5900
				54	3	1	37	3	7500
				62	4	0	33	1	7100
				61	4	1	33	2	7000
				58	3	1	38	3	6600
				61.3	3.9	0.6	32.4	2.0	6666 Ave.

Table 7. Average Total and Differential Counts as Recorded on Human Subjects on Experimental Diets.

Subject	Weight in pounds	Age	Leucocytes: Polymorphonuclear % Neut.	Eosin.	Baso.	Lympho-cytes %	Mono-cytes %	Total counts	
I	131	28	64.5	2.5	.0	29.0	3.0	6350	
II	120	24	54.0	2.5	1.0	35.5	2.0	6050	
III	127	24	64.0	4.0	.5	28.0	2.0	5850	
IV	118	20	71.0	2.0	.0	31.5	1.5	5100	
	(Standard	Diet)	63.3	2.7	.37	31.2	2.1	5837	Average
I	131	28	56.5	2.0	.5	39.5	2.0	7450	
II	120	24	48.0	2.5	1.0	56.0	2.0	7500	
III	127	24	49.5	3.0	.0	45.0	1.5	7000	
IV	118	20	49.5	3.0	.5	43.5	3.0	5650	
	(High Fat	Diet)	50.8	2.8	.5	43.4	2.1	6900	Average

VARIATION IN COUNTS MADE ON FOUR SUBJECTS WHILE ON EXPERIMENTAL DIETS

Total Leucocyte Count. The subjects were given a balanced diet with an excessive amount of fat added, making approximately a 40 per cent fat intake. The total leucocyte counts were taken three to four hours following the meal. The counts were greater in all cases. This was a rise in the average total count from 5,800 to 6,900, as may be seen in Table 7.

Differential Leucocyte Count. The most significant variation in blood counts of the subjects fed a high fat diet was the decided increase in lymphocytes in all cases, with the exception of one. This subject had a smaller increase, which may be accounted for by the incomplete food consumption.

Downey (1938) states that the cytoplasm of the lymphocyte has highly refractile droplets which are lipii in nature. Since, from the current study there was an increase in the lymphocytes, a relationship between these factors seems likely.

Table 8. A Comparison of the Average Blood Counts of Albino Rats as Found by Various Investigators.

Investigator	No. of Cases	Age in days	Leucocytes: Polymorphonuclear % Neut.	Eosin	Baso.	Lympho-cytes %	Mono-cytes %	Unknown cells %	Total counts
Jelly 1909*	?	?							10,000
Rivas 1914*	4	full-grown							11,450
Falconer 1926	10	*	24.8	7.3	?	56.0	14.0	28.2	10,291
Adams Shevket 1929	6	180	33.5	2.8	0.2	45.4	1.2	17.8	12,540
Lineberry 1946	6	110	32.4	3.2	7.2	48.2	2.8	17.1	11,680

* These investigations were cited by Donaldson (1924).

RESULTS OF STUDY WITH EXPERIMENTAL ANIMALS

OBSERVATION OF THE NORMAL

Average Leucocyte Counts in Rats on Standard Diet

From Table 8 it will be noted that the total leucocyte counts made in this experiment, agree with those found by previous investigators. The total leucocyte count range in this study was 8,500 to 14,100 with an average of 11,680, while Adams and Ahevket (1929) found a range of 7,800 to 17000 with an average of 12,540.

Average Differential Leucocyte Count on Rats on Standard Diet

The average differential leucocyte count on the six Albino rats of this study are close to those obtained by Falconer (1926), and Adam and Shevket (1929). However, a slightly higher basophile average was noted in this study than those given in Table 8. Donaldson (1924) reported a slightly higher basophile count than do the later investigators. Donaldson cited, from his observation and those of others, that the percentage value of the polymorphneuclar neutrophiles was approximately one-half that of the small lymphocytes in the normal rat picture.

RESULTS OF FEEDING ON EXPERIMENTAL DIETS

Total Leucocyte Count

High Carbonhydrate Intake. An examination of the results of Table 8 reveals only a moderate change in the total leucocyte count during the

high carbohydrate diets. There was a slight decrease from the values obtained while the animals were on the standard diet. It may be accounted for by the lack of sufficient water intake of the experimental animals while on this particular diet.

From Chart I, comparison of results obtained when rats were on the high carbohydrate diet with that of the standard display reveals a slight variation in the average total count percentage.

High Lipid Intake. The average total count obtained during the high fat feeding period is definitely increased over the value obtained while the animals were fed the standard diet. In each individual case there was a marked increase in the total count after a high fat meal, as was also true in human subjects.

Differential Leucocyte Count

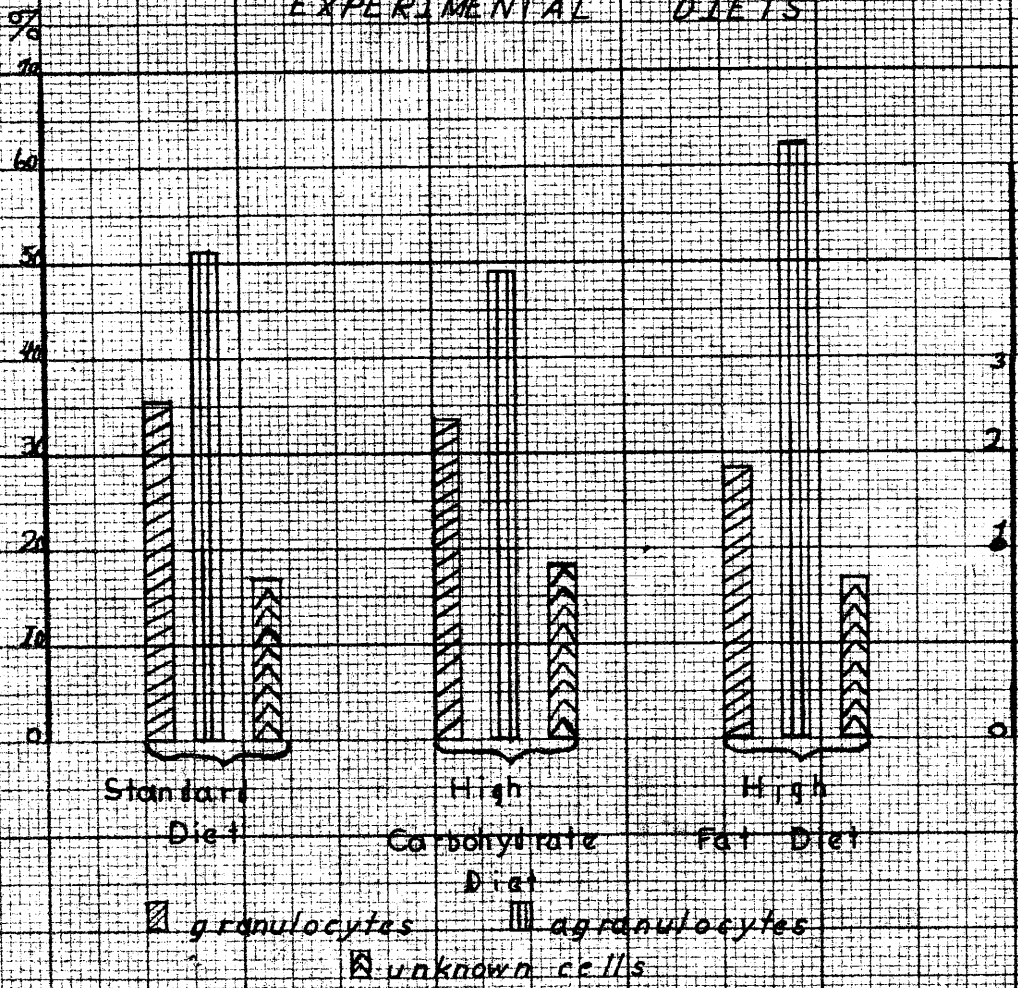
High Carbohydrate Intake. Table 9 shows a rather close agreement between differential counts obtained when the rats were on a high carbohydrate or on the standard diet. Chart I shows very little variation in the granulocyte and agranulocyte percentages as determined in the two periods.

High Lipid Intake. Observation of the differential counts resulting after a high lipid intake (Chart I) displays the fact cited by Donaldson (1924) that granulocyte percentages are approximately one-half that of the agranulocytes.

It is also interesting to note that the unknown cells present in rat blood made no significant change during any dietary period.

CHART I

RAT AVERAGE CELLULAR COUNTS DURING EXPERIMENTAL DIETS



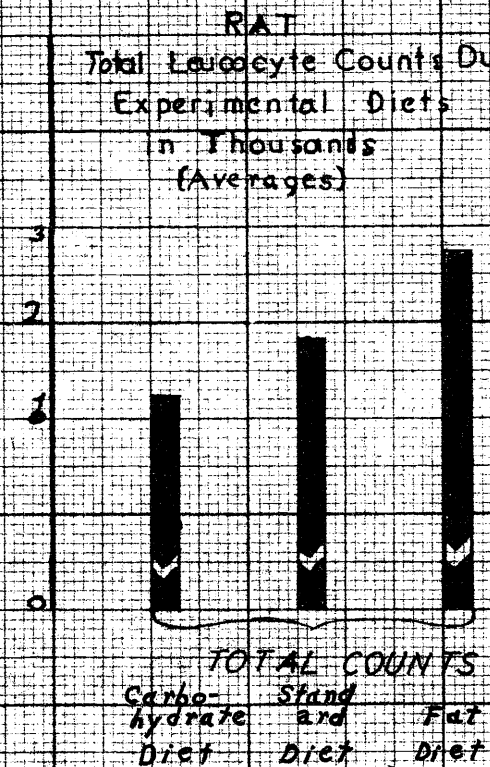


Table 9. Average Total and Differential Counts as Recorded on Six Albino Rats while on Experimental Diets.

Rat No.	Weight in grams	Age in days	Dietary	Leucocytes: Polymorphonuclear % Neut.	Eosin.	Baso.	Lympho-cytes %	Mono-cytes %	Unknown cells %	Total counts	
24	170	109	fat	29.0	2.5	.5	56.0	3.0	9.5	12,350	
12	194	109	"	28.0	2.0	.5	57.5	2.5	9.5	15,150	
14	172	109	"	23.5	2.0	.5	58.0	2.5	22.0	12,500	
56	230	119	"	24.0	6.0	.5	54.5	5.5	15.0	13,650	
22	194	109	"	24.8	2.5	0	57.5	2.0	14.5	11,250	
54	167	119	"	26.0	1.5	0	59.5	3.0	15.0	11,550	
			fat	25.2	3.0	.42	56.5	3.0	11.9	12,733	Average
24	170	109	CHO*	25.5	3.5	1.0	49.0	1.0	20.0	11,900	
12	194	109	"	33.5	4.0	0	45.5	3.5	13.0	13,750	
14	172	109	"	26.5	4.0	0	42.5	4.5	2.5	11,500	
56	230	119	"	34.0	6.0	.5	48.0	2.5	10.5	11,800	
22	194	109	"	25.0	5.0	0	46.0	5.0	15.0	9,900	
54	167	119	"	30.0	4.0	.5	39.0	4.0	20.0	9,050	
			CHO	29.9	4.5	.33	44.5	4.5	17.5	11,272	Average
			Standard	32.4	3.2	.72	48.2	2.8	17.1	11,866	Average

* CHO an abbreviation for Carbohydrate

Variation in Ratio of Gain in weight in Rats

The gain in weight of the rats during the Steenbock V ration was fairly constant. Observation of Chart II, shows a decided fall in the weight during the high carbohydrate feeding. This tendancy may be accounted for by the fact that the minerals were supplied in the water, and the water consumption was very low. These minerals were later placed in the food mix and water consumption became adequate. A second reason for this poorer weight gain was the lower coloric value per gram of this diet. This will be noted in Table 2. The average food consumption on the carbohydrate diet was not sufficient to compensate for the difference. The initial rise in ratio in gain of weight coincides with the difference in coloric value of the two diets.

Plasma Lipid Content of Rat Blood

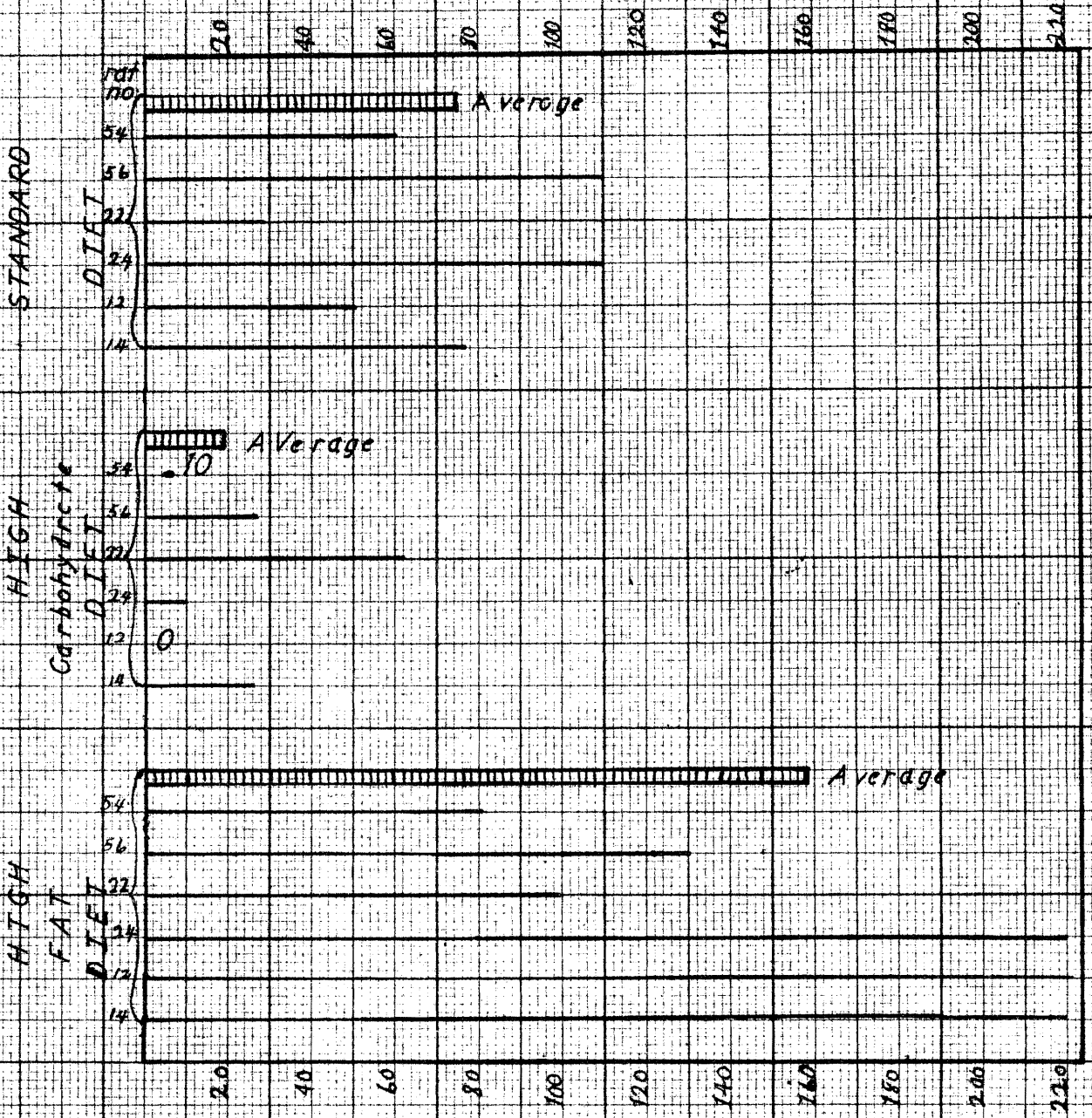
From the results of the Allen plasma determination, the rat plasma lipids increased 73.5 per cent after control high fat diets. The standard diet average was 18.6 milligrams of fat per 100 milliliters of plasma. Bloor (1943) also found that fat diets influence the lipid content of the blood, but not the cholesterol. However, he pointed out further evidence that cholesterol was not concerned in fat metabolism.

MICRO-STAINING OF THE CELLULAR LIPIDS

A micro-staining technic for lipid granules in leucocytes was used in staining slides of rat blood and human blood. In the slides made from

CHART II

RATIO OF GAIN IN WEIGHT OVER THE AVERAGE FOOD CONSUMPTION FOR EACH RAT'S EXPERIMENTAL DIET



The efficiency of the diet is determined by dividing gain in weight of each rat while on each of the test diets by the average amount of food eaten and multiplying by 100.

Each column represents the average value for each rat.

rat blood, the lipid content of the leucocyte from rats on the standard diet seemed to be in uniformly distributed granules. The lipid content of the leucocyte in blood from animals on the high fat diet revealed a similar picture, however, there was a much deeper stain appearing on or in the neuclei of the cells. A similar picture was found in human blood specimens.

In both instances, the mononuclear cells seemed to contain the most visible granules. However, cell differentiation was somewhat difficult in this staining technic. Simpson (1921) also furnished strong evidence of the probability that these cells contained lipids.

SUMMARY

Total and differential leucocyte counts were made on 30 college women of ages of 18 to 28 years. The total counts were determined in duplicate. One to twenty dilution of the blood was made with 1 per cent glacial acetic acid and the counts were made on a Spencer (Bright-Line) haemacytometer. The differential counts were made with commercially prepared Wright's Stain. The Arneth neutrophilic counting scheme was used.

From the 30 women, four were chosen for a six weeks total and differential leucocyte count determination. Each week the total and differential leucocyte counts were determined and recorded, and averages for the group were made. From these the variation that occured was observed. Records were kept of individual data which were thought to be pertinent to this study.

Four of the 30 college women were chosen for high fat diets. These subjects were given a meal of high lipid content and total and differential counts were made before and after such feedings.

Six Albino rats were fed control diets for a period of five weeks. On the fifth and seventh day of feeding the total and differential counts were determined and recorded. Daily weights, food consumption and other data were recorded. Plasma lipid determinations were made at the end of the feeding experiment.

From this study the following observations were made:

1. The average total white blood count as determined in this study on thirty randomly selected college women was 6,786 per cubic milliliter, with a standard deviation of 1,184. The average differential neutrophilic count was 65.5 per cent with a standard deviation of 7, while the average lymphocyte count was 28.4 per cent with a standard deviation of 5.

2. The relationship between increase of total counts and excess weight seemed to be apparent; however, the total counts did not correlate proportionally with the increase in weight.

3. Each individual's normal total count range had considerable fluctuation, yet each subject possessed a particular range.

4. The individual variations in leucocyte counts over a period of six weeks were influenced by the perons's physical health conditions and menstrual period.

5. The average total leucocyte count increased approximately eight per cent after high fat feeding in the rat experiments, and in

human subjects, it was an increase of 18 per cent.

6. Differential counts of human subjects and of experimental animals showed an increase in lymphocytes of 27 per cent.

7. Mico-staining of the cellular lipids revealed only minor changes in the cells effected by the high lipid intake. The nucleus stained slightly heavier as a result of the high fat diet.

8. The Plasma lipid content in rats increase 73.5 per cent in the six Albino rats following high lipid intake.

The number of experimental animals and human subjects was too small to draw conclusive evidence. However, it would seem the lymphocytes may serve as active agents in fat transport.

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APPENDIX

REAGENTS (SOLUTIONS)

Solution used in White Blood Count
(1 % aqueous Solution)

Glacial Acetic Acid---------- 3 cc.
Distilled water---------- 300 cc.
Gentian Violet---------- 10 to 12 drops
Trace of $CuSO_4$---------- preservitive

Alkaline Reagent for Allen Blood fat determination

Sodium carbonate---------- 110 grams
Sodium salicylate dissolved in---------- 200 grams
Water volumetrically to---------- 1 liter
Sodium hydroxide (50%)---------- 30 milliliters
(equal parts by weight with H_2O)
Normal butyl alcohol---------- 100 milliliters

Sudan III Staining Solution

Sudan III---------- 1 gram
Alcohol (96%)---------- 200 cc.
Boil over water bath for 5 min. in loosely stoppered liter flask-
Thoroughly shaken and filtered while hot-
Refrigerate 24 hrs. & Filter
Distilled H_2O added drop by drop to reduce the alcoholic contration to 80%
Keep at room temperature 24 hrs. Filter
Prepare the staining Sol. freshly from this stock solution by adding drop by drop equal amounts of distilled water. A colloidal Sol.

Acid Alcohol

Hydrochloric Acid---------- 0.5 cc.
Alcohol (40%)---------- 100.0 cc.