STUDIES ON EFFECTS OF VITAMIN C-DEFICIENCY ON THE NUMBER OF RED AND WHITE CELLS AND THE HEMOGLOBIN IN GUINEA PIG BLOOD

by

MARY SCHROLLER KORDISCH

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INTRODUCTION

Scurvy has been known to mankind since the sixteenth century. Prior to the twentieth century, little research had been done on this Vitamin C-deficient condition. Some of the earliest investigations dealt with the effects of Vitamin C-deficiency upon blood vessels, with little attention being paid to the actual blood constitutents. Later experiments dealt with the effects of the deficiency on bones, teeth, tendon, and muscle--all mesodermal in origin--and all subject to definite histological changes. This study was undertaken to determine whether or not the red and white blood cells and the hemoglobin undergo any variation in guinea pigs maintained on a diet deficient in Vitamin C.

Treulsen (1943) studied the effect of Vitamin C-deficiency upon skeletal muscle in guinea pigs and found there are definite histological effects which were: a gradual degeneration of muscle fibers, accompanied by a nuclear proliferation and a thinning or wrinkling of the muscle fibers, and finally a complete break down of the fibrillar structure regardless of the use of the muscle. At the same time, Ascham and Geddes (unpublished) studied the ascorbic acid content in the blood stream of these animals at the various stages of scurvy. Blood samples were taken on approximately every seventh day and the amount of ascorbic acid present calculated. This experiment revealed little difference between the Vitamin C-content of the blood of the normal animals and that of the experimental animals. In this experiment, as in preceding ones, the experimental guinea pigs,

in comparison to the normals, had little blood during the later stages of scurvy.

Hess and Fish (1914) and Brandt (1919) reported little decrease in the number of red blood corpuscles in human scurvy. Hryniewicz and Lawrynowicz (1927) likewise found no appreciable change in the number of erythrocytes. Later, Browning (1931) found a discrepancy in the numbers of red blood cells as reported by different observers. The opinion of the majority of these workers was that alterations, if any, are slight and transient. This was in agreement with an earlier paper by Wasserman (1918), who found the condition variable, with the number of red blood cells at times as abnormally low as 2,000,000 cells per cubic millimeter, but at other times, especially during convalescence, as high as 7,000,000 cells per cubic millimeter.

Sigal (1939) found general agreement that Vitamin C-deficiency causes a decrease in the number of erythrocytes. Wales, quoted by Jackson and Harley (1900), and Mether, Minot, and Townsend (1930) also found a decrease in the number of red blood cells following the removal of Vitamin C from the diet. During the first ten days of deletion of Vitamin C, Sigal found only a slight decrease in the number of erythrocytes and the hemoglobin; but during the deletion period from 10 to 21 days, anemia became severe and was accompanied by an increase in the number of leucocytes and a decrease in the number of erythrocytes and hemoglobin.

The change in leucocytes has been found to be slight and variable. Laboulbene (1900) found an increase in white corpus-

cles in human scurvy, and Wasserman (1918) observed sometimes a relative, sometimes an absolute increase in the number of lymphocytes, with an increase also of eosinophil cells. A slight increase in small lymphocytes has been reported by most workers, including DeMare and Brancato (1929), and Gasperi (1926). The number of leucocytes has been found to be below normal by Werkman and coworkers (1923), De Mare and Brancato (1929), and Gasperi (1926); and above normal by Meyer and McCormick (1928), and Sigal (1939). Findlay, however, has suggested that the absolute increase in polymorphonuclear leucocytes noted by Meyer and McCormick may have been due to a bacterial infection superimposed upon the vitamin C-deficient animals.

Most of the work on hemoglobin has been done in connection with erythrocytes; and in nearly every instance, a decrease in the percentage has been recorded. Gasperi (1926) and Hryniewicz and Lawrynowicz (1927) reported no appreciable changes in the hemoglobin content; while Findlay (1921), Meyer and McCormick (1928), Mether and coworkers (1930), and Sigal (1939) have all reported a decrease in the hemoglobin and the color index.

Randoin and Michaux (1929) considered that the hemorrhagic lesions associated with scurvy are due not only to the increased permeability of the capillaries, but also to an increasing dilution of the blood. They showed that in guinea pigs, on a synthetic Vitamin C-free diet, the water content of the blood remained normal during the first three weeks, and then began to rise rapidly from 80 per cent to 91 per cent up to the time of the death of the animal (28 to 32 days).

The literature shows disagreement among these various workers. No change, an increase, and a decrease in the number of erythrocytes, leucocytes, and the per cent of hemoglobin has been reported. This study was started to see what effect scurvy had on the number of red and white cells and the hemoglobin in the blood of guinea pigs; and to see if the findings could be correlated with any of these previous conclusions.

MATERIALS AND METHODS

Seven lots of guinea pigs (varying in number from seven to 32) were used as the subjects of this study. The number of red and white corpuscles and the hemoglobin of the blood was measured on various diets. The four diets used were Rockland guinea pig pellets with a grass supplement, the Sherman, La Mer, and Campbell (1922) diet with a grass supplement, the Sherman, La Mer, and Campbell (1922) diet with a Cebione (Vitamin C) supplement, and the Sherman, La Mer, Campbell (1922) diet, with Cebione given only to the controls. The number of leucocytes was determined by means of a Thoma (1/10) pipette, hydrochloric acid (N/10), and a haemocytometer (Levy chamber with improved Neubauer ruling). The hemoglobin index was measured by utilising some of the above solution in the Haden-Hausser hemoglobinometer. The number of erythrocytes was determined by using a Thoma pipette (1/100), Hayem's solution, and a haemocytometer.

EXPERIMENTAL DATA

Experiment I

Thirty-two animals were isolated from the colony, and on April 3, 1943, they were placed directly on the Sherman, La Mer, and Campbell (1922) Vitamin C-free diet. Vitamin C was supplied daily by feeding the animals lettuce and grass. During the first two weeks that the animals were on this diet, the number of red and white corpuscles were counted and the per cent of hemoglobin was determined. At this time a general emaciation began to occur in the animals. As this condition obviously was not normal, the animals were put back on to the regular diet of Rockland guinea pig pellets (Vitamin C-fortified) and grass. New foodstuffs were ordered and plans were made for the second experiment. The cause of the infection was attributed to the sudden change of the diet, for as soon as the animals were removed from the diet of pellets and greens, a general state of emaciation was evidenced by anarexia and alopecia.

Experiment II

This experiment was started on May 25, 1943. Twenty-three guinea pigs were removed from the diet of Rockland Vitamin C-fortified pellets and put on the Sherman, La Mer, and Campbell diet (1922), with Vitamin C administered daily in the form of Cebione apportioned according to body weight (.66 milligrams daily per 100 grams of body weight). These animals were left on this diet for five days when the first blood counts were made.

On May 30, 1943, the animals showed clinical evidences of illness, which at first were similar to symptoms of scurvy. Numbers 8, 9, and 13 were sacrificed, but post-mortem examination revealed no hemorrhagic lesions in the intestinal mucous membranes or in the intercostal regions of the chest; neither did they appear to have scorbutic symptoms in the conjunctiva of the eye; nor were there any structural defects of the teeth evidenced. Instead, autopsy revealed a generalized septecemia presented by multiple abscesses scattered throughout the viscera. A culture was made of the liquifaction necrosis of the liver and found to be teeming with some streptococci bacteria. The experiment was discontinued.

Experiment III

Twenty-three animals, all apparently in good health, were separated from the stock animals and placed on the Sherman, La Mer, and Campbell (1922) Vitamin C-free diet on June 8, 1943. The plan was to make three blood counts on each of these animals while they were on this diet and then to divide the colony into two parts. Ten guinea pigs remained on the Sherman, La Mer, and and Campbell (1922) Vitamin C-free diet with ascorbic acid administered daily in the form of Cebione pills; and the other 13 animals had Vitamin C removed from their diet.

The first of the three blood measurements of hemoglobin and red and white cell numbers was made on June 11, 1943; and the last one on July 2, 1943. At this time the experiment was discontinued, because six animals had died and others had started to show symptoms of illness accompanied by a loss of weight.

Experiment IV

On July 8, 1943, a fourth series of animals was obtained. This lot consisted of 15 mature guinea pigs, ranging in weight from 275 to 600 g, and four older ones weighing from 750 to 1,000 g. All were fed a normal diet consisting of guinea pig pellets and additional greens in the form of lettuce and spinach until they became accustomed to their environment.

On July 22, they were divided into two lots and all were fed the Sherman, La Mer, and Campbell (1922) Vitamin C-free diet. One lot was composed of seven medium sized animals and two large animals designated as controls. The regular diet of the controls was supplemented daily by Vitamin C in the form of Cebione to the amount of .66 milligrams per 100 g of body weight. (This has been calculated to be a sufficient amount for the maintenance of a normal guinea pig body). The second lot, composed of eight medium sized animals and two large animals, was designated as experimentals and they were deprived of Vitamin C after this date.

The numbers of red and white corpuscles were counted and hemoglobin measurements were made on the experimental animals approximately every third day. The same measurements were made on the controls once a week.

When the above measurements had been made and tabulated, it was noticed that Vitamin C-deficiency generally caused a decrease in the number of erythrocytes, although there were individual differences. After the curves had been plotted of the erythrocyte number, the general trend was downward in the deficient animals, for 9 out of 10 animals had curves of a descending nature following

the removal of Vitamin C from the diet (Fig. 1). Five control animals had curves with a slight upward trend, three had curves of a descending nature, and one remained nearly the same (Fig 2). Two of the three animals (12 and 13) that had curves of the descending nature, died with bacterial infection.

The numbers of leucocytes were variable, for some animals upon the Vitamin C-deficient diet reacted one way and others the opposite. Six out of 10 animals showed that the number of leucocytes was below normal when Vitamin C was removed from their diet; but three had a higher number of white corpuscles and the other remained about the same (Fig. 3). In comparison, four of the normals had a larger number of leucocytes, three had a smaller number of leucocytes and the other two maintained approximately the same number (Fig. 4).

Probably, the most noticeable result was the decrease in hemoglobin, for as soon as Vitamin C was removed from the diet, there was a definite reduction in hemoglobin with as much as a 30 per cent decrease in some cases. Nine deficient animals, at the close of the experiment, had lower hemoglobin readings while the tenth remained about the same (Fig. 5). In comparison, five normal guinea pigs had hemoglobin readings that were slightly higher than the original readings, one had the same reading, and the other three had a drop in hemoglobin percentage; however two of these animals (12 and 13) died early with bacterial infection (Fig. 6).

During this experiment as in preceding ones, there occurred a definite circulatory change, as the Vitamin C-deficiency increased. Whether it was a hydremic effect cannot be said because no

measurements were taken. The first time blood was taken from the animals, it could be taken with ease from any peripheral vessel of the ear. As the experiment continued, blood, in some cases, could not be obtained from the marginal vessels; but had to be taken from a portion of the body nearer the heart.

Whether the change was due to a collapse of the peripheral blood vessels, a decrease in the volume of the blood, or a shift of the volume of the blood from the peripheral portions to the central, more vital portions, is not known. It is merely known that a definite circulatory change occurs.

The animals were apparently normal in these first four series until the regular Sherman, La Mer, and Campbell (1922) diet was introduced and ascorbic acid was administered in the form of "Cebione". Abnormalities and diseased animals soon resulted.

When the animals in series two appeared abnormal, numbers 13 and 14 were posted. Post-mortem examination revealed multiple foci of abscesses scattered throughout the mesenteries, spleen, pancreas, and intestine which is an indication of generalized septecemia. Pneumonia was suspected so the lungs were examined but they were of a normal consistency, texture, and color. There was no evidence of consolidation, hepatization, or edematous swelling which would have indicated a pneumonic condition. Therefore, the early death of these animals was not due primarily to the deficiency of Vitamin C, but from secondary factors envolved.

Paralleling this Vitamin C-deficiency, there was a lowered resistance of the animal's body and generalized metabolic reduction which made the animals susceptible to a secondary invasion

of bacteria.

From research and laboratory experience, it is known that the body is a constant reservoir for bacteria at all times. In a state of health these bacteria remain dormant, but in a vitamin-deficiency, these bacteria take up an active state and the animals soon become diseased.

Table 1. Showing the number of erythrocytes per cubic millimeter of guinea pig blood in the animals of Experiment IV.

Animal Number	Counts of erythrocytes							
	Vitamin C		Deficient in Vitamin C					
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1	5,860	5,690	5,540	5,230	5,500	5,500	4,800	died
2	6,820	6,930	6,430	6,340	6,930	6,730	6,260	died
3	6,190	6,100	5,760	6,410	4,870	4,760	died	
4	5,320	5,830	6,160	5,230	6,070	5,220	died	
5	5,860	5,650	5,750	5,620	5,730	5,470	5,320	died
6	4,600	4,790	5,180	5,100	4,850	died		
7	5,860	6,040	5,760	4,500	5,750	3,090	3,900	died
8	6,590	5,810	6,140	6,430	6,340	6,450	died	
18	5,970	5,980	6,170	5,550	5,380	5,900	6,020	died
19	6,820	6,100	5,900	5,140	died			
	Normal Intake of Vitamin C							
9	5,440	5,390	5,430	5,270				
10	6,230	6,010	5,980	5,410	5,740			
11	5,180	5,960	5,960	5,530	5,280			
12	4,900	5,800	5,890	4,010	4,530	4,310	died	
13	6,090	5,910	4,950	5,830	died			
14	6,210	4,870	4,570	4,630	died			
15	7,870	5,550	6,760	5,870	5,910			7,570
16	5,100	5,640	5,420	5,230				5,250
17	5,930	5,510	5,920	5,590				5,700

Table 2. Showing the number of leucocytes per cubic millimeter of guinea pig blood in the animals of Experiment IV.

Animal Number	Counts of leucocytes							
	Vitamin C		Deficient in Vitamin C					
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1	3,250	5,150	5,050	7,000	4,200	3,500	4,000	died
2	7,200	6,450	6,800	6,950	7,850	8,450	10,600	died
3	6,400	6,550	5,750	5,750	4,550	3,800	died	
4	7,850	5,100	6,350	3,800	4,200	5,050	died	
5	10,500	8,300	11,700	6,350	6,450	5,250	6,800	died
6	10,300	6,700	7,300	9,650	8,600	died		
7	5,800	7,250	5,350	7,950	8,350	9,850	5,050	died
8	4,950	8,250	5,650	9,450	6,100	4,500	died	
18	12,400	13,600	11,500	10,550	7,500	11,000	7,950	
19	7,300	7,600	8,000	9,500	died			
	Normal intake of Vitamin C							
9	6,200	5,000	8,000	7,000				
10	5,700	6,000	5,750	5,450	6,000			
11	7,600	7,600	8,400	9,850	11,400			
12	6,300	6,800	6,600	4,850	3,750	3,050	died	
13	5,700	3,600	2,900	1,300	died			
14	7,750	6,600	6,850	4,450	died			
15	11,400	8,350	6,500	7,700	5,900			8,800
16	8,850	11,500	8,900	11,350				8,500
17	8,050	8,300	9,450	15,850				10,600

Table 3. Showing the per cent of hemoglobin in guinea pig blood in the animals of Experiment IV.

Animal Number	Per cent of hemoglobin							
	Vitamin C		Deficient in Vitamin C					
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1	87	91	87	78	71	66	63	died
2	93	87	81	81	84	86	87	died
3	81	84	78	68	76	63	died	
4	81	82	78	81	78	80	died	
5	82	82	84	73	71	78	75	died
6	80	82	80	78	75	died		
7	82	78	81	86	84	78	60	died
8	87	85	78	78	87	84	died	
18	80	84	81	80	73	83	73	died
19	81	75	73	81	died			
			Normal intake of Vitamin C					
9	78	78	75	81				
10	82	84	81	80	84			
11	86	81	77	84	87			
12	81	71	78	78	77	77	75	died
13	68	67	71	73	died			
14	84	81	78	78	died			
15	80	81	81	81	84			91
16	87	89	84	84				81
17	87	83	78	81				87

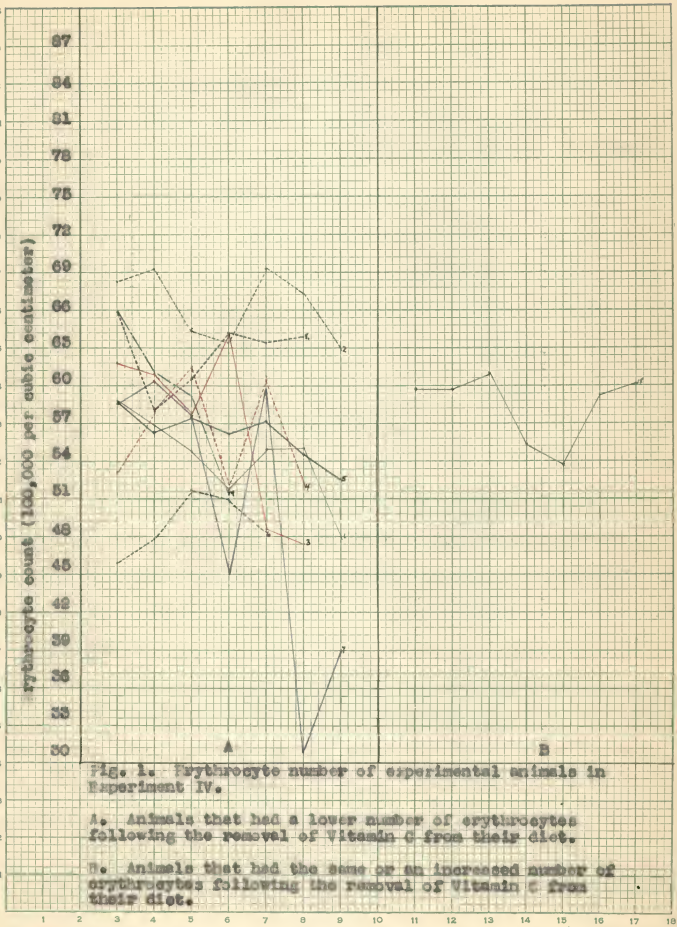

Fig. 1. Erythrocyte number of experimental animals in Experiment IV.

A. Animals that had a lower number of erythrocytes following the removal of Vitamin C from their diet.

B. Animals that had the same or an increased number of erythrocytes following the removal of Vitamin C from their diet.

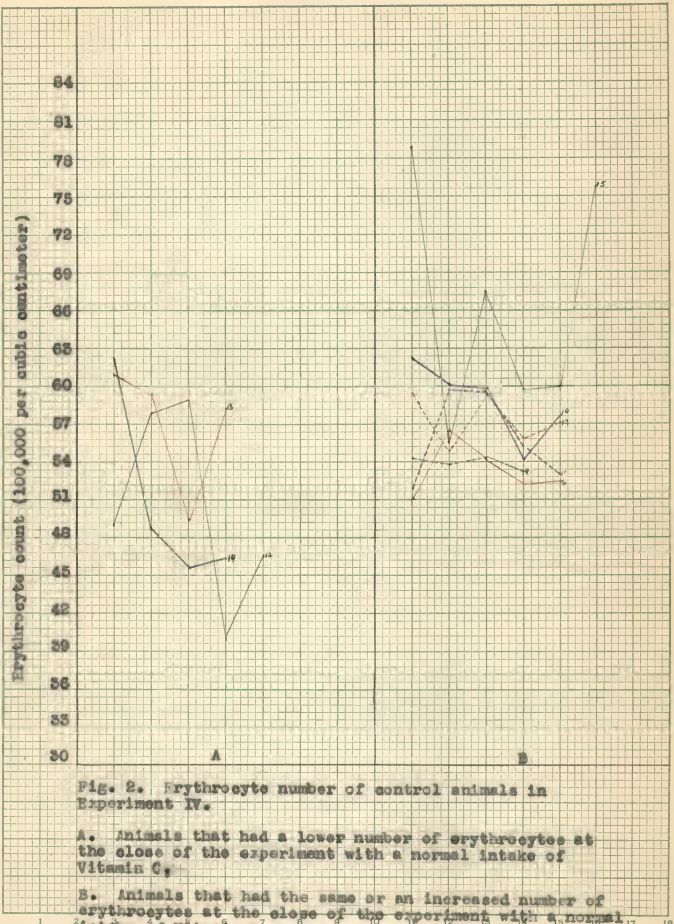

Fig. 2. Erythrocyte number of control animals in Experiment IV.

A. Animals that had a lower number of erythrocytes at the close of the experiment with a normal intake of Vitamin C.

B. Animals that had the same or an increased number of erythrocytes at the close of the experiment with a normal intake of Vitamin C.

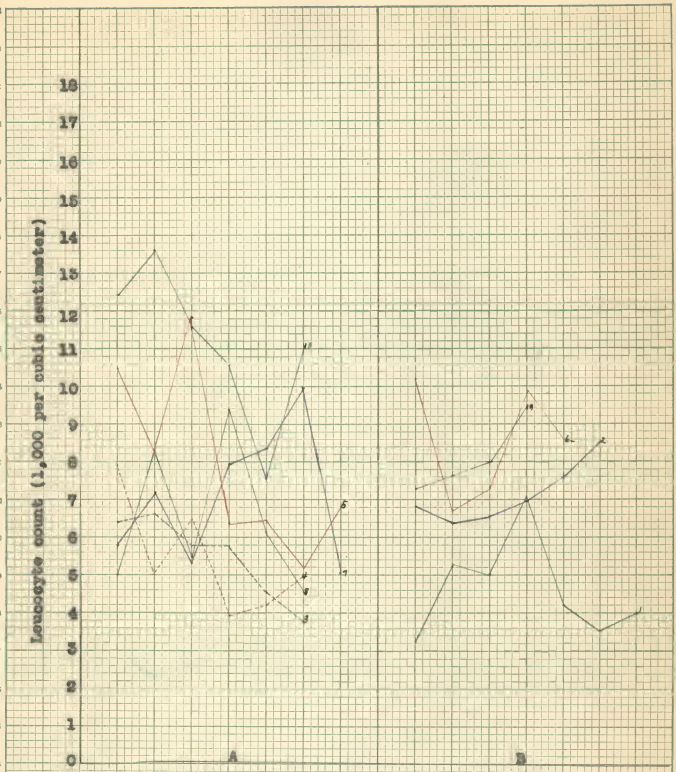

Fig. 3. Leucocyte number of experimental animals in Experiment IV.

A. Animals that had a lower number of leucocytes following the removal of Vitamin C from their diet.

B. Animals that had the same or an increased number of leucocytes following the removal of Vitamin C from their diet.

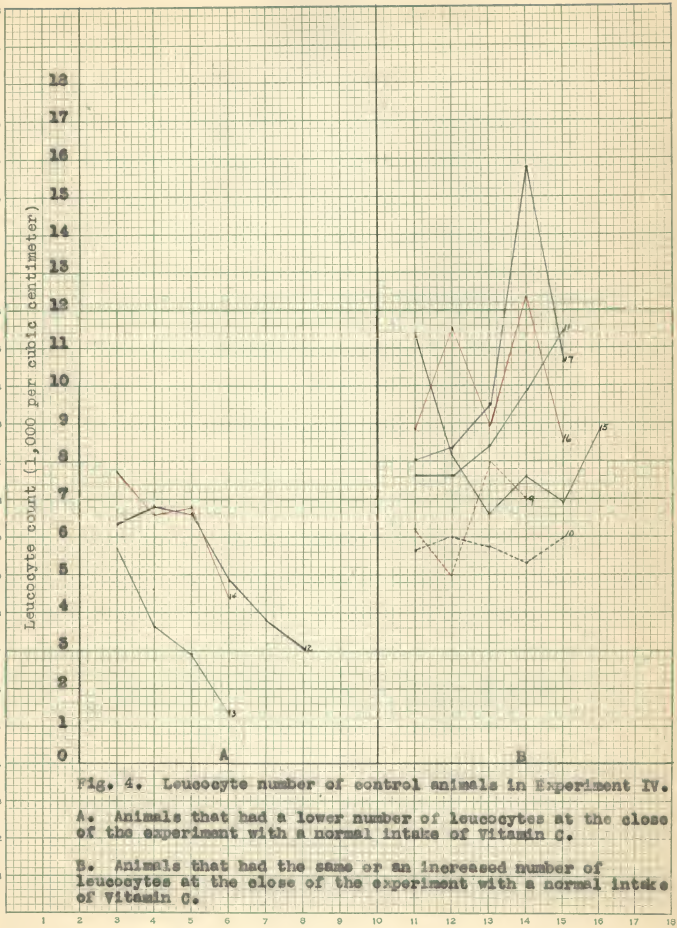

Fig. 4. Leucocyte number of control animals in Experiment IV.

A. Animals that had a lower number of leucocytes at the close of the experiment with a normal intake of Vitamin C.

B. Animals that had the same or an increased number of leucocytes at the close of the experiment with a normal intake of Vitamin C.

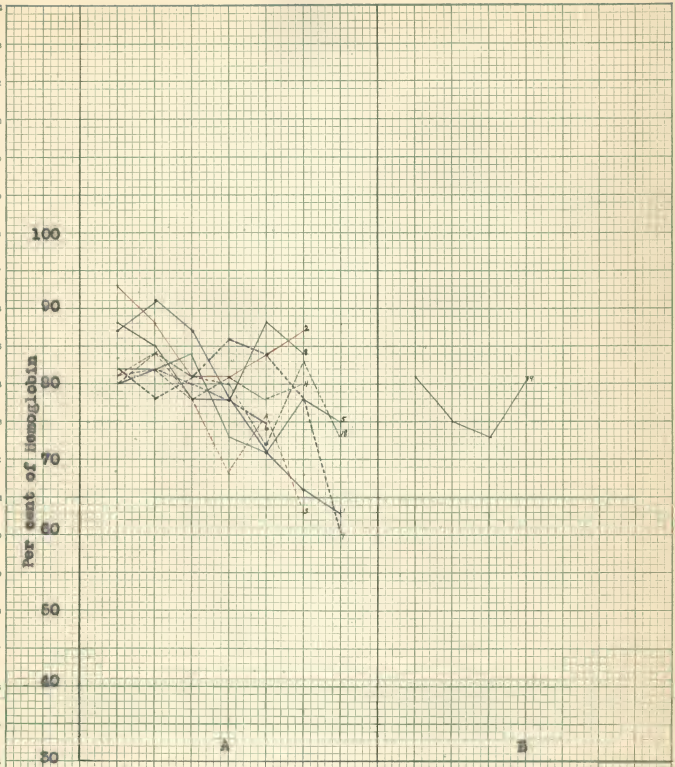

Fig. 8. Hemoglobin of experimental animals on Experiment IV.

A. Guinea pigs of Experiment IV that had a lower hemoglobin percentage following the removal of Vitamin C from their diet.

B. Guinea Pigs of Experiment IV that had the same or an increased hemoglobin percentage following the removal of Vitamin C from their diet.

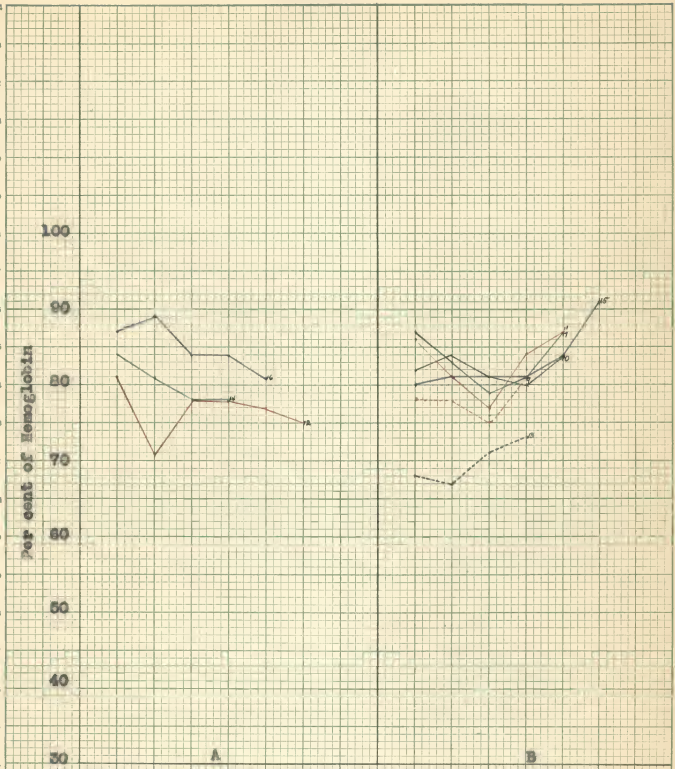

Fig. 6. Hemoglobin of control animals in Experiment IV.

A. Guinea pigs of Experiment Four that had a lower hemoglobin percentage at the close of the experiment with a normal daily intake of Vitamin C.

B. Guinea pigs of Experiment Four that had the same or an increased percentage of hemoglobin at the close of the experiment with a normal daily intake of Vitamin C.

Experiment V

Eight animals were chosen for this experiment, six large females and two males. The plan of this experiment was similar to preceding ones. The animals were first placed on the stock diet of Rockland Vitamin C-fortified guinea pig pellets and gradually the Sherman, La Mer, and Campbell (1922) Vitamin C-free diet was introduced until the animals were entirely on the latter diet. After the guinea pigs had been on this second diet for two weeks, their hair became shaggy, sparsely scattered, and they appeared dirty and greasy. In addition, they had lost considerable weight.

The guinea pigs were again examined; it was uncertain whether the disorder was due to infection or nutrition, or a combination of the two. Food experts were consulted and new skim milk was ordered. In addition, it was decided to use a supplement of dried yeast (three per cent of the diet weight per day). After four days under these conditions, the animals showed no apparent improvement, and had a greater loss of hair and weight. This experiment was then discontinued.

Experiment VI

Twelve animals, all apparently in good condition, were placed on the experiment. This lot included four large males, four medium sized males, and four medium sized females. These guinea pigs soon showed signs of infection again, so the entire colony was powdered for lice, and all the cages were disinfected with pine oil.

Two more animals were posted, and it was decided again that the animals were suffering from a generalized septecemia. All the animals were given doses of anti-bacterial serum (Bovine origin). The dosage administered varied from 1 to 3cc depending upon the size and condition of the animal; the serum was given subcutaneously and massaged thoroughly to afford immediate absorption and aleviate the possibility of any unfavorable reaction. In the evening, the eyes of all the guinea pigs were bathed with a two per cent solution of boric acid in an effort to remove the exudate which had accumulated in the canthi. The eyes of the guinea pigs also showed the presence of opacities brought on by the presence of cataracts which were of a superficial nature. The animals showed a slow daily improvement, following the serum injection, but the experiment was discontinued.

Experiment VII

This series of animals consisted of nine middle-sized guinea pigs ranging in weight from 480 g to 770 g. From March 28 to June 8, 1944, they were fed the normal diet of Rockland guinea pig pellets and greens. The numbers of red and white

corpuscles were tabulated and hemoglobin measurements made.

On June 9th, these animals were placed on the Sherman, La Mer, and Campbell (1922) Vitamin C-free diet with a supplement of greens. June 22nd, they were placed back on the pellets again and three more measurements of hemoglobin and corpuscle numbers were made. They were placed on the Sherman, La Mer, Campbell (1922) Vitamin C-free diet again on July 8th. Vitamin C was administered in the form of lettuce and spinach. On every third day, erythrocyte and leucocyte counts were made and hemoglobin percentages determined.

On July 21, 1944, the guinea pigs were left on the Sherman, La Mer, and Campbell (1922) Vitamin C-free diet; but the Vitamin C was administered daily in the form of Cebione according to body weight (.66 milligrams per 100 g of body weight). Three more measurements of hemoglobin and numbers of erythrocytes and leucocytes were taken while the animals were on this diet.

On August 5th, the animals were divided into two lots. Numbers 4 and 5 were designated as normals, and they were placed on the Sherman Vitamin C-free diet with Cebione administered daily in proper amounts. Animals number 1, 2, 3, 7, and 8 were called the deficient or experimental animals. Counts of the blood corpuscles and hemoglobin measurements were made on these animals on approximately every second day, until all the experimental animals had died with scurvy. This experiment was completed with no complications resulting from infection. Animals numbered 6 and 9 died early in the experiment.

Erythrocytes. The removal of Vitamin C brought about a

decrease in the number of erythrocytes as the animal approached a death due to scorbutus. During the first three weeks of the Vitamin C-deficiency, the guinea pigs at some time showed an increased number of red blood cells; but it seemed to be a matter of individual difference as to which week, the greatest number of erythrocytes per individual were present. The maximum number of red corpuscles (in three animals out of five) was generally tabulated at the end of the first week. After the crest had been reached, a general decline in the number of erythrocytes ensued until the animal succumbed. The decreases (from the first day the animal was deprived until the last day it lived) recorded were No. 1, 1,120,000 cells per cubic millimeter; No. 2, 2,300,000; No. 3, 1,710,000; and No. 8, 1,910,000 cells per cubic millimeter. In comparison, the two control guinea pigs showed neither a rise nor a fall in the number of erythrocytes (Fig. 7).

Leucocytes. The number of leucocytes was variable; but, in general, the guinea pigs had an increased or decreased number of white corpuscles depending on the length of time they had been deprived of Vitamin C. During the first week, four out of five guinea pigs had an increase in the number of leucocytes; during the second week, four guinea pigs out of five had a decreased number of leucocytes; during the third week, four out of five animals had an increase in the number of leucocytes; and during the fourth week, the number of leucocytes of each animal increased until the time the animal died. The highest number of white corpuscles generally occurred a few hours previous to the death of the animal. The normal animals maintained a uniform number of leucocytes throughout the experiment (Fig. 8).

Hemoglobin. As in Experiment IV, after the numbers of red and white cells had been tabulated and the hemoglobin measurements had been completed, the most noticeable change was the drop in hemoglobin percentage. As the degree of scurvy increased, the percentage of hemoglobin decreased; and at the termination of the experiment, every Vitamin C-deficient guinea pig had a marked decrease in the hemoglobin reading. The percentage decreased as follows: No. 1, 11 per cent; No. 2, 33 per cent; No. 3, 31 per cent; No. 7, 25 per cent; and No. 8, 18 per cent. In comparison, at the close of the experiment, the normal guinea pigs had hemoglobin readings that were slightly higher. No. 4 had a three per cent higher reading, and No. 5 had a one per cent increase (Fig. 9).

Circulatory Change. In the foregoing experiments, there appeared to be a definite circulatory change as the period of Vitamin C-deficiency increased; and the blood needed for the last measurements could no longer be taken from the peripheral vessel of the ear, but in four animals out of five, blood had to be taken from a vessel nearer the central portion of the body of the guinea pig. In contrast, blood for the cell counts and hemoglobin measurements of the normal guinea pigs could be drawn as easily the last day of the experiment as the first.

Table 4. Showing the number of erythrocytes per cubic millimeter of guinea pig blood in the animals of Experiment VII.

Number of Count	Number of erythrocytes (divided by 1,000) per animal						
	Rockland guinea pig pellets and greens						
	(1)	(2)	(3)	(7)	(8)	(4)	(5)
1	6,190	6,560	5,560	5,910	5,890	5,970	5,760
2	5,890	6,190	6,210	5,600	6,520	5,970	5,210
	Sherman, La Mer, and Campbell (1922) diet and greens						
3	5,890	6,800	5,750	5,850	6,220	5,630	5,000
4	6,040	6,020	5,720	5,910	6,330	5,760	5,600
5		5,900		5,790			
	Sherman, La Mer, and Campbell (1922) diet and Cebione						
6	5,950	5,900	5,750	5,800	6,200	5,600	5,360
7	5,900	6,070	5,550	5,850	6,300	5,620	5,700
8	5,950	6,000	5,650	5,810	6,220	5,640	5,450
	Sherman, La Mer, and Campbell (1922) diet						
	No Cebione					Cebione	
9	5,180	6,450	5,900	5,840	7,090	5,400	5,670
10	5,680	5,920	5,860	7,110	6,210	5,560	5,570
11	5,620	5,940	5,520	5,380	6,820	5,600	5,540
12	5,810	5,980	5,410	5,530	6,490	5,480	5,400
13	5,290	5,490	4,670	4,890	5,970	5,550	5,490
14	6,530	5,590	6,010	5,320	5,550	5,630	5,500
15	5,200	5,210	5,510	5,520	5,590	5,680	5,450
16	6,950	5,360	5,090	4,840	5,180	5,600	5,550
17	5,120	5,860	5,160	4,750		5,650	5,650
18	6,060	4,760	5,320	3,710		5,580	5,580
19	4,060	4,150	3,190			5,600	5,680

Table 5. Showing the number of leucocytes per cubic millimeter of guinea pig blood in the animals of Experiment VII.

Number of Count	Number of leucocytes per animal						
	Rockland guinea pig pellets and greens						
	(1)	(2)	(3)	(7)	(8)	(4)	(5)
1	8,800	7,000	10,300	9,800	9,700	7,400	4,000
2	11,900	7,500	8,900	9,100	7,800	7,500	4,800
	Sherman, La Mer, and Campbell (1922) diet and greens						
3	11,200	7,800	8,000	8,900	7,750	7,700	4,700
4	10,800	7,350	8,550	9,400	8,100	7,800	6,200
5		6,750		9,100			
	Sherman, La Mer, and Campbell (1922) diet and Cebione						
6	10,400	7,400	9,300	9,800	7,600	7,500	5,900
7	10,100	7,600	8,750	9,300	7,000	7,400	5,600
8	10,300	7,200	9,200	9,600	7,400	7,500	5,100
	Sherman, La Mer, and Campbell (1922) diet						
	No Cebione					Cebione	
9	10,500	9,550	8,600	8,600	8,750	7,200	5,550
10	11,500	10,350	11,100	13,400	10,200	7,500	5,650
11	13,300	12,700	9,250	12,800	9,600	7,250	5,350
12	7,750	7,500	11,850	11,500	13,000	7,350	5,300
13	8,750	9,550	11,000	10,950	9,850	7,200	5,300
14	10,800	6,050	8,800	8,750	8,650	7,600	5,700
15	10,600	8,200	11,150	10,100	12,000	7,400	5,600
16	12,100	8,350	11,050	11,100		7,500	5,700
17	13,750	8,650	10,500	14,750		7,400	5,550
18	13,300	8,650	11,950	17,150		7,550	5,550
19	14,400	9,100	12,200			7,400	5,750

Table 6. Showing the percent of hemoglobin in guinea pig blood in the animals of Experiment VII.

Number of Count	Percent of hemoglobin per animal						
	Rockland guinea pig pellets and greens						
	(1)	(2)	(3)	(7)	(8)	(4)	(5)
1	87	80	86	83	87	85	87
2	73	83	85	75	83	80	80
	Sherman, La Mer, and Campbell (1922) diet and greens						
3	83	86	80	85	93	78	78
4	83	87	86	84	84	83	87
5		86		84			
	Sherman, La Mer, and Campbell (1922) diet and Cebione						
6	84	84	84	84	86	94	87
7	86	84	90	84	83	87	87
8	87	87	91	87	86	87	85

	Sherman, La Mer, and Campbell (1922) diet						
	No Cebione					Cebione	
9	86	86	86	80	86	83	86
10	81	87	84	71	87	84	87
11	78	81	74	68	83	84	87
12	75	81	78	78	81	84	87
13	81	75	85	80	80	87	87
14	81	84	83	79	75	87	87
15	83	81	83	78	68	87	84
16	78	60	81	60		84	87
17	82	60	74	80		87	84
18	87	58	63	80		87	87
19	75	53	55			87	87

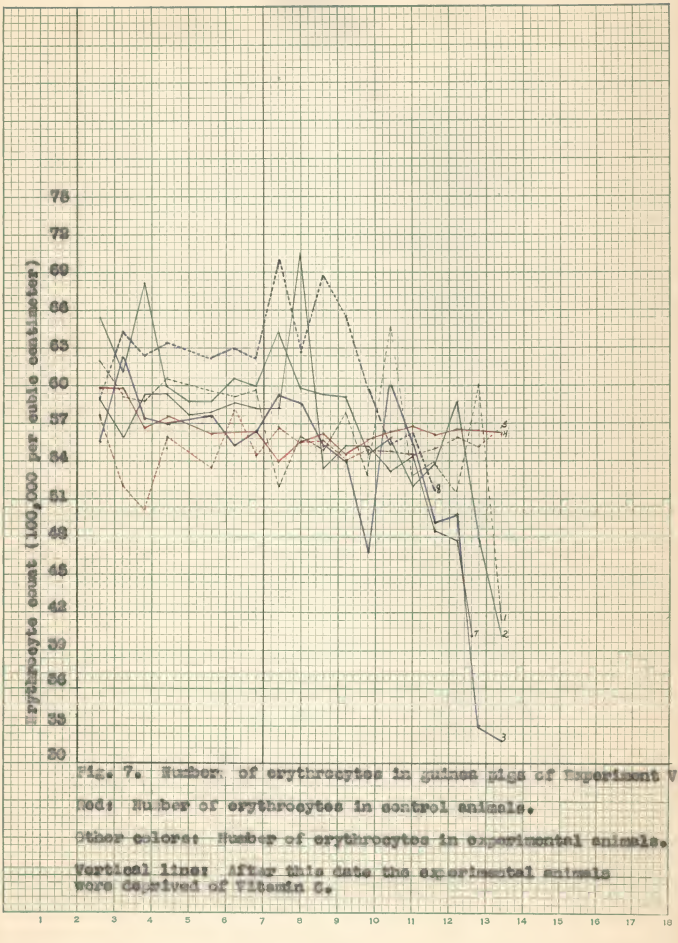

Fig. 7. Number of erythrocytes in guinea pigs of Experiment VI.

Red: Number of erythrocytes in control animals.

Other colors: Number of erythrocytes in experimental animals.

Vertical line: After this date the experimental animals were deprived of Vitamin C.

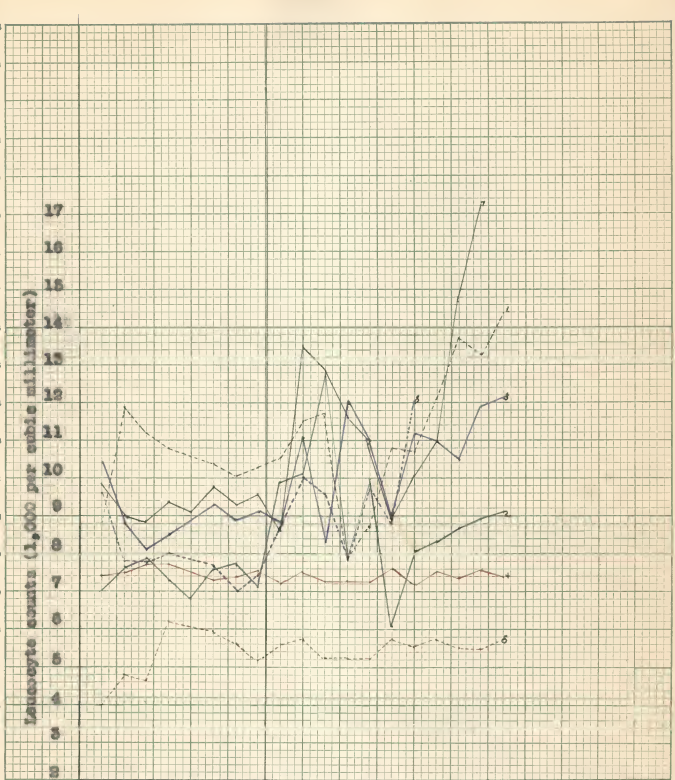

Fig. 8. Number of leucocytes in guinea pigs of Experiment VII.

Red: Number of leucocytes in control animals.

Other colors: Number of leucocytes in experimental animals.

The vertical line: After this date the experimental animals were deprived of Vitamin C.

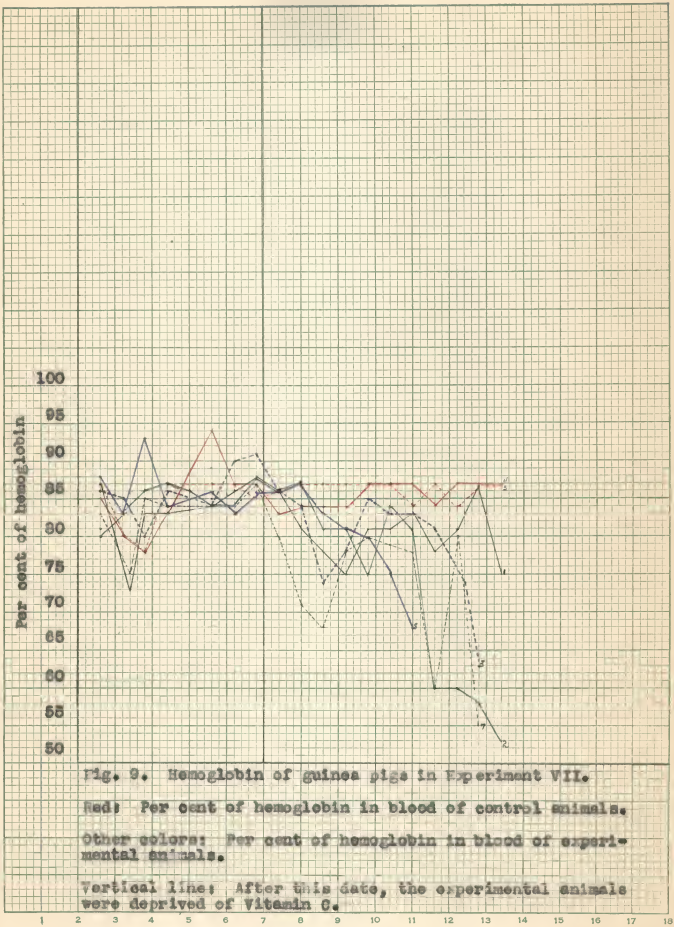

Fig. 9. Hemoglobin of guinea pigs in Experiment VII.

Red: Per cent of hemoglobin in blood of control animals.

Other colors: Per cent of hemoglobin in blood of experimental animals.

Vertical line: After this date, the experimental animals were deprived of Vitamin C.

DISCUSSION

Erythrocytes. The results derived from these experiments correspond closely to the findings reported by other investigators. Like those of Mether and coworkers (1930), Gasperi (1926), or Findlay (1921), it was found that Vitamin C-deficiency generally causes a decrease in the number of erythrocytes; because 14 out of 15 Vitamin C-deficient animals, in experiments four and seven, had a lower number of red corpuscles previous to their death from scurvy than they had on the day ascorbic acid was removed from their diet. In these same two experiments on the final days, five control guinea pigs had a slight increase in the number of erythrocytes, three had a decreased number (two of these died early with a bacterial infection), and the other three animals retained approximately the same number throughout the experiment.

In neither experiment did the number of red blood cells drop as low as 2,000,000 cells per cubic millimeter as recorded by Wasserman (1918). The lowest count in the present experiments was 3,090,000.

Leucocytes. With respect to the number of leucocytes, the effect produced by the Vitamin C-deficiency seemed to be a matter of individual difference. The results were similar to those of Werkman and coworkers (1923), De Mare and Brancato (1929), and Gasperi (1926), who found the number of leucocytes to be below normal after Vitamin C was removed from the diet; but the conclusions also agree with those of Meyer and McCormick (1928) and Sigal (1939), who reported an increase in the number of leucocytes

as the period of deficiency increased. The difference was determined by the period of deletion of Vitamin C. After the first few days of deletion, there occurred a rise in the number of leucocytes until about the seventh or tenth day; then, by the end of the second week of the deletion a decrease generally occurred. (Two guinea pigs had an increased number of leucocytes, five had approximately the same number, but eight had a decreased number). During the third week of deletion, an increase in the number generally occurred. (Nine experimental animals had an increased number of white blood cells, four had a decreased number, and two maintained approximately the same number).

Most of the animals died during the third week. Those that lived to the fourth week of deletion, had variable counts. (Four of the experimental animals had a decreased number of white corpuscles and nine animals had an increased number). The highest number of leucocytes recorded during the life of the guinea pig was generally recorded a few hours previous to the time the animal died as a result of scurvy.

Hemoglobin. In both Experiment IV and VII, the hemoglobin had the lowest reading at the close of the experiment. Fourteen out of 15 animals had a decrease in the percentage of hemoglobin as the deficiency increased. Some had readings lowered as much as 33 per cent. The results of these experiments disagree with the findings of Casperi (1926), and of Hryniewicz and Lawrynowicz (1927), who reported no appreciable changes in the hemoglobin content following the removal of Vitamin C from the diet; but, the findings are in close agreement with those of Findlay (1921),

Meyer and McCormick (1928), and Mether and coworkers (1930) who reported a decrease in the hemoglobin percentage as the Vitamin C-deficiency increased.

These results closely agree with the conclusions of Sigal (1939) who stated that during the first 10 days of deletion, there occurred a slight decrease in the number of erythrocytes and hemoglobin percentage; but during the deletion period from 10 to 21 days, anemia had become severe and was accompanied by a decrease in hemoglobin and an increase in the number of leucocytes.

SUMMARY

1. A study was made of 126 guinea pigs to determine effects of Vitamin C-deficiency upon the blood of the animals.

2. In the various stages of scurvy, the number of erythrocytes varied considerably in the different guinea pigs, but the general rule was a reduction of erythrocytes as the Vitamin C-deficiency increased.

3. There was a tendency for variations to occur in the number of leucocytes depending upon the length of time the animal had been deprived of Vitamin C. During the first week of Vitamin C-deficiency, the number of leucocytes increased; during the second week, the number decreased; during the third week, the number of leucocytes generally began to increase and continued to do so until the animal died as a result of the deficiency.

4. The Vitamin C-deficiency caused a decrease in the percentage of hemoglobin.

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