THE FORMED ELEMENTS OF THE BLOOD OF OKLAHOMA COLLEGE WOMEN

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PREFACE

The increasing importance of an accurate knowledge of the formed elements of the blood has made the scientific world conscious of a need for reliable standards. The counting of the erythrocytes, measurement of hemoglobin content and the determination of the packed cell volume of the blood are routine clinical procedures in the differentiation of the various types of anemia. Obviously, before an intelligent interpretation of the results of these procedures can be made in anemic patients, a definite knowledge of the values in the normal person is necessary. These values are also physiological measurements used as criteria in the determination of the nutritional status of an individual.

Since the values given as normal in the literature vary widely, it seems advisable to analyze samples of blood from persons living in a number of localities which differ in climate and altitude. From such a comparative study, adaptation of universal standards may ultimately be possible, or the establishment of separate regional standards may be necessary. Heretofore the college girl has been considered as an adult and judged by adult standards. Since many girls enter college between the ages of sixteen and twenty years, it may be a fallacy to classify her with mature women. This study of the formed elements of the blood presents observations on the hemoglobin content, the number of red cells, and the volume of packed cells of the blood of 101 Oklahoma college women whose ages range from seventeen to twenty-three years, inclusive.

HISTORICAL REVIEW

Some of the most important physical characteristics of the erythrocytes and their variations in disease have been known since the first days of the microscopic study of the blood. The development of hematology as we know it today began in the middle of the nineteenth century when Vierordt (56), in 1851, first enumerated the red blood cells and thus stimulated the work in the counting of cells. With Welcher (45), a student of his, he made a study of the red cells of the blood of four subjects. Mallassez (25) published a monograph on the enumeration of the erythrocytes in 1873. A few years later, in 1877, a counting chamber was devised by Gower (11) which remained the standard piece of apparatus until recent years. The original crude and laborious methods were rapidly improved, and now the counting of the blood cells is a routine clinical procedure.

The blood pigments were first brought to the attention of the hematologist with the discovery of hemoglobin by Funke (9), in 1851. He was able to isolate the pigment in crystalline form. Welcher (46) is credited with the first clinical estimation of the hemoglobin content of the red blood cells. This determination was made in 1854 by comparing a fixed dilution of the unknown blood with dilutions of normal blood. Interest in hemoglobin spread and soon quite complete analysis of its crystals were made. Hoppe-Seyler (23) was responsible for the establishment of the importance of the blood pigments in tissue metabolism. His early work, in the years 1871-1875,

showed that hemoglobin combined with oxygen in the lungs to form oxyhemoglobin, which in turn gave up its oxygen to the tissues and became reduced hemoglobin again. Soon after Hoppe-Seyler's work, Haldane (18) devised a method for measuring the gases of the blood. In 1899-1900, he observed that on the addition of potassium ferricyanide to a solution of oxyhemoglobin, the oxygen was quantitatively released. The amount of oxygen thus given up by the oxyhemoglobin was measured in a gas analysis apparatus. Haldane found that one gram of hemoglobin released 1.34 cubic centimeters of oxygen. This method of analysis was adapted by Van Slyke (54) to his apparatus for the analysis of the blood gases. The improved apparatus of Van Slyke is in wide use today and provides an accurate, rapid and simple method for determining the hemoglobin content of the red cells and for checking other apparatus and methods.

Parallel to this development of the determination of hemoglobin by measurement of the blood gases was the development of the colorimeteric method. Hoppe-Seyler (22), in 1892, was the first to describe carbon monoxide hemoglobin and to make use of this stable combination for the estimation of the hemoglobin content of the red cells. He devised a "double pipet" for comparing the unknown carbon monoxide hemoglobin solution with a standard solution prepared from hemoglobin crystals. The method never came into general use because of the technical difficulties involved. In 1900, Haldane (18) suggested a much simpler method for comparing carbon dioxide solutions by using the apparatus employed by Gower (39) to

compare oxyhemoglobin solutions with picrocarmine standards. This apparatus was later employed by Sahli (38), in 1909, who prepared an acid hematin solution by adding dilute hydrochloric acid to the blood. In 1918, Palmer (34) advocated a method for general use in hemoglobin determinations in which the blood was treated with an ammonia solution and an illuminating gas. This carbon dioxide hemoglobin was then compared with a carbon dioxide hemoglobin standard. Osgood and Haskins (30), in 1923, modified the acid-hematin method of Sahli and prepared a new standard to be used in comparing the unknown acid hematin solution. This acid hematin method affords a quick and relatively simple method for determining the hemoglobin content of the blood and is used extensively today.

With the advent of the above mentioned methods for counting the erythrocytes and for determining their hemoglobin content, clinical application of these estimations were made quickly. Johannes Duncan (8) was the first person to recognize the possibility of a relationship between the size of the cells and their hemoglobin content and the possible variation of these with disease. In 1875, he demonstrated the relation between the decrease in the hemoglobin content and the size of the erythrocyte in a case of chlorosis. Welcher (45), in 1854, determined the volume of the red cell, with the aid of plastic models, and found that the corpoacle was smaller in a case of chlorosis than it was in normal subjects.

The next step in the development of hematology came in the late nineteenth century with the work of Bliebtreu and

Bliebtreu (32). These workers were responsible for the first practical method for the clinical determination of the volume of the red cells. Credit for the introduction of the hematocrit method for the determination of the volume of the red cells belonged to Hedin (21), Daland (7) and Gaertner (10), whose works were reported in 1890-1892. In these early methods the cells were separated from the plasma of the blood by centrifuging a given quantity of blood for a definite time. Caski (6), in 1922, was the first to emphasize centrifugation to a constant volume rather than for a definite length of time.

By the end of the nineteenth century, methods were available for the determination of the volume and the diameter of the cells, and a few students of the anemias knew of the more important changes which might occur in the size and hemoglobin content of the cells. In 1903, Capps (5) reported studies on one hundred and seventy-five persons, ten of whom were normal. These studies included estimations of the hemoglobin content of the cell by the Fleischl method; red cell counts; cell volume determinations by the hematocrit method; computation of the average diameter of the cell, based on the measurement of one hundred cells with the eyepiece micrometer; and the calculation of the color index and the volume index. Had the methods used been as accurate as those of today, undoubtedly Capps would have discovered everything that is now known of the value of these indices. With the technic used he could not have obtained full packing of the red cells. He centrifugated at 10,000 revolutions per minute for three minutes, and relied on the speed of manipulation to secure packing of the

cells before coagulation occurred. His hemoglobin estimations were not expressed in absolute values and cannot be transformed into them. His normal standards were based on the study of only four men and six women. Notwithstanding these facts, he arrived at the following general conclusions which have been substantiated by other workers who used more accurate technics:

> 1. "The centrifuge accurately determines the mass of the red corpuscles, but cannot be relied upon to estimate the number of red cells, because the volume of the cell undergoes variation in disease.
>
> 2. The volume of the individual erythrocyte is best obtained by using the centrifuge in conjunction with the hemacytometer. . .
>
> 3. The cell volume is invariably increased in pernicious anemia and usually more so than the hemoglobin content of the cell. . ."

Early workers found a great deal of variation in the actual values for hemoglobin, red cell counts and cell volumes, obtained by the above procedures, from studies of the blood of apparently normal subjects. These differences were due to the inaccuracy of the methods employed, to the variety of methods used, and to the small number of observations made. Because of the differences in the methods used by the various workers, their data cannot be pooled to establish standard values.

Although an accurate knowledge of the number of red cells is of fundamental importance, the present accepted standards for this value were based on insufficient and inaccurate data. The generally accepted standard for the normal erythrocyte count in adults is based on determinations made by Vierordt (56) and Welcher (45) on four subjects in 1854. Only in the last decade has the wisdom of drawing conclusions from data taken on only a few subjects been questioned, and has an attempt

been made to supply an adequate number of observations

The normal hemoglobin standards for women of from 13.0 to 14.0 grams per one hundred cubic centimeters of blood, which appear in most of the textbooks of clinical diagnosis, were based on Leichenstern's (39) study. In 1878 he made sixty-one determinations of the hemoglobin of normal individuals of different ages by the spectrophotometric method. He found the average for the women to be 13.10 grams and for the men to be 14.00 grams of hemoglobin per one hundred cubic centimeters of blood. The accuracy of the determinations made with the spectrophotometer depends on the correctness of the absorption ratio, which ratio varies with the different observers. Therefore, the values obtained by the different workers by this method are not comparable. Various standards which have been based on determinations made with an hemoglobinometer appear in the literature. The number of entirely different values used as normal, or one hundred percent, in the calibration of these instruments indicates the inconsistency of the early data obtained with them.

Most of the textbooks of physiology and clinical pathology give the normal volume of the red corpuscles as forty percent of the volume of whole blood. The early values found in the literature range from thirty-nine cubic centimeters of whole blood, reported by Haden (15), to forty-one cubic centimeters, reported by Bonninger (4). In most of the studies before 1929, centrifugation to a constant volume was not used in the determination of the volume of the erythro-

cytes. Even though these early studies were inaccurate, the results were fairly consistent.

The knowledge gained from these early hematological investigations was purely academic. The procedures were little employed by the medical profession until sometime later, early in the present century. Within the past ten years they have grown to be of great clinical importance especially in the diagnosis of the anemias. Although anemia has been recognized clinically for years, our present understanding of its real nature and its variations has come largely from the laboratory studies of the erythrocytes. An accurate differentiation of the various types of anemia depends to a great extent on the determination of the physical and chemical properties of the red cells, estimation of their hemoglobin content and their volume. Hematology is not only important in the field of medicine but is recognized to be of fundamental importance in the science of nutrition. Secondary anemia may be due to a nutritional deficiency of iron. Since it is known that the hemoglobin molecule contains four molecules formed of an iron-pyrrol compound known as hematin, the estimation of the hemoglobin content of the cell may be used as an indirect measure of the iron content. It has been shown that diet plays an important part in the therapy of the anemias.

REVIEW OF THE LITERATURE

1. Erythrocyte Counts

Many studies on the number of red cells in a given volume of blood have been reported. In this review of the literature, however, only those studies on normal women from seventeen to thirty years of age, inclusive, will be considered.

A survey of the literature shows that counts of the erythrocytes were made on normal women of this age group as early as 1920. Many counts had been reported earlier, but the ages of the subjects were not given. Bierring's (3) study of three Swedish women, in 1920, gave an average erythrocyte count of 4.24 million cells per cubic millimeter of blood. Gram and Norgaard (13), in 1923, made a similar study in Denmark on six women in this age group. The average number of red cells was found to be 4.59 million per cubic millimeter of blood. Two other studies which were made in Denmark have been reported. The first of these was that of Bie and Möller (2). In this study determinations were made on ten Danish women, and a mean count of 4.74 million was found. The second study was that of Rud (37) in which an average of 4.80 million cells per cubic millimeter of blood was reported. The earliest study on this age group of women in the United States was reported by Haden (15). His work was done during the years of 1923 and 1924 on twelve women residing in Missouri. The results of these determinations indicated an average erythrocyte count of 4.26 million per cubic millimeter of blood.

The first accurate study of this age group of women which included a large number of observations was reported by Osgood

and Haskins (31) in 1926. These authors presented determinations on one hundred women residing in Oregon, all of whom were nurses with the exception of a few medical students. A mean red cell count of 4.84 million was found for the twelve women of the group who were eighteen years of age. For the eighty-eight subjects who were from nineteen to thirty years old, inclusive, an average of 4.79 million cells per cubic millimeter of blood was reported. The average of the entire series was 4.80 million, and ninety percent of the cases fell between 4.3 and 5.3 million cells per cubic millimeter of blood.

Wintrobe (48) in summarizing the data of the hematological studies reported prior to 1930 in both the United States and Europe, has reported that the average for the 186 women included in these studies was 4.78 million cells per cubic millimeter of blood. In 1930, Wintrobe (49) presented the results of his own study of fifty women students of Newcomb College, New Orleans, Louisiana. The subjects were from eighteen to thirty years old, inclusive. The methods employed seemed to be quite accurate and all of them presented a relatively low probable error. An average erythrocyte count of 4.93 million per cubic millimeter of blood was found.

Seeking to establish normal standards for red cell values for residents of Colorado, Andresen and Mugrage (1) carried on studies for over three years on a large group of subjects. Included in the study were determinations of the number of the red cells of the blood of forty women from the ages of twenty to forty-five years, inclusive. Their findings, published in 1936, showed that the mean red cell count for the women was

4.63 million. Their data is of particular significance as it is the most comprehensive study yet reported on women residing at an altitude of 5000 feet or more,

Two other studies were reported in the literature in which the ages of the subjects were not available. The first was that of Haden (17) published in 1932. Determinations of the erythrocyte counts of thirty women residing in Detroit, Michigan were made. The average number of red cells of the group was reported as 4.38 million per cubic millimeter of blood. The second study was that of Wintrobe (51) in which 101 women of Baltimore, Maryland served as subjects. The results of the determinations, reported in 1933, gave a mean red cell count of 4.84 million per cubic millimeter of blood for this group of subjects. The data from these studies of red cell counts of women have been summarized and are presented in table 1.

TABLE 1

Mean Values for the Number of Red Cells of the Blood of Women from 18 to 30 Years Old, Inclusive, (Reported Since 1920)

Authority and the Location	Date	Number of Subjects	Red Cells in millions per c. mm.
Bierring (3) Sweden	1920	3	4.24
Rud (37) Denmark	1922	8	4.80
Bie and Möller (2) Denmark	1922	10	4.74
Gram and Norgaard (13) Denmark	1923	6	4.59
Haden (15) Missouri	1923	12	4.26
Osgood and Haskins (31) Oregon	1926	100	4.80
Wintrobe (49) New Orleans	1930	50	4.93
Haden (17) Detroit	1932	30	4.38
Wintrobe (51) Baltimore	1933	101	4.84
Andresen and Mugrage (1) Denver, Colo.	1936	40	4.63

2. Hemoglobin Content of the Blood

The inaccuracy found in the early data from studies of the number of red cells is also evident in the data from the early estimations of the hemoglobin content of the erythrocytes. Due to the lack of standard methods and to the insufficient number of observations, the results of these first determinations are very unsatisfactory.

One of the earliest studies on the hemoglobin content of the red cells of the blood of women whose ages were from eighteen to thirty years, inclusive, was that of Williamson (47), reported in 1916. Nine hundred determinations of the hemoglobin content of the cells were made, with the spectrophotometer, on a large group of subjects of both sexes and of all ages. Williamson reported that the forty women from sixteen to sixty years of age, inclusive, had a mean hemoglobin value of 15.55 grams per one hundred cubic centimeters of blood.

From 1921 to 1923, inclusive, Haden (15) made hemoglobin determinations on fifty-two normal individuals. Of this group, twelve were women of from twenty to forty years of age. With Haldane ferracyanide method adapted by Van Slyke, Haden found that the average hemoglobin value for these women was 13.34 grams per one hundred cubic centimeters of blood.

Several European studies on the hemoglobin level of women, eighteen to thirty years old, inclusive, were reported about this same time. In 1923, Gram and Norgaard (13) determined the hemoglobin of six Swedish women of this age group by means of the Autenrieth-Konigsburger colorimeter and found

that the average hemoglobin of these women was 12.82 grams per one hundred cubic centimeters of blood. Bie and Möller (2) reported a study of ten women of Denmark in 1922. They made their determinations by means of a Meisling colorimeter. The mean hemoglobin value obtained by these workers for the women studied was 13.30 grams per one hundred cubic centimeters of blood. Rud (37) reported an average of 12.28 grams of hemoglobin per one hundred cubic centimeters of blood for hemoglobin determinations made on nine Danish women, whose ages ranged from eighteen to thirty years, inclusive. His work was done by the colorimetric method for measuring hemoglobin, and he used the same type of apparatus as that of Gram and Norgaard.

In order to summarize the hemoglobin values that have been reported recently, a comprehensive analysis of the data on the hemoglobin content of the erythrocytes of women from eighteen to thirty years of age, inclusive, has been made. Only those subjects who were residing in the United States and only those reports which have been made since 1926 have been included in this analysis. The results are presented in table 2.

TABLE 2

Mean Values for the Hemoglobin Content of the Blood of Women Residing in the United States, (Reported Since 1925)

Authority and Location	Date	Number of Subjects	Method Used	Hemoglobin, in grams per 100 cc.
Osgood and Haskins (31) Oregon	1926	100	Osgood and Haskins acid-hematin	13.69
Wintrobe (49) New Orleans	1930	50	Newcomer hemoglobinometer, checked by oxygen capacity	13.76
Haden (17) Detroit	1932	30	Oxygen used	13.37
Wintrobe (51)	1933	101	Same as New Orleans study	14.41
Andresen and Mugrage (1) Denver, Colo.	1936	40	Acid hematin, checked by oxygen capacity	14.45

3. Volume of Packed Cells of the Blood

The earlier determinations of the volume of the erythrocytes by the hematocrit method have been criticized because of their inaccuracy, and in many reviews of these studies the figures are not given because they are considered to be of no value. Bonninger (3) was the first to study a comparatively large number of subjects. In 1919, he reported determinations of the volume of the red cells on sixty individuals. An average of 41.00 cubic centimeters of packed cells per one hundred cubic centimeters of whole blood was given for the women of the group. His determinations were far from accurate. Reich (32) was apparently the first to use oxalated blood for hematological studies. In 1921, he reported values on thirty-three subjects with various diseases, fifteen of whom he inadvisedly used as normals. Since the methods were inaccurate and the sex of his subjects was not available, his results are not to be given here. During the years of 1922 and 1923, Haden (15) made determinations of the packed red cell volumes of twelve women. He gave an average of 39.7 cubic centimeters of cells per one hundred cubic centimeters of whole blood for this group. The accuracy of Haden's figures was questioned by Osgood (32) because the determinations were made by centrifuging for a definite time interval and not to a constant volume of the cells.

The later and more accurate studies began with the work of Osgood and Haskins (31) in 1926. A mean of 41.00 cubic centimeters of whole blood was reported by these workers for determinations on one hundred women of Portland, Oregon.

The following year a study of southern women appeared in the literature. This was the work of Wintrobe (49) which included red cell volume determinations on fifty New Orleans college students. The author felt that the available hematocrit tubes were inaccurate and therefore devised his own by cutting off a Mohr pipet and carefully recalibrating it. A mean of 39.5 cubic centimeters of packed cells per one hundred cubic centimeters of whole blood was obtained in this study. From a study of thirty women of Detroit, in 1932, Haden (17) concluded that the packed cell volume per one hundred cubic centimeters of blood for this group was 39.8 cubic centimeters. In 1933, Wintrobe (51) made a comprehensive study of 101 women residing in Baltimore, Maryland. He indicated that the mean volume of packed red cells for this study was 42.00 cubic centimeters per one hundred cubic centimeters of whole blood. Mugrage and Andresen (1), working with forty women of Denver, Colorado, reported the results of their study in 1936. In determining the packed red cell volume they used the Van Allen hematocrit tube at first but later changed to the Wintrobe tubes because they felt that the latter were more accurate. Their results show an average packed cell volume of 43.22 cubic centimeters per one hundred cubic centimeters of blood. Murphy (28) reported, in 1931, a relatively small number of determinations of the packed cell volume on twenty-one women of Boston, Massachusetts. He observed a mean of 41.20 cubic centimeters of packed red cells per one hundred cubic centimeters of whole blood. A summary of these later studies has been made and the results are presented in table 3.

TABLE 3

Mean Values for the Packed Cell Volume of the Blood
Of Normal Women of the United States
(Reported Since 1922)

Authority and Location	Date	Number of Subjects	Volume of Packed Red Cells in cc. per 100 cc.
Haden (15) Missouri	1923	12	39.70
Osgood and Haskins (31) Oregon	1926	100	41.00
Wintrobe (49) New Orleans	1930	50	39.50
Murphy (28) Boston	1931	101	41.20
Haden (17) Detroit	1932	30	39.80
Wintrobe (51) Baltimore	1933	101	42.00
Mugrage and Andresen (1)	1936	40	43.22

EXPERIMENTAL PROCEDURE

1. Subjects

The subjects of this investigation of the hemoglobin, erythrocytes, and cell volume of the blood were 101 women enrolled as freshmen in the Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma. The ages of these subjects, who were selected at random, ranged from seventeen to twenty-three years, inclusive.

In addition to the above study of the formed elements of the blood, twenty-six determinations were made over a period of thirty-four days on a graduate student. This phase of the study was conducted to observe the normal day-to-day intravariation of hemoglobin, the number of red cells, and the packed cell volume. Tests were made throughout one complete menstrual cycle of the individual.

All of the determinations were made during the months of February and March in the year 1939.

2. Methods

The blood was taken, usually between the hours of eight and twelve in the morning, from the finger by use of a spring lancet. Freely flowing blood was used in all of the determinations. After the capillary was punctured, the first drop of blood was discarded because of its possible dilution with tissue fluid. The blood was then allowed to drip into a small parafin cup. The samples of blood were taken either directly from the finger or from the cup.

The activities of the subjects prior to the examination were not controlled, however, all of the subjects were re-

quired to rest for at least ten minutes before the blood was taken. The date of the beginning of the last menstrual period was recorded, even though menstruation has been shown to have no significant effect on hematological values (24).

For the determinations of the red cell counts, at least two pipets were prepared from each sample of blood. A 1:200 dilution was made with Hayem's diluting fluid in Thoma and Trenner pipets certified by the National Bureau of Standards. Four counts were made on each subject, two from each pipet. A certified Levy-Hausser counting chamber which had the improved Neubauer ruling was employed throughout. In counting the cells the following procedure was used:

1. The pipets were shaken by the approved method for at least five minutes.
2. The fluid in the capillary of the pipet was discarded before the counting chamber was filled. Special care was taken to fill the chamber exactly.
3. The cells in five large squares, each of which contained sixteen small squares, were counted and totaled.
4. The total was multiplied by the factor 10,000 to obtain the number of cells in one cubic millimeter of blood.

The mathematical derivation of the factor, 10,000 is as follows:

> Each small square of the chamber is 1/400 square millimeters in area; therefore, one large square is 1/25 square millimeters; and five large squares, the area actually counted, are 1/5 square millimeters in area. The correction factor for area is 5. Since the blood was originally diluted 1:200, the necessary correction for dilution is 200. The counting chamber is 1/10 millimeter in depth, therefore, this correction factor for volume is 10. The total correction which must be made to the cells counted in five large squares, in order to obtain the number of cells in one cubic millimeter of blood, is 5 x 200 x 10 or 10,000.

No attempt was made to secure counts varying by any definite figure, but careful technic was accepted as the criterion of checks. The criterion that duplicate counts must not vary by more than 200,000, which is usually accepted, seems to have been arbitrarily established.

The values reported in the Results and Discussion of this paper are the averages of the four counts obtained by the above procedures on each subject.

Hemoglobin determinations were made by the acid hematin method described by Newcomer (29). A one percent solution of hydrochloric acid was used as the hypotonic diluent, and two pipets were prepared from each sample of blood. Each dilution was compared colorimetrically with a brown glass standard, which had been prepared by the Bausch and Lomb Company to match the acid hematin color. All readings were made with a Bausch and Lomb colorimeter in a dark room. A blue filter was used in the eyepiece of the colorimeter to eliminate the matching of the yellow color. A daylight base lamp provided the constant source of light. The average of the colorimeter readings for each subject was converted to grams of hemoglobin per one hundred cubic centimeters of blood by reference to the conversion table supplied by the company from which the standard was purchased.

To prevent coagulation of the blood to be used in the determination of the packed red cell volume, a very small amount of heparin was mixed with the blood in the parafin cup. The heparin was obtained from the Connaught Laboratories, University of Toronto. The anticoagulant properties of

heparin are believed to be due to its ability to inhibit the activation of prothrombin to thrombin. Since heparin is isotonic, it does not alter the size of the cells, and since approximately only a 0.1 percent solution is necessary to prevent coagulation of the blood, it is considered the ideal anticoagulant. Some workers have encountered difficulty in using heparin because it did not dissolve readily, but the finely powdered and highly purified form which was used in this study was very satisfactory and dissolved without an excess of stiring.

Van Allen (53) hematocrit tubes were used in all determinations of the packed red cell volume. The tubes were filled to the 100 percent mark with the heparinized blood. The blood was then drawn slightly above the mark to prevent leakage when the tubes were sealed and suspended on the rubber cushion of the special spring type holder. The tubes were placed in the centrifuge in such a manner that their axes were perpendicular to the axis of rotation while centrifuging. A large type international centrifuge was used at an estimated speed of 3000 revolutions per minute. Readings of the height of the column of red cells were made at the end of thirty minutes of centrifugation and at fifteen-minute intervals thereafter until no further change in the height of the column occurred. Two determinations were made on each subject and the mean of these is reported in the Results and Discussion of this paper.

RESULTS AND DISCUSSION

The data obtained from this study of the red cell counts, hemoglobin and the packed cell volume of the blood of 101 Oklahoma college women are presented in table 4. The corpuscular constants, mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration, calculated from these absolute values are also given in table 4.

The corpuscular constants were calculated from the following formulas:

(1) MEAN CORPUSCULAR HEMOGLOBIN, IN MICROMICROGRAMS ($\gamma\gamma$)

$$\frac{\text{Hemoglobin, in grams per 1000 cc. of blood}}{\text{Red blood cells, in millions per c.m. of blood}}$$

(2) MEAN CORPUSCULAR VOLUME, IN CUBIC MICRONS ($c.\mu.$)

$$\frac{\text{Packed cell volume, in cc. per 1000 cc. of blood}}{\text{Red cells, in millions per c.m. of blood}}$$

(3) MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION, IN PER CENT

$$\frac{\text{Hemoglobin, in grams per 100 cc. of blood}}{\text{Packed cell volume, in cc. per 100 cc. of blood}} \times 100$$

TABLE 4

Red Cell Counts, Hemoglobin Content, Packed Cell Volume
And Corpuscular Constants of the Blood of 101
Oklahoma College Women

Subj.	Age	Red Blood Cells, Millions per c.mm. of blood	Hemoglobin, gms. per 100 cc. of blood	Volume, cc. per 100 cc. of blood	Mean Corpuscular		
					Hemo-globin	Vol.	Hemoglobin Concentration
					(rr)	(c.µ)	(%)
1	17	3.90	12.91	38.00	39.0	100.0	40.0
2	"	4.00	13.89	42.50	34.7	86.8	32.7
3	"	4.06	12.91	34.50	30.2	74.4	37.4
4	"	4.14	12.51	33.50	30.2	80.9	37.3
5	"	4.26	12.28	32.40	28.8	76.0	37.9
6	"	4.35	14.55	-----	33.4	-----	----
7	"	4.63	13.89	39.20	30.0	84.7	35.4
8	"	4.74	14.90	42.60	31.4	89.9	35.0
9	"	4.77	9.95	36.00	20.8	75.5	27.6
10	"	4.78	14.03	40.70	29.4	85.1	34.5
11	18	3.92	11.29	41.00	26.2	107.1	26.9
12	"	3.96	13.75	40.50	34.5	102.3	34.0
13	"	4.06	13.19	38.00	32.5	93.6	34.7
14	"	4.17	13.05	39.00	31.3	93.5	33.5
15	"	4.23	12.17	39.00	28.8	92.2	31.2
16	"	4.23	14.03	43.00	33.2	101.6	32.6
17	"	4.23	12.05	37.10	28.5	87.7	32.5
18	"	4.25	12.77	37.90	30.0	89.2	33.7
19	"	4.26	12.63	41.20	29.7	96.7	30.6
20	"	4.31	12.05	40.00	27.6	92.8	30.1
21	"	4.32	12.91	40.08	29.9	94.4	31.6
22	"	4.33	11.82	36.00	27.3	83.1	32.8
23	"	4.34	12.91	43.80	29.8	100.9	29.5
24	"	4.35	13.19	37.80	30.3	86.9	34.9
25	"	4.35	12.91	41.40	29.7-	95.2	31.2
26	"	4.36	14.03	38.50	32.2	88.3	36.4
27	"	4.36	12.51	39.50	28.7	90.6	31.7
28	"	4.36	12.40	37.40	28.4	85.8	33.2
29	18	4.38	13.19	40.00	30.1	91.3	33.0
30	"	4.43	14.73	38.00	33.2	85.8	38.8
31	"	4.48	13.47	42.20	30.0	94.2	31.9
32	"	4.52	12.28	39.60	27.2	87.6	31.0
33	"	4.54	13.47	40.50	29.7	89.2	33.0
34	"	4.60	14.73	44.80	32.0	97.4	32.9
35	"	4.62	13.19	48.00	28.5	103.9	27.5
36	"	4.62	13.05	41.50	28.2	89.8	31.4
37	"	4.62	14.38	42.50	31.1	91.9	33.8
38	"	4.63	14.38	42.00	31.0	90.7	34.2
39	"	4.69	14.38	44.50	30.6	94.9	32.3
40	"	4.70	14.73	44.00	31.4	93.6	33.5
41	"	4.70	13.89	39.50	29.6	84.0	35.2
42	"	4.71	14.55	44.80	30.9	95.1	32.5

(TABLE 4 CONTINUED)

Subj.	Age	Red Blood Cells, Millions per c.mm. of blood	Hemoglobin, gms. per 100 cc. of blood	Volume, cc. per 100 cc. of blood	Mean Corpuscular Hemoglobin	Mean Corpuscular Volume	Mean Corpuscular Hemoglobin Concentration
					(γγ)	(c.μ.)	(%)
43	18	4.73	13.47	46.10	28.5	97.7	29.2
44	"	4.74	13.33	42.20	28.1	89.0	31.6
45	"	4.75	11.82	39.00	24.9	82.1	30.3
46	"	4.78	14.20	40.80	29.7	85.4	34.8
47	"	4.79	11.19	38.00	23.4	79.3	29.4
48	"	4.85	12.77	41.50	26.3	85.5	30.8
49	"	4.85	11.48	48.50	23.7	93.8	25.2
50	"	4.86	15.25	40.50	31.4	83.3	37.6
51	"	4.98	13.61	43.50	27.8	88.9	31.3
52	"	4.90	14.03	42.50	28.6	86.7	33.3
53	"	4.92	14.38	47.00	229.2	95.5	30.6
54	"	4.98	12.28	44.00	24.6	88.4	27.9
55	"	5.10	12.91	38.70	25.4	75.9	33.4
56	"	5.10	16.46	43.80	32.2	85.9	37.6
57	19	3.82-	13.05	39.80	35.02	104.2	32.8
58	"	3.11	13.05	37.00	31.8	90.0	35.3
59	"	4.28	13.75	40.00	32.1	93.4	34.4
60	"	4.32	13.33	37.50	30.9	86.8	35.5
61	"	4.33	12.91	35.00	29.8	80.8	36.7
62	"	4.34	12.77	41.00	29.4	94.5	31.3
63	"	4.34	13.33	40.50	30.7	93.3	32.9
64	"	4.35	12.77	39.20	29.5	90.1	32.6
65	"	4.35	13.05	41.20	30.0	94.7	31.7
66	"	4.37	12.40	41.10	28.4	94.0	30.2
67	"	4.37	10.44	39.80	23.9	91.1	26.2
68	"	4.39	12.28	44.40	28.0	101.1	27.6
69	"	4.41	14.03	41.00	31.9	92.8	34.2
70	"	4.41	13.47	41.50	30.5	94.1	32.4
71	"	4.47	14.03	39.50	31.4	88.4	35.5
72	"	4.55	13.47	39.50	29.6	86.8	34.1
73	"	4.56	13.04	38.60	28.6	48.6	33.8
74	"	4.56	13.75	39.00	30.2	85.5	35.2
75	"	4.58	15.78	42.90	34.4	93.7	36.8
76	"	4.58	11.59	33.00	24.8	72.0	35.1
77	"	4.60	13105	41.60	28.4	90.4	31.4
78	"	4.63	12.91	39.50	27.8	85.3	32.7
79	"	4.64	13.75	40.00	29.6	86.2	34.4
80	"	4.65	11.94	39.00	25.7	83.9	30.6
81	"	4.65	13.47	40.60	29.0	87.3	33.2
82	"	4.65	14.03	40.50	30.2	97.0	34.6
83	"	4.66	15.43	45.10	33.2	96.8	34.2
84	"	4.76	12.40	36.50	26.1	63.4	34.0
85	"	4.78	13.19	36.30	27.6	75.9	36.3
86	"	4.84	14.20	41.80	29.4	86.4	34.0

(TABLE 4 CONTINUED)

Subj.	Age	Red Blood Cells, Millions per c.mm. of blood	Hemoglobin, gms. per 100 cc. of blood	Volume cc. per 100 cc. of blood	Mean Corpuscular Hemoglobin	Mean Corpuscular Volume	Mean Corpuscular Hemoglobin Concentration
					(rr)	(c.μ)	(%)
87	19	4.85	14.20	45.00	29.3	93.8	31.6
88	"	5.34	11.94	43.00	22.4	80.5	22.8
89	"	5.34	13.05	40.80	24.4	76.4	32.0
90	20	5.71	13.75	40.00	37.1	107.8	34.4
91.	"	4.14	12.28	33.20	29.6	80.2	37.0
92	"	4.33	12.17	36.50	28.1	84.3	33.4
93	"	4.43	15.60	43.50	25.2	98.2	35.6
94	"	4.56	13.61	39.00	29.9	85.5	34.9
95	"	4.84	14.73	43.00	30.5	88.8	34.2
96	"	5.19	15.78	42.00	30.4	80.9	37.6
97	21	4.16	14.20	43.00	24.2	103.4	33.0
98	"	4.40	12.28	39.20	27.9	89.1	31.3
99	"	4.48	13.33	37.20	29.8	83.0	35.8
100	22	4.37	15.08	39.60	34.5	90.6	38.1
101	23	4.21	12.77	40.50	30.4	77.2	31.5

From table 4 it can be seen that there is an intervariation in all of the red cell values of the Oklahoma women. The range for the red cell counts is 3.71 to 5.34 million per cubic millimeter of blood, and the mean is 4.51 million. For the hemoglobin values a range of 9.95 to 16.46 grams per one hundred cubic centimeters of blood was observed, and the average was 13.31 grams. The total range for packed cell volume was 32.4 to 48.0 cubic centimeters per one hundred cubic centimeters of blood, and the mean was found to be 40.35 cubic centimeters.

A statistical analysis of the data for the total 101 subjects and for the four age groups, seventeen, eighteen, nineteen, and twenty to twenty-three years was made. The results are presented in table 5. The calculations were made from the following formulas:

(1) Mean = $\frac{\text{Total of items}}{\text{Number of cases}}$ or $\frac{\text{Sum of X}}{n}$

(2) Standard Deviations = $\sqrt{\frac{\text{Sum of } X^2}{n} - m^2}$

(3) Coefficient of Variation = $\frac{\text{Standard deviation}}{m} \times 100$

(4) Standard Error of Mean = $\sqrt{\frac{\text{Standard Deviation}}{(n-1)}}$

(5) Standard Error of Standard Deviation = $\sqrt{\frac{\text{Standard Deviation}}{2(n-1)}}$

X denotes--item

n denotes--number of cases

m denotes--mean

TABLE 5

Results of a Statistical Analysis of the Data on the Formed Elements of the Blood of 101 Oklahoma College Women

Age Groups	No. of Subj.	Range	Mean and Standard Error	Standard Deviation & Standard Error	Coefficient of Variation (%)
			Hemoglobin, in grams/100cc.		
17-23	101	9.95-16.46	13.31 ± 0.12	1.15 ± 0.81	8.64%
17	10	9.95-14.90	13.18 ± 0.45	1.37 ± 0.32	10.44%
18	46	11.19-16.46	13.29 ± 0.16	1.07 ± 0.11	8.05%
19	33	10.44-15.78	13.21 ± 0.18	0.97 ± 0.12	7.34%
20-23	12	12.17-15.78	13.80 ± 0.36	1.21 ± 0.26	8.76%
			Red Cells, in millions/c.mm.		
17-23	101	3.71- 5.34	4.51 ± 0.03	0.03 ± 0.02	6.65%
17	10	3.90- 4.78	4.36 ± 0.13	0.38 ± 0.03	8.71%
18	46	3.92- 4.10	4.54 ± 0.04	0.30 ± 0.03	6.60%
19	33	3.82- 5.34	4.54 ± 0.06	0.32 ± 0.04	7.05%
20-23	12	3.71- 5.19	4.40 ± 0.11	0.36 ± 0.08	8.18%
			Volume, in cc./100cc.		
17-23	101	32.4-48.0	40.35 ± 0.31	3.10 ± 0.22	7.68%
17	10	32.4-42.6	37.7 ± 1.18	3.37 ± 0.84	8.93%
18	46	36.0-48.0	41.3 ± 0.41	2.74 ± 0.28	6.64%
19	33	36.3-45.1	40.0 ± 0.48	2.55 ± 0.32	6.37%
20-23	12	33.2-43.5	39.2 ± 0.91	3.02 ± 0.64	7.70%
			Mean Corpuscular Hemoglobin, in γγ		
17-23	101	20.8-39.0	29.4 ± 0.19	1.89 ± 0.13	6.42%
17	10	20.8-39.0	30.7 ± 0.44	4.36 ± 0.31	14.20%
18	46	32.4-34.5	29.3 ± 0.30	2.04 ± 0.21	6.96%
19	33	22.4-34.4	29.2 ± 0.49	2.63 ± 0.33	9.00%
20-23	12	24.2-37.1	29.8 ± 0.49	3.27 ± 0.69	9.74%
			Mean Corpuscular Volume, in c.μ		
17-23	101	63.4-107.8	89.1 ± 0.80	8.05 ± 0.57	9.03%
17	10	74.4-100.0	83.7 ± 0.18	8.94 ± 2.24	10.68%
18	46	75.9-107.1	91.1 ± 0.96	6.41 ± 0.67	7.03%
19	33	63.5-104.2	88.1 ± 1.33	7.00 ± 0.88	7.94
20-23	12	72.2-107.8	88.7 ± 2.86	9.44 ± 2.01	10.64%
			Mean Corpuscular Hemoglobin Concentration in %		
17-23	101	25.2-40.0	33.1 ± 0.29	2.90 ± 0.20	8.76%
17	10	27.6-40.0	35.3 ± 1.24	3.51 ± 0.88	9.94%
18	46	25.2-38.8	32.3 ± 0.38	2.61 ± 0.27	9.08%
19	33	26.2-38.8	33.0 ± 0.59	0.06 ± 0.39	9.27%
29-23	12	31.3-38.1	34.8 ± 0.38	1.29 ± 0.26	3.71%

The frequency distribution of the hemoglobin, in grams per one hundred cubic centimeters of blood; of the number of erythrocytes, in millions per cubic millimeter; of the volume of packed red cells, in cubic centimeters per one hundred cubic centimeters of blood, and of the corpuscular constants, mean corpuscular hemoglobin, in micromicrograms; mean corpuscular volume, in cubic microns; mean corpuscular concentration, in percent; are given in charts 1, 2, 3, 4, 5, and 6, respectively.

CHART 1

Histogram of the Hemoglobin Content of the Blood of 101 Oklahoma College Women

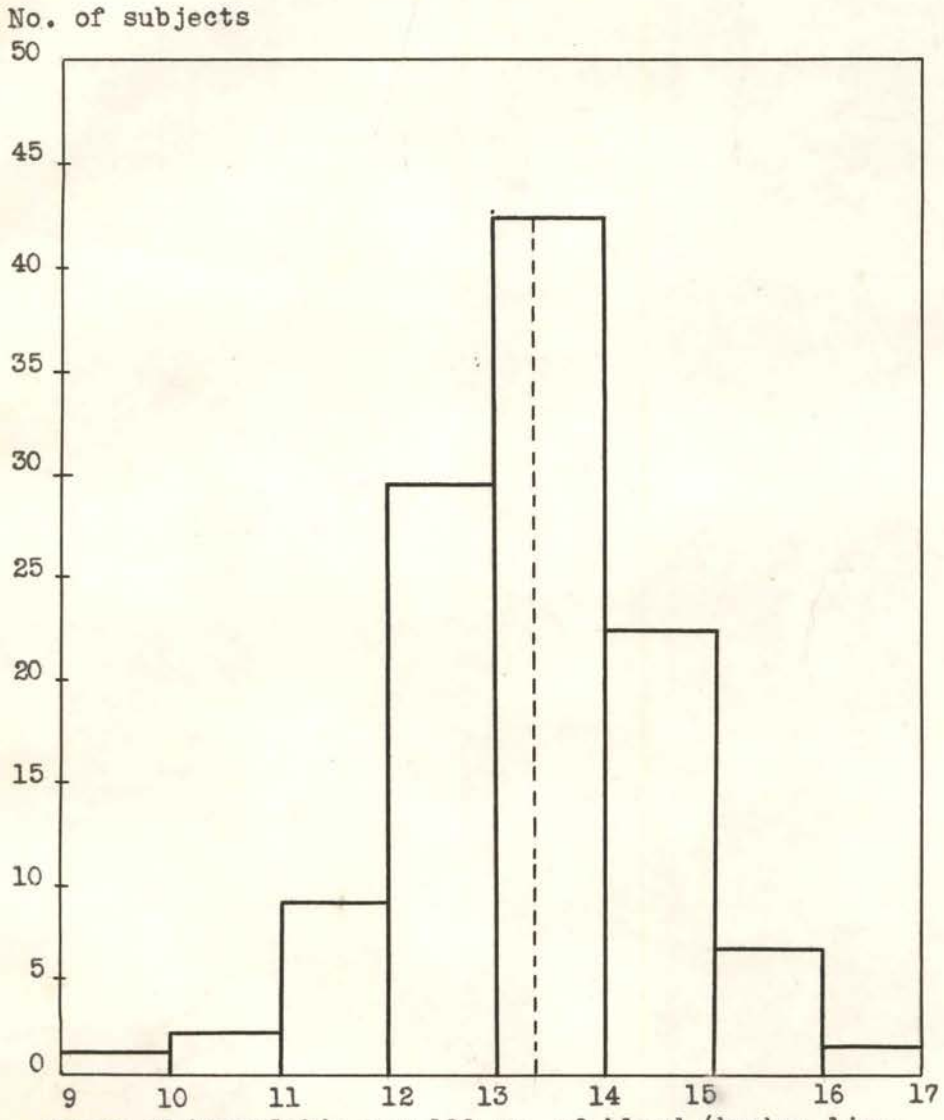
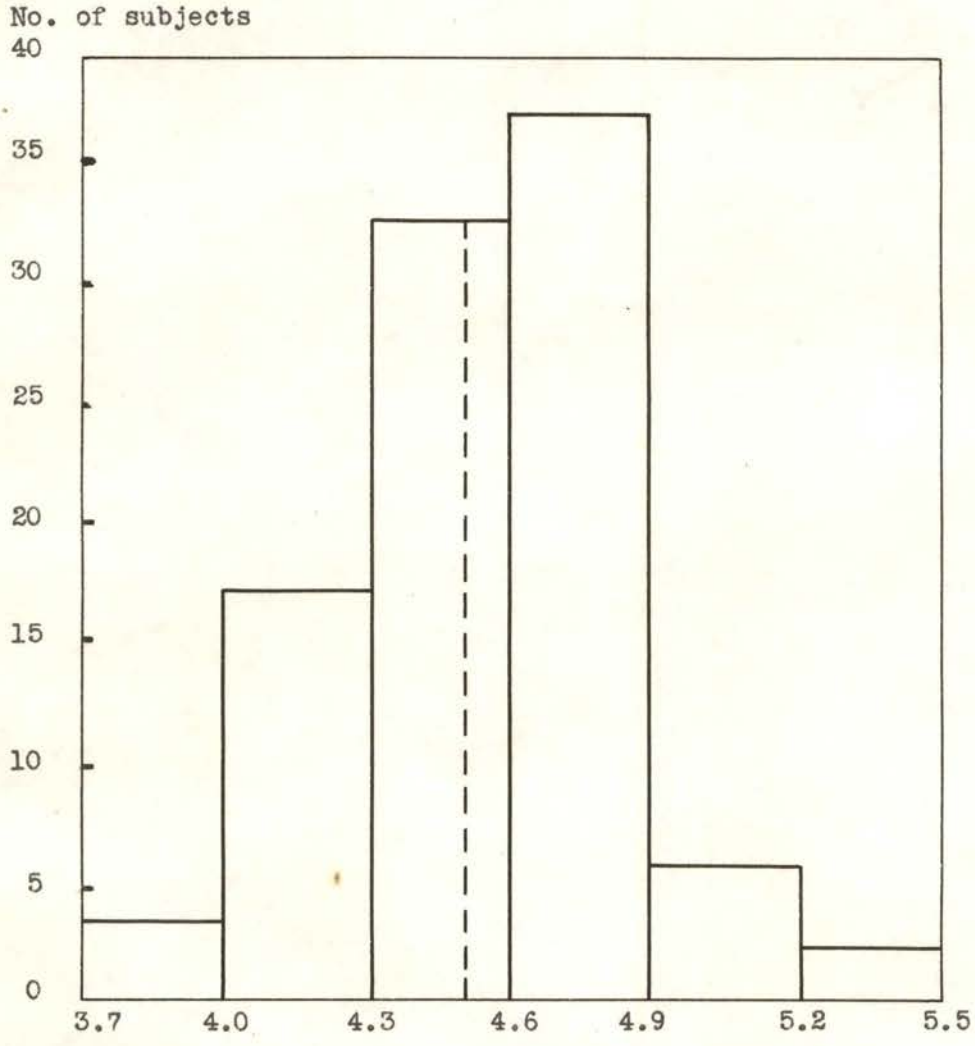


CHART 2

Histogram of the Number of Red Blood Cells in the Blood of 101 Oklahoma College Women



Millions of cells per cu. mm. of blood (broken line indicates the mean)

CHART 3

Packed Cell Volume of the Blood of 101 Oklahoma College Women

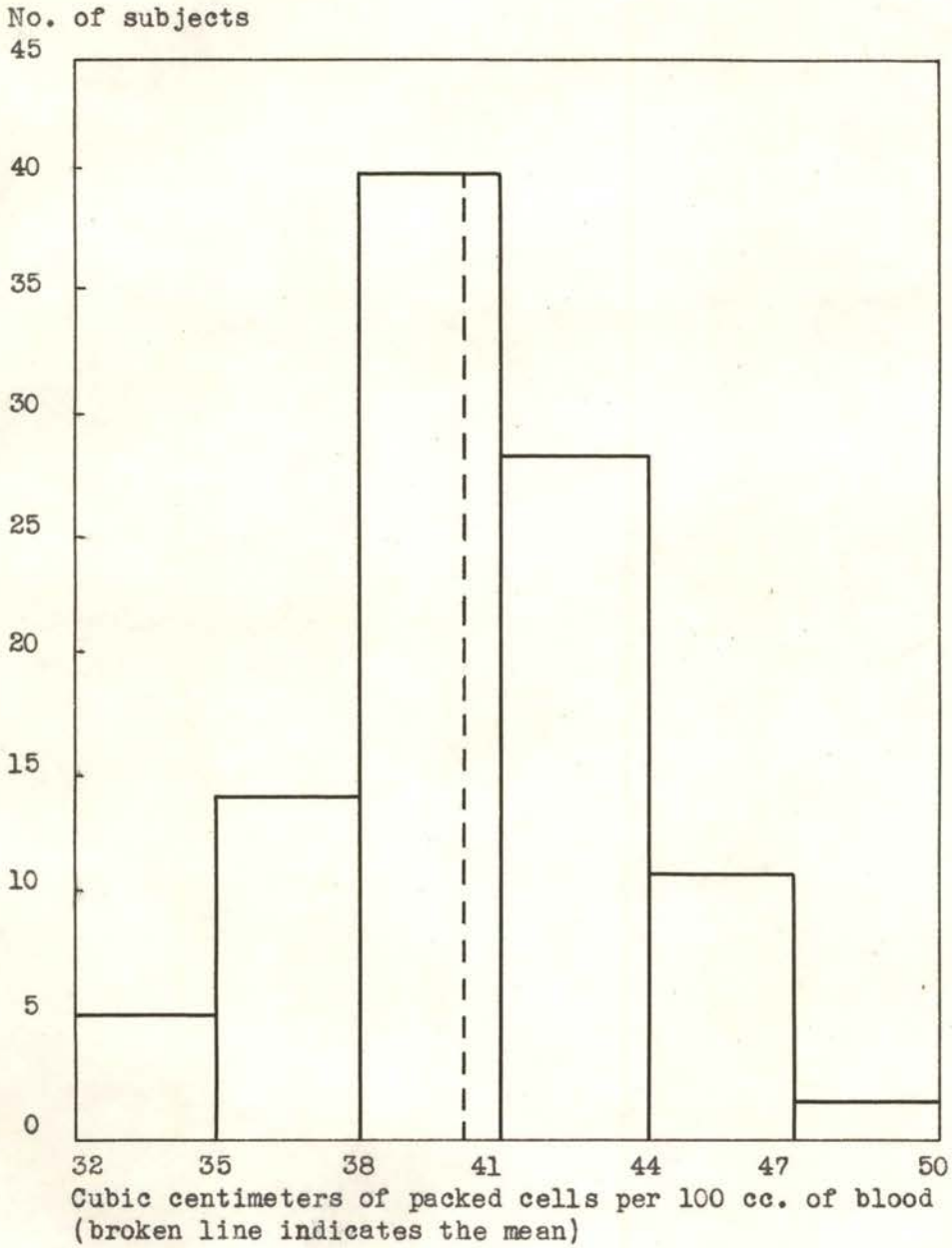


CHART 4

Histogram of Mean Corpuscular Hemoglobin of the Blood of 101 Oklahoma College Women

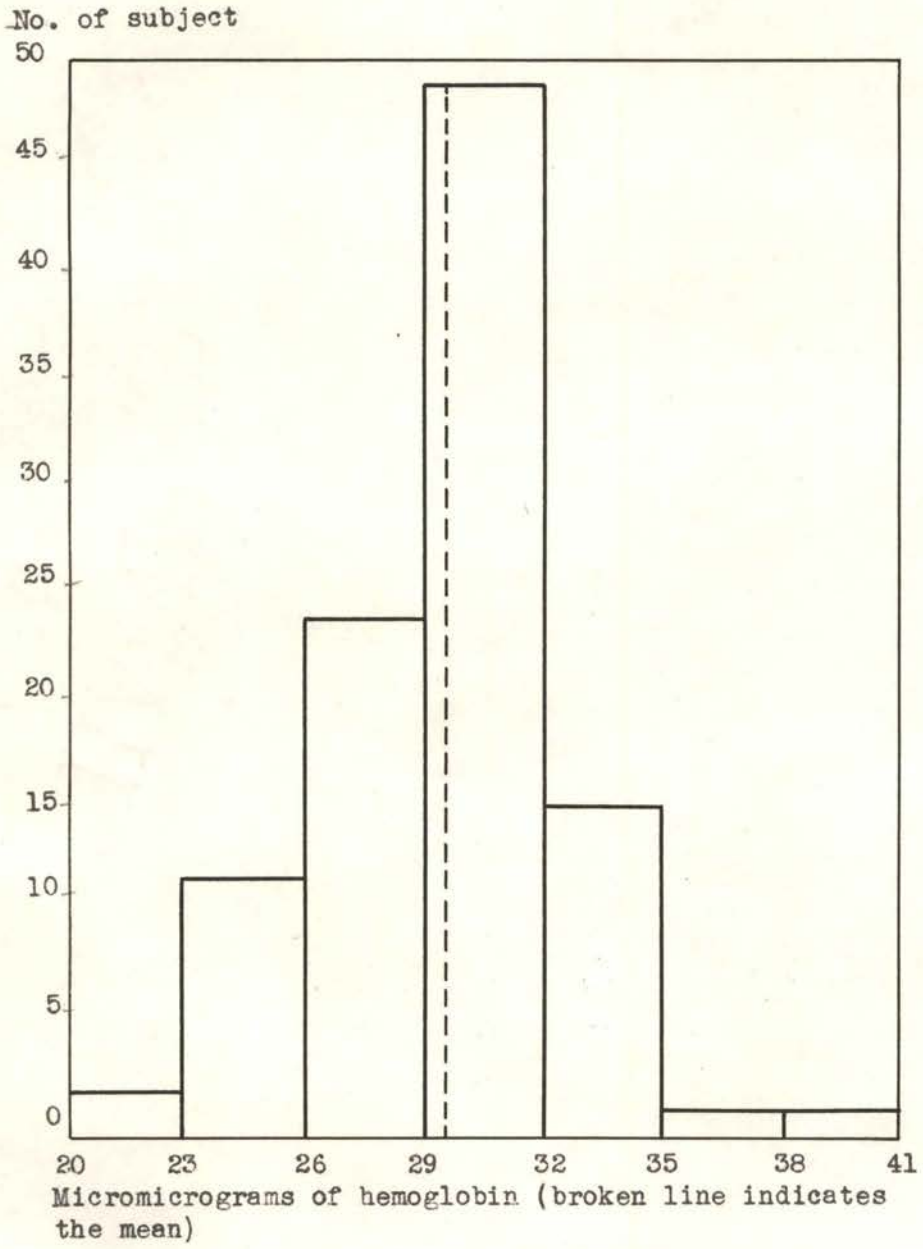


CHART 5

Histogram of the Mean Corpuscular Volume of the Blood of 101 Oklahoma College Women

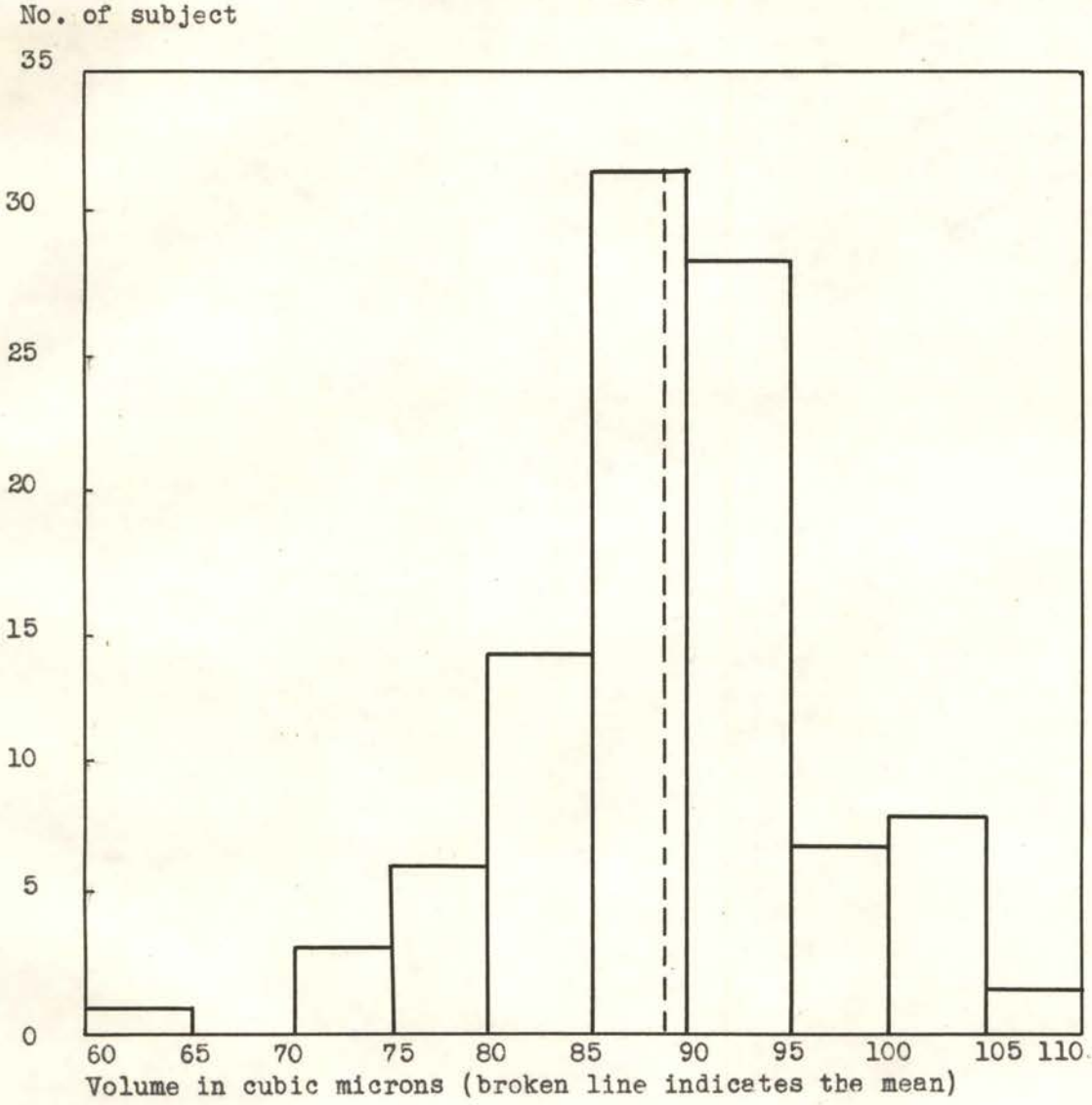
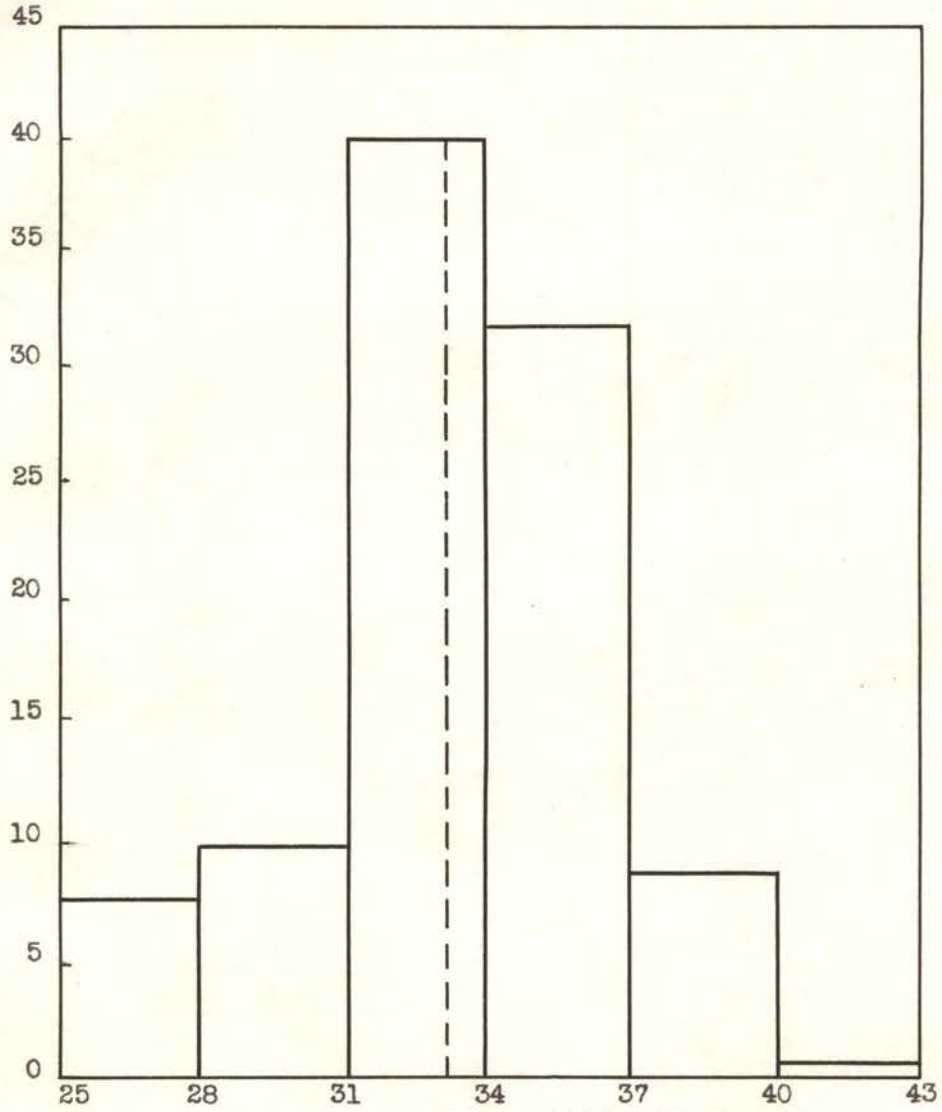


CHART 6

Histogram of the Mean Corpuscular hemoglobin Concentration of the Blood of 101 Oklahoma College Women



The eighteen and nineteen-year-old groups are the only ones containing enough subjects to be considered as entities for comparative purposes. A mean value of 4.54 million erythrocytes per cubic millimeter of blood was identical for each of these groups. For the eighteen-year-old group, the mean hemoglobin value was 13.29 grams per hundred cubic centimeters of blood and for the nineteen-year-old group it was 13.21 grams. These mean values differ by only 0.08 grams, a difference which is not significant since it is within the range of experimental error of the method. There is a wider variation in the mean values for the packed cell volume. For the eighteen-year-old group the mean is 41.3 cubic centimeters of packed cells per one hundred cubic centimeters of blood. This difference is not significant since the significance of of difference calculated by the formula, $\sqrt{\frac{\sigma_1^2}{N_1} - \frac{\sigma_2^2}{N_2}}$ is only .018, a figure less than three times the difference in the means. On the basis of these findings it seems doubtful if the difference in the mean values for the hemoglobin content of the cell, the number of erythrocytes and the packed cell volume obtained in this study can be explained on the basis of age.

Since the values for the number of red cells and the hemoglobin of the two groups are so nearly identical, it is to be expected that their mean corpuscular hemoglobin would be almost the same. The value for this constant in the eighteen-year-old group is 29.3 micromicrograms and 29.2 micromicrograms for the nineteen-year-old group. Likewise, there is a wider variation in the mean corpuscular volume

which is obviously to be expected by examination of the formula from which the constant was calculated. The mean corpuscular hemoglobin concentration for the eighteen-year-old group is 32.3 percent and 33.0 percent for the nineteen-year-old group. This difference is accounted for by the fact that there is practically no difference in the hemoglobin values for the two groups but the cell volume of the younger subjects is larger.

As a whole the values for the two age groups agree very closely and it may be concluded that there is no significant variation in the red cell values of women who are eighteen years of age and those who are nineteen years old.

A comparison of the mean values obtained from this study with those from other recent studies on women of the United States is presented in table 6.

TABLE 6

Number of Red Cells, Hemoglobin Content, Packed Cell Volume and Values for Corpuscular Constants of the Blood of Normal Women in the United States.

Authority & Location	No. of Subjects	Hemoglobin in gms. per 100cc.	Cell Vol. in cc. per 100 cc.	Red Cells in Millions per c.mm.	Corpuscular Constants		
					Hb. (γγ)	V_ol. (c.μ)	Hb. Conc. (%)
(31) Osgood and Haskins, Oregon (1927)	100	13.70	42.43	4.80	28.5	88.3	32.2
(49) Wintrobe and Miller, New Orleans (1930)	50	13.76	39.50	4.93	28.0	84.1	33.1
Haden, (17) Detroit (1932)	30	13.37	39.80	4.38	30.5	91.0	33.5
(51) Wintrobe, Baltimore (1933)	101	14.10	42.00	4.82	29.2	87.1	33.5
(1) Mugrage and Andresen, Denver (1936)	40	14.45	43.22	4.63	31.2	93.3	33.4
Barber, Oklahoma (1939)	101	13.31	40.35	4.51	29.4	89.1	33.1

As a whole the values for the Oklahoma women are slightly lower than any reported in recent years with the exception of those given by Haden (17). However, the value for packed cell volume is somewhat higher than the average given by both Haden (17) and Wintrobe (49). The differences in the mean values for cell counts, hemoglobin and packed cell volume of these studies given in table 6 cannot be explained on the basis of any one factor. Very little, if anything is known of the different factors which control the intervariations observed in the red blood cell values of healthy individuals in different parts of the country. Wintrobe (52), from an analysis of the data obtained from a number of accurate hematological studies in different parts of the United States and Europe, states that there is no significant geographical variation in the values for normal blood. It is generally accepted, however, that with an increase in altitude there is a progressive rise in the hemoglobin content of the blood and in the number of red cells. Mugrage and Andresen's (1) study seem to support these observations.

The ages of the women of this study fall within the lower limits of the age groups reported in the literature. Ten percent of the subjects were seventeen years of age, forty-six percent were eighteen years of age, thirty-three percent were nineteen years of age, and twelve percent were twenty to twenty-three years of age. Variation in the red cell values of subjects of different ages has been reported.

In 1916, Williamson (47) investigated the influence of age on the hemoglobin content of the blood and reported slight variations in the values for a group of women whose ages ranged from seventeen to fifty-five. Haden (14) stated that he believed the variations in red cell values with age reported were incorrect and that the differences were due not to age but to the lack of uniform methods. Osgood and Haskins (31) gave separate values for the red cell counts, hemoglobin, and the packed cell volume for the girls of their group who were eighteen years of age. These values were: number of cells, 4.84 million per cubic millimeter of blood; hemoglobin, 14.11 grams per one hundred cubic centimeters of blood, and packed cell volume, 41.16 cubic centimeters per one hundred cubic centimeters of blood. In comparing these figures with those for the group of eighty-eight women over nineteen years of age, it was observed that they were higher in every case except packed cell volume, which was slightly lower. Since the group of subjects who were eighteen years old was small, any conclusion as to the effect of age on red cell determinations would not be justified. In this present study no marked variation in the red cell values in the four age groups was indicated.

In comparing the average values for the corpuscular constants of the blood of the Oklahoma women with the others reported, a wider variation in the average mean corpuscular volume is found. Mugrage and Andresen (1) feel that the variation in this value cannot be explained on the basis of

the effect of altitude. To justify their conclusion they point out that the mean value of 91.2 cubic microns obtained in their study of forty men and forty women is slightly lower than that obtained by Haden (17) in a similar study of persons living at a much lower altitude. However, Mugrage and Andresen's value is higher than that suggested by Wintrobe (52) as a world average. The average mean corpuscular volume of the Oklahoma women is slightly higher than Wintrobe's (52) suggested average of 87.0 cubic microns for women but falls within his normal range of 82.0 to 92.0 cubic microns.

Further confirmation is given to the previous observation that the value for the hemoglobin concentration in the individual cell is remarkably constant in the blood of all normal persons. The mean of 33.1 percent obtained in this study is identical with that found by Wintrobe (49) in his study of southern women and is in close agreement with the others reported. As a world average for women, Wintrobe (52) suggests 33.4 percent for the mean corpuscular hemoglobin concentration of the individual cell.

The value obtained as the average mean corpuscular hemoglobin is mid-way between the mean given by Mugrage and Andresen and that given by Wintrobe as the general average. Since the mean for the number of red cells is considerably lower than any reported, with the exception of Haden's, and the mean for the hemoglobin is only slightly lower than the others, it is to be expected that the mean corpuscular hemoglobin of this study will be higher.

A comparison of the values found in this study with those suggested by Wintrobe as world averages for women is given in table 7.

TABLE 7

Comparison of the Data Obtained by Wintrobe With Those of the Present Study

	Mean	Range*	Maximum	Minimum
Red Blood Cells				
Wintrobe	4.8	4.2 to 5.4		
Barber	4.51	3.91 to 5.10 (94%)	5.34	3.71
Hemoglobin				
Wintrobe	14.00	12.00 to 16.00		
Barber	13.31	11.01 to 15.60 (94%)	16.46	9.95
Cell Volume				
Wintrobe	42.00	37.00 to 47.00		
Barber	40.35	34.15 to 46.55 (96%)	48.00	32.4
Mean Corpuscular Hemoglobin				
Wintrobe	28.8	27.00 to 31.00		
Barber	29.4	25.62 to 33.18 (92%)	39.00	20.80
Mean Corpuscular Volume				
Wintrobe	87.00	82.00 to 92.00		
Barber	89.10	75.00 to 105.2 (96%)	107.8	63.40
Mean Corpuscular Hemoglobin Concentration				
Wintrobe	33.4	32.0 to 36.0		
Barber	33.1	29.3 to 36.9 (84%)	40.0	25.2

* Range for this study represents the limits of plus and minus two standard deviations from the mean with the percentage of the cases which fall within these limits.

At the present time studies of the hemoglobin content, the number of erythrocytes, and the packed cell volume are being made on the blood of college women in Minnesota, Wisconsin, Kansas, and Iowa. These investigations are not complete but progress reports have been released. The ages of the subjects in these studies range from seventeen to twenty-five, with a majority of the cases falling within the eighteen and nineteen-year-old groups.

A comparison of the results given in these preliminary reports and the values obtained in this study of Oklahoma college women has been made and the results are given in table 8.

Table 8

Comparison of the Data Obtained in Studies of College Women in Minnesota, Wisconsin, Kansas and Iowa with those obtained in the study of Oklahoma College Women

Location	No. of Cases	Hemoglobin, in gms.	No. of Cases	Red Blood Cells, in Millions	No. of Cases	Vol. in cc.
Minnesota	84	13.30	84	4.42	84	38.49
Wisconsin	158	13.07	62	4.56		
Kansas	77	12.92	77	4.76		
Iowa	300	13.29	235	4.58	152	40.75
Oklahoma	101	13.31	101	4.51	101	40.35

In every case the values for the Oklahoma women agree very closely with those given in table 7. When these studies

are complete, the data can no doubt be pooled to establish universal standards for college women or regional standards can be set up if a need for them is indicated.

The literature concerning the daily intravariation in the values for the red cell counts, the hemoglobin content of the cell, and for the packed cell volume presents a conflicting picture. Investigation of this subject has been made in different directions such as, diurnal variation in these red cell values, the effect of menstruation, and age on hematological values, and observations on day-to-day variation in individuals. Smith (41) has observed that there is no significant diurnal variation in these red cell values. A great deal of work has been done on the effect of menstruation and there have been recorded pre-menstrual and menstrual rises and falls in the number of the erythrocytes of the blood. The literature on the intravariation in the hemoglobin content of the cell and the cell volume is limited, however.

Riech and Green (36) in 1932 presented valuable data on determinations made on six women. The number of red cells and the hemoglobin content of the blood were determined twice each week over a period of three months. From the results they obtained, these workers concluded that there was no orderly variation in the hemoglobin content of the blood and in the number of corpuscles that could be attributed to menstruation.

In 1936, Smith (42) made daily determinations of the number of erythrocytes, the hemoglobin content of the cell and the packed red cell volume of six subjects over a

period which included sixteen menstrual cycles. The results of this study showed that: (1) there is an intra-variation in the daily red cell count (the curves plotted from the data possessed waves of varying lengths with small fluctuations from day to day); (2) there is no parallelism in the fluctuations of the number of cells, the total volume of cells and the hemoglobin content, and (3) the part of the curve during the menstrual period was not different from that during the inter-menstrual portion of the cycle.

As a part of an iron balance study, Leverton and Roberts (24) made a very thorough investigation of the effect of menstruation on the number of red cells and the hemoglobin content of the blood of four college women. The tests were made daily over a period of 110 days on two subjects and over a period of 140 days on the other two women. In the entire series the differences between the averages for the menstrual cycle and the menstrual period within the cycle did not exceed the error of the experimental method. There was no consistent effect of the process of menstruation on these daily red cell values. The standard deviations for the entire series were 0.9 grams of hemoglobin per one hundred cubic centimeters of blood and 0.31 million red cells per cubic millimeter of blood. Leverton and Roberts concluded, that although marked variations may occur in the hemoglobin content and the number of erythrocytes of the blood, they do so irrespective of the different phases of the menstrual cycle.

The results of the daily determinations of the number of erythrocytes, of the hemoglobin content of the blood and the packed red cell volume of one individual are given in table 9. The corpuscular constants calculated from these values are also presented in this table.

TABLE 9

Daily Variations in Red Cell Values of One Individual

Day of Menstrual Cycle	Red Cells, in millions per c.mm	Hb. in Gms. per 100cc.	Packed cell vol. in cc. per 100cc.	Mean Corpuscular Hb. (μμ)	Mean Corpuscular Vol. (c.μ.)	Mean Corpuscular Hb. Conc. (%)
12	4.59	11.82	43.00	25.8	93.7	27.5
13	4.56	12.91	42.25	28.3	92.6	30.5
14	4.64	12.17	42.00	26.2	90.5	29.0
15	4.68	12.17	39.00	28.5	83.3	31.2
16	4.62	13.19	41.00	28.5	88.7	32.2
17	4.70	13.19	40.10	28.0	85.3	32.6
19	4.64	13.89	43.25	29.9	93.2	32.1
20	4.56	13.84	42.75	30.6	93.8	32.4
21	4.05	12.51	38.65	30.9	95.4	32.4
22	3.82	13.61	40.00	35.6	104.71	43.0
23	4.48	13.61	-----	30.4	-----	----
24	4.37	13.05	37.00	29.9	84.70	35.3
26	4.65	12.17	38.95	26.2	83.8	31.2
27	4,22	12.17	38.25	28.8	90.6	31.8
28	4.54	12.51	40.00	27.6	88.1	31.3
1	4.41	13.19	-----	29.7	-----	-----
2	4.51	12.61	40.25	30.3	89.2	33.8
3	4.51	13.75	40.50	30.5	89.9	34.0
6	4.36	10.44	42.80	23.9	98.2	21.4
8	4.44	12.91	41.00	29.1	92.3	31.5
9	4.38	13.89	41.45	31.7	41.6	33.5
10	4.35	13.33	41.50	30.6	95.4	32.1
13	4.44	13.33	41.00	30.0	92.3	32.5
14	4.32	12.51	40.90	28.9	94.7	30.6
16	4.54	13.47	40,50	29.7	89.2	33.2
17	4.22	13.33	40,85	31.6	96.8	32.6
Mean ----	4.44	12.95	40.7	29.0	91.7	31.7
Standard Deviation-	0.17	0.73	2.45	2.70	4.97	2.71
Coeffieient of Variation-	3.8%	5.6%	6.0%	9.3%	5.4%	8.5%

The values obtained from the daily determinations of hemoglobin, number of red cells and packed cell volume of the subject studied are plotted in charts 7, 8, and 9, respectively. In charts 10, 11, and 12, the curves of the daily values for the corpuscular constants are plotted.

An examination of these charts reveals that there is a daily intravariation in the red cell values. The menstrual period showed no marked effect on the blood picture. There was a decided drop in the hemoglobin level of the blood the day following the cessation of menstruation which cannot be explained. As a whole the values remained consistently high, increasing in the case of the hemoglobin, during the menstrual period. The curves are very similar to those plotted by Smith (42).

The standard deviation for the hemoglobin values during the entire series was found to be 0.70 0.1 grams per one hundred cubic centimeters of blood; and for the number of erythrocytes, 0.17 0.02 million cells per cubic millimeter of blood. These deviations are somewhat lower than those reported by Leverton and Roberts (24). The standard deviation for the packed cell volume of this study was 2.45 0.34 cubic centimeters per one hundred cubic centimeters of whole blood. The coefficients of variation for the data on the determinations made in this phase of the present study are within the limits accepted for data on physiological measurements. For the number of erythrocytes the coefficient of variation was 3.89 percent; for hemoglobin, 5.61 percent, and for packed red cell volume 6.02 percent.

CHART 7

Daily Hemoglobin Levels of One Individual

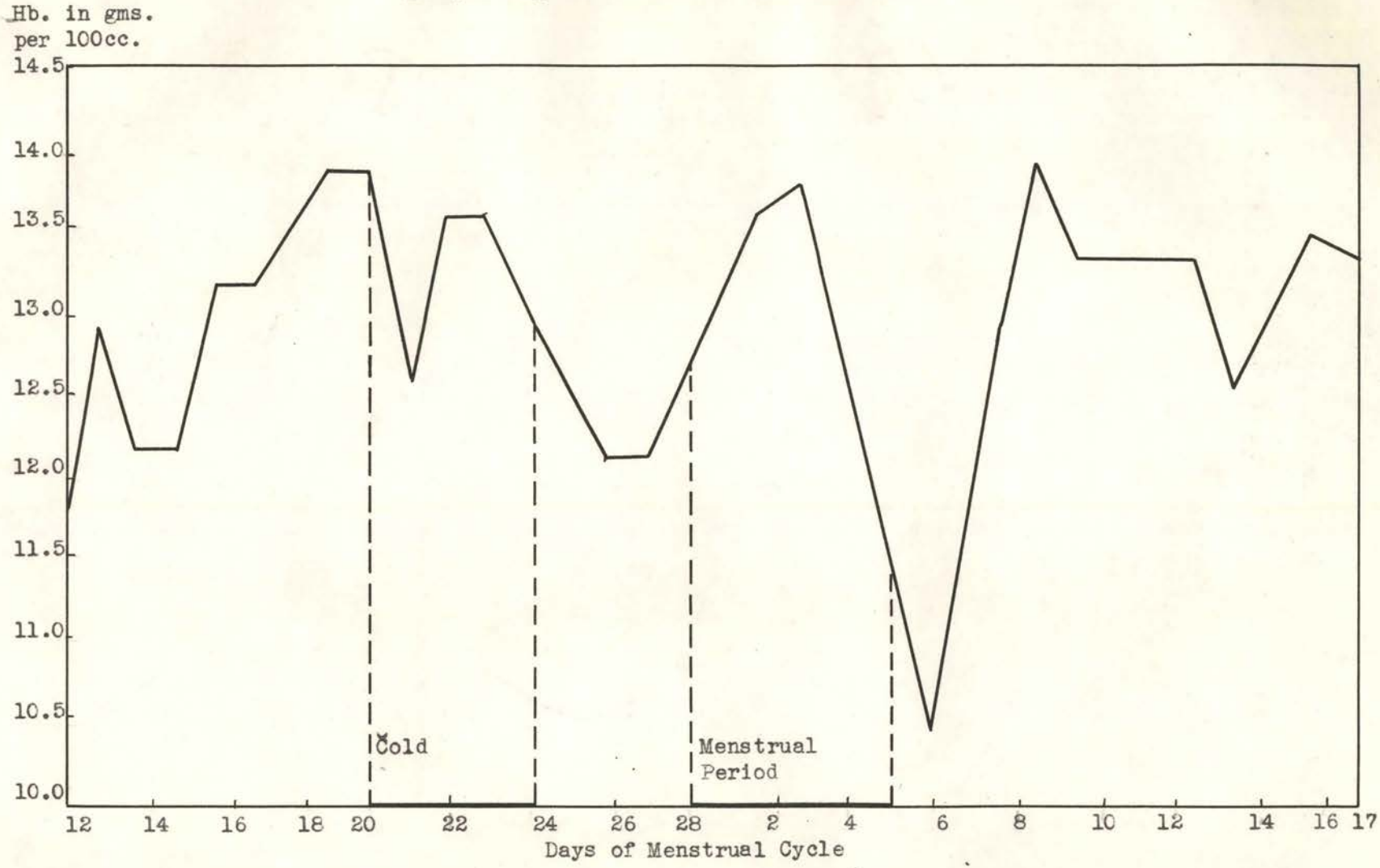


CHART 8

Daily Determinations of the Number of Erythrocytes

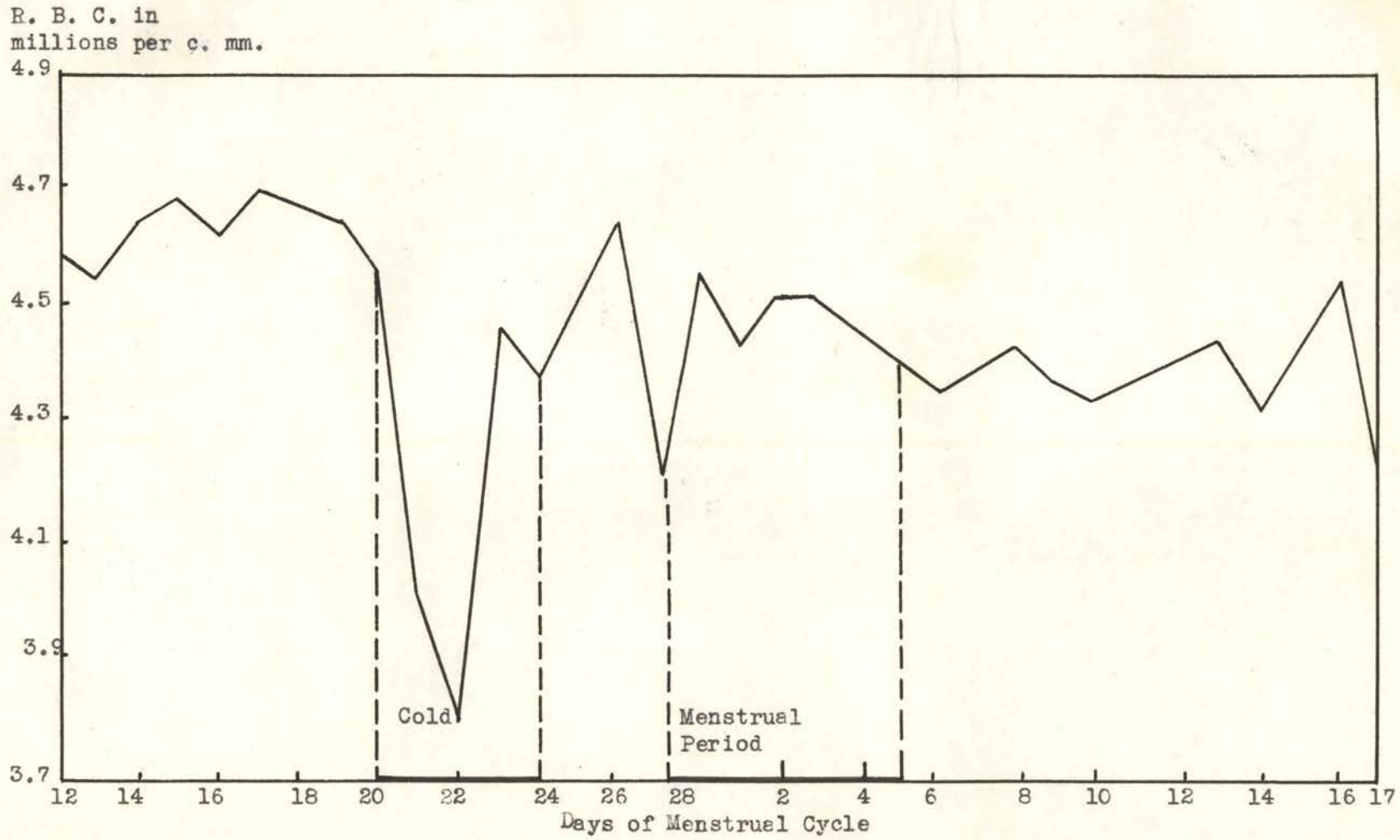


CHART 9

The Daily Packed Cell Volume of One Individual

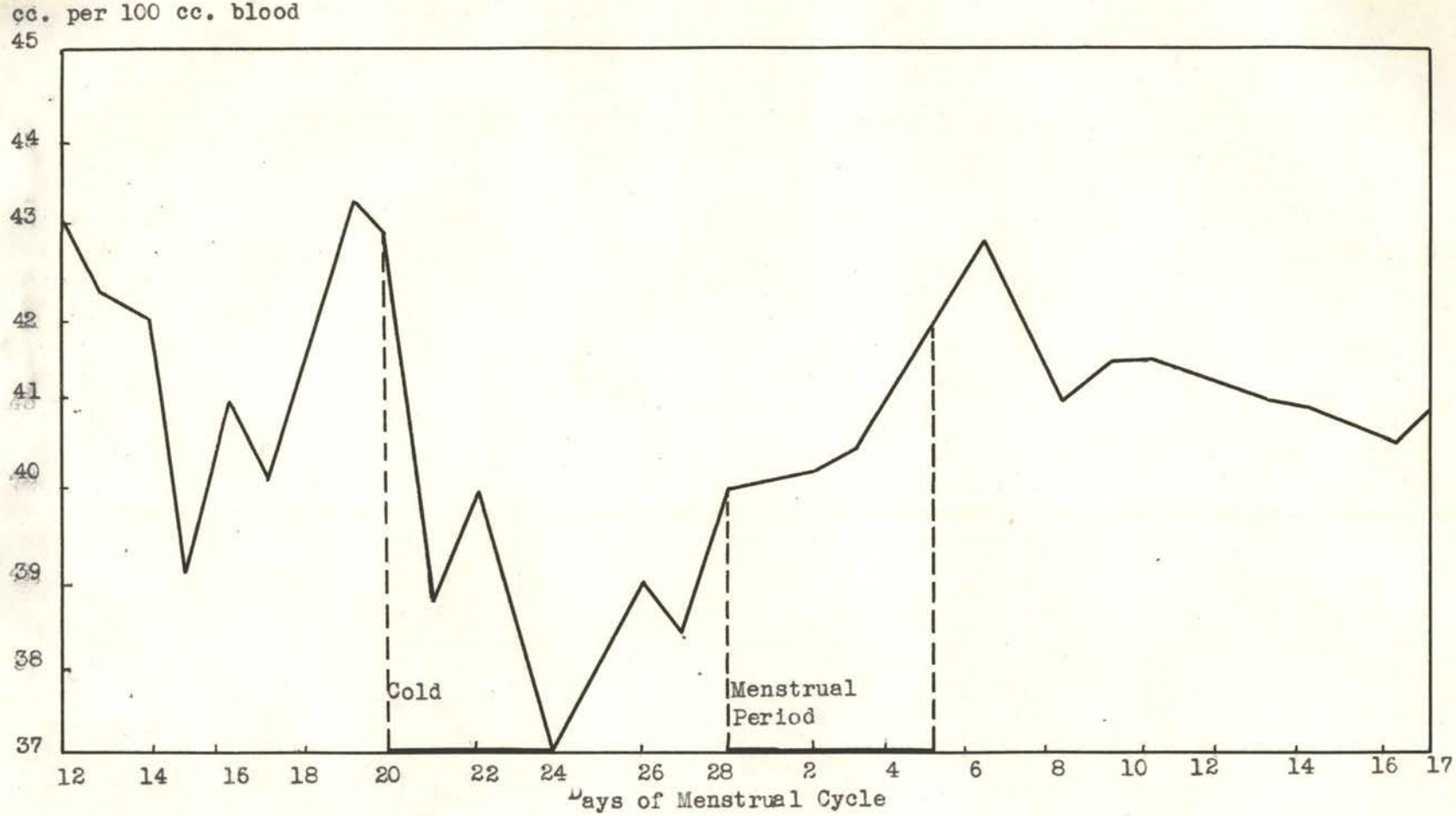


CHART 10

Daily Mean Corpuscular Hemoglobin

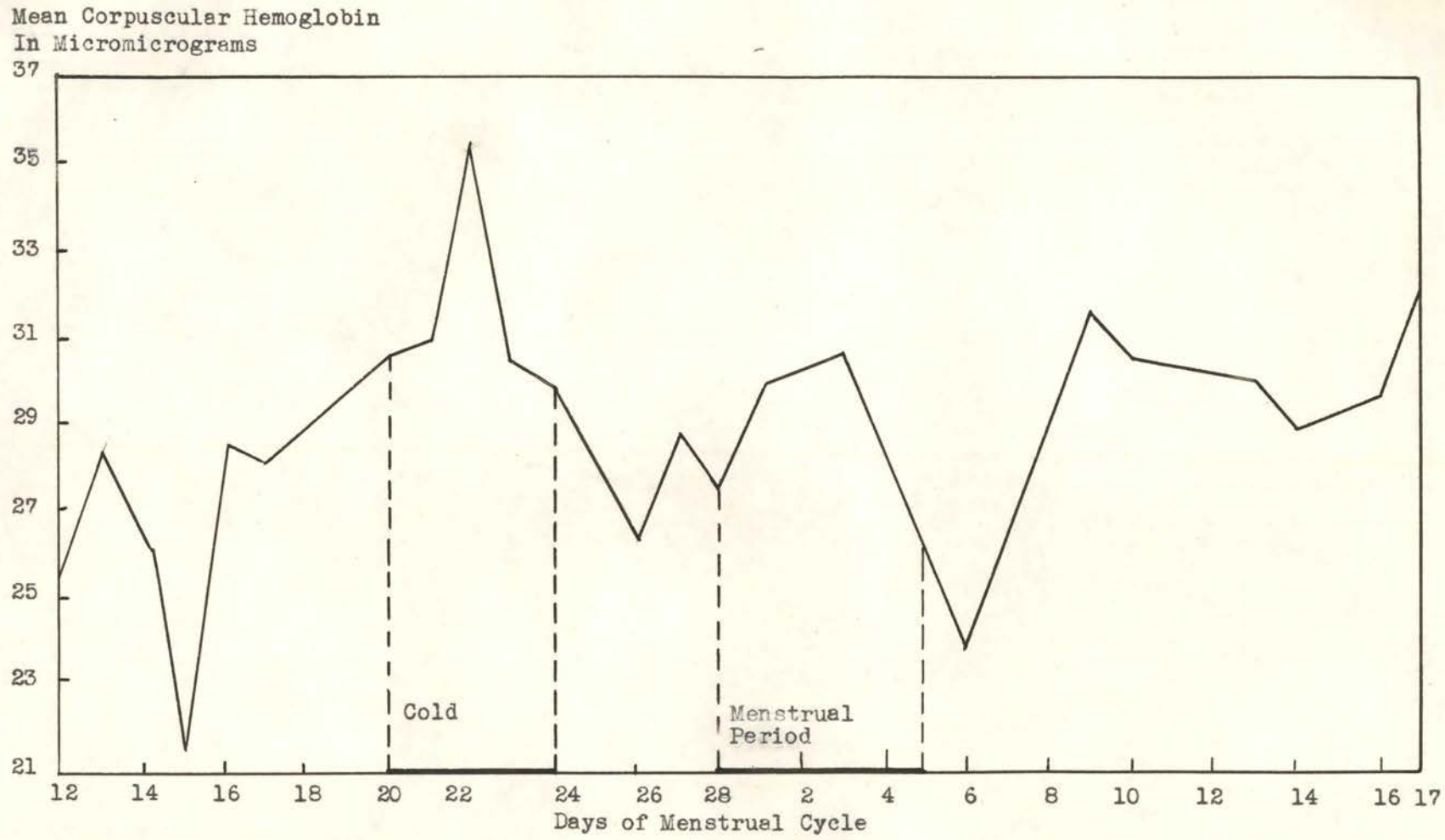


CHART 11

Daily Mean Corpuscular Volume

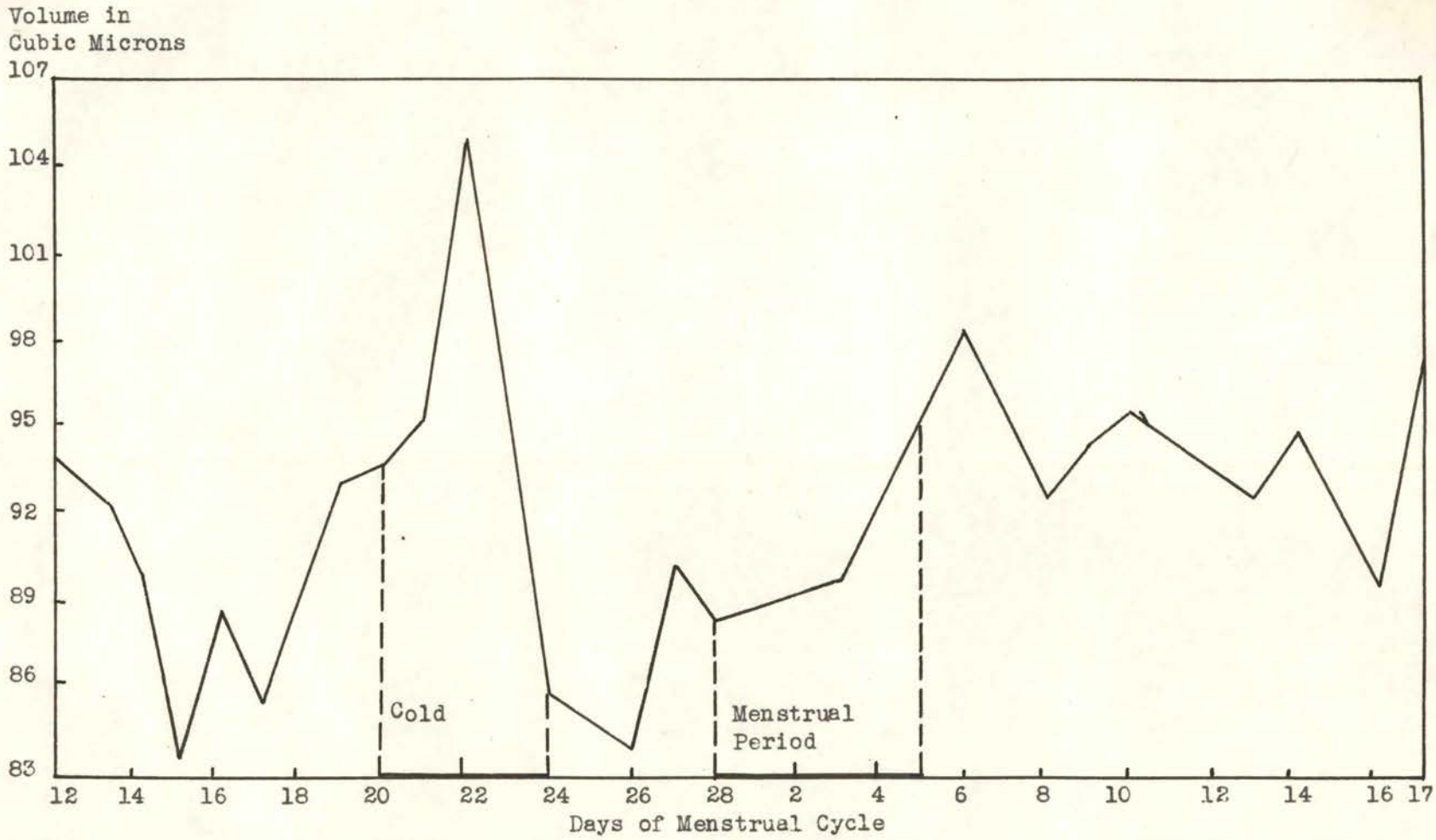
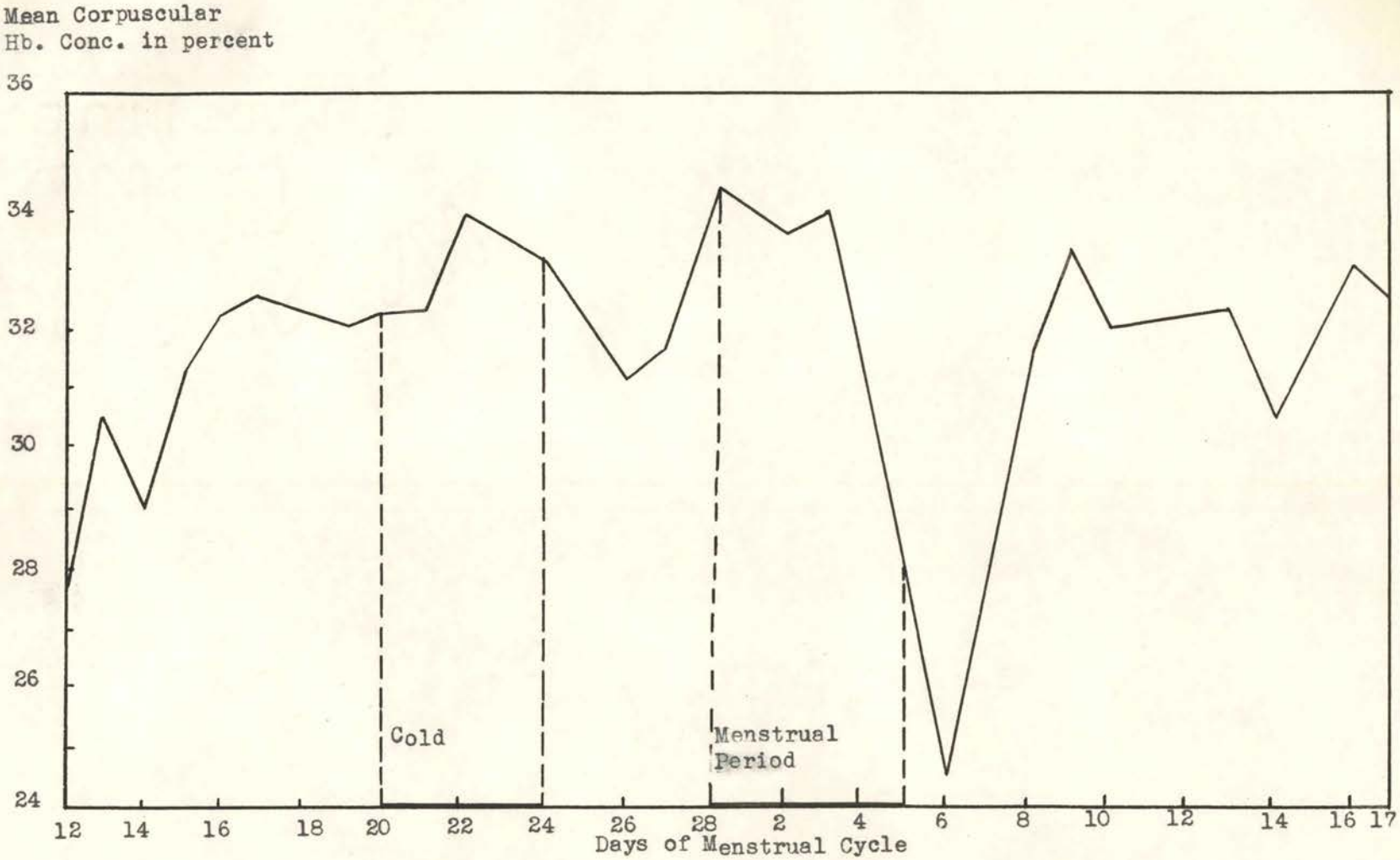


CHART 12

Daily Mean Corpuscular Hemoglobin Concentration



Marked fluctuations in the curves plotted for the corpuscular constants were found, but the portion of the curves during the menstrual period were not different from those of the inter-menstrual part of the cycle. In the mean corpuscular volume curve, there seems to be a slight consistent drop during the actual menstrual period but on no day of the period was the value lower than some women found in other portions of the cycle. The standard deviation for the mean corpuscular volume of the entire series was found to be 4.97 ± 0.73; for the mean corpuscular hemoglobin, 2.70 ± 0.38 micromicrograms, and for mean corpuscular hemoglobin concentration of the individual cell, 2.71 ± 0.40 percent. The coefficients of variation for these values are: 9.31% for mean corpuscular hemoglobin, 5.42% for mean corpuscular volume, and 8.51% for mean corpuscular hemoglobin concentration.

An interesting observation brought out in this phase of the study is the possible effect a cold may have on the red cell values. On the ninth day of the study the subject had a moderately severe cold. The following day the red cell count fell from the value of 4.56 million of the preceding day to 4.05 million and continued to decrease until the thirteenth day. Then regeneration apparently began since the count increased rather rapidly. Parallel with the fall in the number of red cells was a decrease in the packed cell volume. On the ninth day the packed cell volume was 42.8 cubic centimeters per one hundred

cubic centimeters of whole blood and the following day it fell to 36.6 cubic centimeters per one hundred cubic centimeters of blood. On the thirteenth day the value for the volume of red cells was not obtained, for no apparent reason the blood coagulated in the hematocrit tubes while centrifugation was carried out and a reading of the height of the column of packed cells was impossible. The volume reached a low of 37.0 cubic centimeters per one hundred cubic centimeters of blood on the fourteenth day and slowly increased to the normals observed before the onset of the cold. Contrary to these findings for the cell count and volume, the hemoglobin level remained consistent.

One of the group of 101 women came to the laboratory while suffering from a cold and determinations were made. The same subject was asked to return for another examination when she had recovered from the cold. The second tests were made three weeks later and an increase in the number of erythrocytes and in the cell volume was noted.

These observations do not present sufficient data to draw conclusions as to the effect of colds on the red blood cell values, but do suggest the possibility of such an effect. Apparently nothing has been published on this subject and very little work has been done on the effect of colds on any physiological function. An extensive investigation in this direction would no doubt reveal many interesting findings.

SUMMARY AND CONCLUSIONS

The quantity of hemoglobin, the number of red cells and the volume of packed cells have been determined accurately on samples of blood from 101 Oklahoma college women ranging in age from seventeen to twenty-three years, inclusive. The corpuscular constants were calculated for each subject.

The subjects were divided into four age groups, seventeen, eighteen, nineteen and twenty to twenty-three and a statistical analysis of the data for each group was made.

Histograms of the data on hemoglobin, red cell counts, packed cell volume and the corpuscular constants show that there is a marked intervariation in these values in normal subjects.

The mean values of 13.31 grams per hundred cubic centimeters of blood for hemoglobin, 4.51 million cell per cubic millimeter of blood for the red cell counts and 40.35 cubic centimeters per one hundred cubic centimeters of blood for the packed cell volume are somewhat lower than the reports given in the literature for women from eighteen to thirty years of age, inclusive. However, these values agree very closely with those given in the preliminary reports of studies on college women in Minnesota, Wisconsin, Kansas, and Iowa.

There was no significant difference in the red cell values of the eighteen-year-old group and the nineteen-year-old group.

The value for the mean corpuscular hemoglobin of 29.4

micromicrograms for this study as well as the mean corpuscular volume of 89.1 cubic microns are higher than the values suggested by Wintrobe as a general average for women.

The figure of 33.1 percent for the mean corpuscular hemoglobin concentration of the individual cell is identical with that reported by Wintrobe in his study of southern women and agrees very closely with the others found in the literature. This observation confirms previous reports that this value is consistent in the blood of the normal person.

Daily determinations of the hemoglobin, red cell counts, and packed cell volume are reported for one individual. Marked fluctuations from day-to-day were observed in the values. There was no marked variation associated with menstruation. The standard deviations and their standard errors of the entire series in this phase of the study were 0.70 ± 0.1 grams for hemoglobin, 0.17 ± 0.02 million for red cell counts, and 2.45 ± 0.34 cubic centimeters for the packed cell volume.

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