THE RELATIVE VITAMIN A CONTENT OF LEAF AND HEAD LETTUCE

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The Relative Vitamin A Content of Leaf and Head Lettuce

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THESIS

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THESIS

INTRODUCTION

Some investigations have yielded results which indicate that the vitamin A content of plant and animal tissues is correlated positively with the degrees of their pigmentation. This being true a fresh vegetable plant which contains small amounts, or none, of the pigments would also be low in vitamin A.

This is an important question with many vegetable crops since they are marketed in both green and blanched states. The older standards of quality for salad crops were mainly appearance, crispness, and palatability. However, there is an increasing tendency to recognize the vitamin content of such crops in determining their quality, thus including their food value in the concept of quality. Hence, if a vegetable which contains small amounts of pigments also contains less vitamin A than those more pigmented, it would rate lower in quality according to the more modern standard.

Since lettuce is an important salad crop, experiments aimed to determine whether or not head and leaf lettuce which differ very materially in the amount of pigment contained also differ in vitamin A content were undertaken and completed. Experiments were also conducted to determine the relative vitamin A content of parts of the same plant which differed greatly in the amount of chlorophyll contained and also to determine the

effect growing plants under glass would have on the vitamin A content. The results are reported herein.

Review of Literature

In 1920 Steenbock and Gross (12) examined a number of plants for their fat-soluble vitamin content and concluded that the leaves are richer in this vitamin than any of the other structures of the plant. They found that the white leaves of cabbage and the somewhat etiolated leaves of lettuce were poorest in vitamin A content of all the plants they examined. They assumed as a working hypothesis for further investigations that where yellow pigments are found in plants one may look for the presence of the fat-soluble vitamin.

Delf (3) is quoted as saying, that same year, that the inner white leaves of cabbage do not contain growth promoting vitamins.

Osborn and Mendell (10) reported experiments in which white rats were fed oily residues from ether extracts of dried spinach leaves, young clover, alfalfa, and grass. The residues when fed daily in quantities equivalent to 1 to 2 grams of the dried plant promoted recovery and renewal of growth in rats that were declining in weight on diets deficient in the fat-soluble vitamin.

Coward and Drummond (1) in 1921 while working with cabbage, etiolated seedlings, dried seed, algae,

and fungi found that dried seed generally are deficient in vitamin A and that the amount does not appear to be increased by germination. Etiolated seedlings and white leaves of cabbage apparently did not synthesize the vitamin. Green leaves from the outside of cabbage heads were found to contain large amounts of vitamin A. Lower plants containing chlorophyll (marine algae) were found to synthesize this dietary factor while those (mushrooms) devoid of pigments which play a role in photosynthesis were found to be almost completely deficient in the vitamin.

In 1921 Drummond, Coward, and Watson (4) stated that the diet of a cow is undoubtedly the chief cause of variation in the amount of vitamin A in the milk and butter. The butter was richer in this vitamin when the cow was on good pasture than when stall-fed on dry feeds of hay, roots, and cake. Even the drying up of the pasture in summer lowered the vitamin A content of the butter. This indicated that drying green plants in sunshine reduced the vitamin A content of the foliage.

Wilson (17) reported in 1922 that rats grew as well when fed on wheat seedlings sprouted in the dark as on those sprouted in the light. The seedlings were dried, powdered, and fed in quantity equal to 5 per cent of the diet. This amount represented a quantity of seed that would be inadequate as a source of vitamin A. He concluded that "vitamin A is produced in the growing plant with or

without accompanying photosynthesis." He criticized Coward and Drummond's work (1) stating that it only showed that green tissue synthesized the vitamin more rapidly than the etiolated tissue and that the quantity of etiolated tissue which they fed the animals was insufficient to show the smaller production in these plants. It was noticed, however, that etiolated tissues were slightly active even in the small quantities used by them.

In 1922 Steenback and Sell (13) found that the green inside leaves of cabbage, which had failed to head properly, contained ten times as much green pigment as leaves on the inside of good heads and were far richer in vitamin A, although the white leaves gave considerable growth when fed in amounts equal to 10 per cent of the diet. They concluded that the fat-soluble vitamin is present in more than the minimal demonstrable amounts in the white leaves of cabbage heads.

The work of Coward (2) in 1923 showed that light is necessary for the synthesis of vitamin A. The synthesis takes place in the light from an electric light bulb. Chlorophyll does not seem to be necessary for the synthesis of this food factor and the synthesis will be carried on in an atmosphere which does not contain either oxygen or carbon dioxide. The almost complete absence of calcium from the water solution did not prevent the formation of vitamin A in the plants. Plants synthesized the

vitamin in sunlight that had passed through a plate glass window and a glass bell jar which probably removed most of the ultra-violet rays. The results from this work reconfirmed her previous work showing that etiolated plants contain much smaller amounts of vitamin A than green plants. This worker commenting on the work of Wilson pointed out the fact that the amounts fed by him were more than the minimal required to produce growth therefore there was no difference in rate of growth on the two kinds of tissues. The conclusions drawn from the experiments reported in Coward's paper show that the conditions for the synthesis of vitamin A are not the same as the conditions necessary for photosynthesis. However, it seems that the conclusions were based on insufficient evidence. The number of animals used in each experiment was usually small and the experiments were very seldom continued for more than four weeks. The plants grown in atmospheres devoid of oxygen and carbon dioxide were likely to be abnormal and the amounts fed were not weighed quantities. It seems that there is need of more investigation along this line before the conditions necessary for the synthesis of this vitamin are definitely known.

In 1925 Steenbock, Sell, and Boutwell (14) reported work in which six samples of peas were investigated and it was found that those of green color and carrying

considerable yellow pigment were far richer in their fat-soluble vitamin content than yellow peas which contained much less yellow pigment. They supported the theory advanced by Steenbock and Gross in 1920, that the fat-soluble vitamin is biologically related to certain yellow plant pigments. They disregarded the green pigments whose presence was much more evident than that of the yellow pigments. It seems that they might as surely be associated with the vitamin A.

The available experimental evidence does not permit a positive conclusion regarding the relationship between the vitamin A content of plant tissues and the intensity of their pigmentation. It can not be said, as has been pointed out by other investigators, that the vitamin is always associated with certain yellow or green pigments. However, it can be said safely that the vitamin is usually very abundant in tissues containing large amounts of such pigments.

Materials and Methods

In determining the vitamin A content of a substance, the general method is to feed a weighed quantity of the material to be tested to a standard animal fed on a basal diet adequate in all other respects for a standard time. The animals used in all the experiments reported in this paper were albino rats of high grade stock and known pedigree. The young animals were from families fed on the

following stock ration:

Whole milk powder	15 per cent
Cracked wheat	25 per cent
Oat meal	25 per cent
Yellow corn meal	25 per cent
Flax seed meal	10 per cent

Fresh lettuce daily, except Sunday (not a weighed amount).

The above ration is the one used by Dr. M. Dye of the Home Economics Department, Michigan State College, in growing stock and experimental animals. It provides enough vitamin A to produce strong, healthy litters and yet not enough to enable the young animals to store large amounts in their bodies.

The test animals were selected at weaning time from uniform litters of from five to ten animals when they were 28 to 30 days old and weighed from 28 to 50 grams each. These were placed in separate wire cages in a well lighted, ventilated, and heated laboratory. Each rat was fed on a well balanced basal diet, complete in every respect except that it contained no vitamin A. This basal diet was composed of the following ingredients:

Irradiated cornstarch	78 per cent
purified casein	18 per cent
salt mixture (McCollum's No.32)	4 per cent
dried yeast	about 500 mg. daily, except Sundays.

Commercial "Cream" cornstarch was activated by exposing it to the actinic rays of a 110 volt, 4 ampere, alternating current Copper Hewitt quartz mercury vapor lamp for 15 minutes at a distance of 14 inches. The cornstarch was put in a layer about one inch deep, in large shallow zinc pans and was stirred well every four or five minutes during the process of irradiation. Enough was irradiated each time to last two weeks. Hess and Wienstock (7) stated that vegetable oil became activated on exposure to the mercury vapor lamp for two minutes, or less, and retained its protective power for at least six months. Dutcher and Kruger (5) showed that dextrine, made from commercial cornstarch possessed marked calcifying properties.after being treated with ultra-violet light. Steenbock and others (16) observed that starch became activated when treated with ultra-violet light while antirachitic activation could not be induced in purified protein. Some investigators dextrinize their cornstarch but in these experiments it was fed raw and was relished by the animals. The activation seemed to be sufficient,for none of the animals showed any symptoms of rickets.

The casein was purified by Sherman and Munsell's (11) method, with slight modifications. 800 grams of finely ground, dry, crude casein was placed in a three liter flask, two liters of ethyl alcohol was added and this was boiled under a reflux condenser for one and one-half hours and quickly filtered, while still hot, through a suction filter.

This operation was repeated twice, the casein thereby receiving three one and one-half hour extractions with boiling alcohol. After having been filtered the third time it was left in the Buchner funnel and washed with two liters of hot alcohol. When the alcohol had ceased dripping from the funnel, the casein was spread in layers about one-half inch deep in shallow pans and left in a warm room until the alcohol evaporated. It was then placed in a gas oven and heated at a temperature of 100 degrees C. for eight hours, being well stirred every two or three hours so that all of it would be exposed to the oxygen of the air while being heated. Finally it was removed from the oven and ground to a fine powder in a Hobart pulverizer.

The salt mixture used in the basal diet was McCollum's number 32 mixture composed of the following ingredients:

Sodium chloride	4.7 per cent
Magnesium sulphate	7.2 per cent
Sodium di-hydrogen phosphate	9.4 per cent
Potassium mono-hydrogen phosphate	25.8 per cent
Mono-calcium phosphate	14.6 per cent
Calcium lactate	35.1 per cent
Ferric citrate	3.2 per cent

The yeast was prepared by crumbling up Fleischman's yeast cake and heating it in an oven at 100 degrees C. for several hours, until perfectly dry, and then grinding it to

a fine powder. The amount fed was not weighed but measured in a ladle that held approximately 500 milligrams.

After being placed on a vitamin A deficient ration the animals continued to grow for from five to eight weeks, usually seven weeks. When their weight remained constant for a week or two, or they were losing weight, they were given the lettuce which was to be tested. Many of the animals at that time had xerophthalmia, an eye disease caused by the deficiency of vitamin A in the diet, before they were given the vitamin containing food. Some of the animals continued to gain in weight after showing definite symptoms of xerophthalmia. All of them showed that they were suffering from a deficiency of this vitamin by the color of th the skin of their ears and tails and by the appearance of their fur in addition to the fact that they had ceased gaining and most of them were losing weight.

One animal from each litter was retained on a vitamin A deficient ration and used as a negative control, or check animal. Sherman and Munsell (11) stressed the importance of negative controls in determining whether the basal diet is free from vitamin A.

The materials tested in these experiments are leaf lettuce (Lactuca sativa var. crispa) .and head lettuce (Lactuca sativa var. capitata). Leaf lettuce of the Grand Rapids Forcing variety was used in all experiments with leaf lettuce, while all the head lettuce was of the Iceberg type. The head lettuce was purchased on the local market, it being impossible to grow satisfactory

heads in this locality either in the field or the greenhouse.

Fresh lettuce was secured from one to three times each week, or as often as necessary to avoid the use of wilted leaves. Data are not available on the effect of storage on the vitamin A content of vegetables. In an effort to eliminate that factor, as much as possible, the leaf lettuce was harvested and stored in an ice box with the head lettuce each time a new supply was purchased. The portion of lettuce that was fed to rats was taken from about an inch margin of green leaves of leaf lettuce and the well blanched, inside leaves of firm heads of lettuce, so that the large midribs would not be contained. The green outside leaves of head lettuce were discarded except in experiment three where both blanched and green parts of this plant were fed. Any variation from the above procedure will be stated later.

Preliminary experiments were carried on to determine the amount of lettuce to feed each day to secure an average weekly gain of three grams. Sherman and Munsell (11) suggested three grams per week as a suitable standard for the gain an animal should make. Eddy (6) shows that work done by Munsell indicates that 600 to 700 mg. of lettuce daily is necessary to produce 25 grams gain in eight weeks. The type of lettuce fed by Munsell was not mentioned. Using Munsell's figures as a basis the animals were placed in eight groups and fed the following amounts: Group 1, 500 mg. leaf lettuce; group 2, 500 mg. head lettuce; group 3, 700 mg.

leaf lettuce; group 4 , 700 mg. head lettuce; group 5, 1 gram leaf lettuce; group 6,1 gram head lettuce; group 7, 2 grams leaf lettuce; group 8, 2 grams head lettuce. The results from this test showed that all the animals had made excessive gains and from the gains made it seemed the amount necessary to secure the desired growth was about 300 mg. So this amount was fed in experiments one and two.

In all experiments an abundance of the basal diet and water were kept constantly before the animals. The lettuce, a fresh supply of food, and fresh water were supplied daily, except Sundays.

PRESENTATION OF DATA

Experiment 1.

This experiment was designed to determine if there is any difference in the vitamin A content of leaf lettuce and the blanched, inside leaves of head lettuce. Each animal receiving lettuce was fed 300 mg. of fresh tissue daily, except Sunday. Thirteen animals were fed leaf lettuce, twelve head lettuce, and eight (negative controls) were given no lettuce. The control animals were the last to cease gaining in weight on the basal diet which indicates that they were probably a little more vigorous than the other animals. The experiment was continued for a period of eight weeks. The data are set forth in tables 1, 2, and 3 and are shown graphically in Figure 1.

Table 1.- Growth of Animals Receiving No Vitamin A.

Animal and Sex	Original weight (grams)	WEEKLY GAINS (in grams)								Total gains	Average weekly gains
		First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Seventh week	Eighth week		
228B-♀	51	1	3	2	-3	-2	-10	Dead	---	-9	-1.50
222B-♀	66	2	3	1	0	1	- 3	-6	-12	-14	-1.75
229H-♀	76	2	1	-1	-1	-1	-20	Dead	---	-20	-3.33
221D-♀	66	0	0	-0	-2	1	4	-2	-12	-11	-1.37
217B-♀	102	7	2	3	5	5	0	-2	-7	13	1.62
216A-♂	88	-2	4	-3	Dead	--	--	--	--	-1	-0.33
216D-♂	90	0	0	0	-1	2	-1	1	-13	-12	-1.71
221C-♂	55	1	1	-1	Dead	--	--	--	---	1	0.33
Totals (grams)	600	11	14	1	-2	6	-30	-9	-44	-53	
Ave.gains (grams)	75	1.37	1.75	0.12	-0.33	1.0	-5.0	-2.25	- 11.0	-6.6	1.06

Table3 Growth of Animals Fed Bond Lettuce for Vitamin A Supply.

Table2 Growth of Animals Fed Head Lettuce for Vitamin A Supply.

Animal and Sex	Original Weight	WEEKLY GAIN OF ANIMALS (in grams) First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Seventh week	Eight week	Total Gain	Av. Weekly Gain
216R-♀	69	-2	7	5	3	8	10	7	5	43	5.4
201C-♀	110	2	4	3	2	-6	10	4	7	26	3.2
217A-♂	68	6	3	3	12	12	8	3	0	47	5.9
217E-♀	77	20	7	7	6	7	4	2	1	54	6.7
217G-♂	78	6	11	4	10	9	8	7	4	59	7.4
221A-♀	75	4	5	2	4	6	2	1	-1	23	2.9
222A-♂	111	4	4	2	3	1	-4	-8	13	15	1.9
222C-♀	107	3	1	1	-2	2	1	-1	0	5	0.6
228C-♂	78	0	-2	-2	1	-7	3	-5	-2	-14	-1.7
228G-♀	71	3	2	0	-2	6	13	5	2	29	3.6
229A-♂	57	8	12	8	4	3	10	5	1	51	6.3
229C-♂	45	6	2	7	6	12	17	10	7	67	8.4
Total (grams)	946	60	56	40	47	53	82	30	37	405	
Average (grams)	78.8	5.0	4.7	3.3	3.9	4.4	6.8	2.5	3.0	33.7	4.2

Tab.3 Growth of Animals Fed Leaf Lettuce for Vitamin A Supply.

Animal and Sex	Original Weight	WEEKLY GAIN OF ANIMALS (in grams) First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Seventh week	Eighth week	Total Gain	Av. Weekly Gain
228F-♀	61	9	14	3	1	6	8	12	7	60	7.5
228E-♀	75	3	2	11	13	8	5	1	4	47	6.0
229F-♀	62	11	8	4	10	12	6	7	0	58	7.2
216C-♂	72	1	22	17	17	9	12	7	12	97	12.1
216F-♂	88	13	9	9	14	12	11	7	5	80	10.0
222D-♀	113	10	7	11	-1	12	-1	9	-4	43	5.4
222E-♀	106	-4	12	11	10	6	2	-1	2	38	4.7
222F-♂	94	2	15	12	13	11	4	2	1	60	7.5
217C-♀	68	15	11	11	8	6	6	4	3	64	8.0
217D-♂	81	8	14	13	13	8	4	4	7	71	8.9
217F-♂	78	19	14	12	10	12	9	5	3	84	10.5
221B-♂	65	17	14	10	13	12	8	8	6	86	10.7
221E-♀	61	13	11	5	6	5	3	1	-2	42	5.2
Total (grams)	1026	117	153	129	127	117	77	66	44	830	
Average (grams)	78.9	9.0	11.8	9.9	9.9	9.0	5.9	5.1	3.4	63.8	8.0

Figure 1

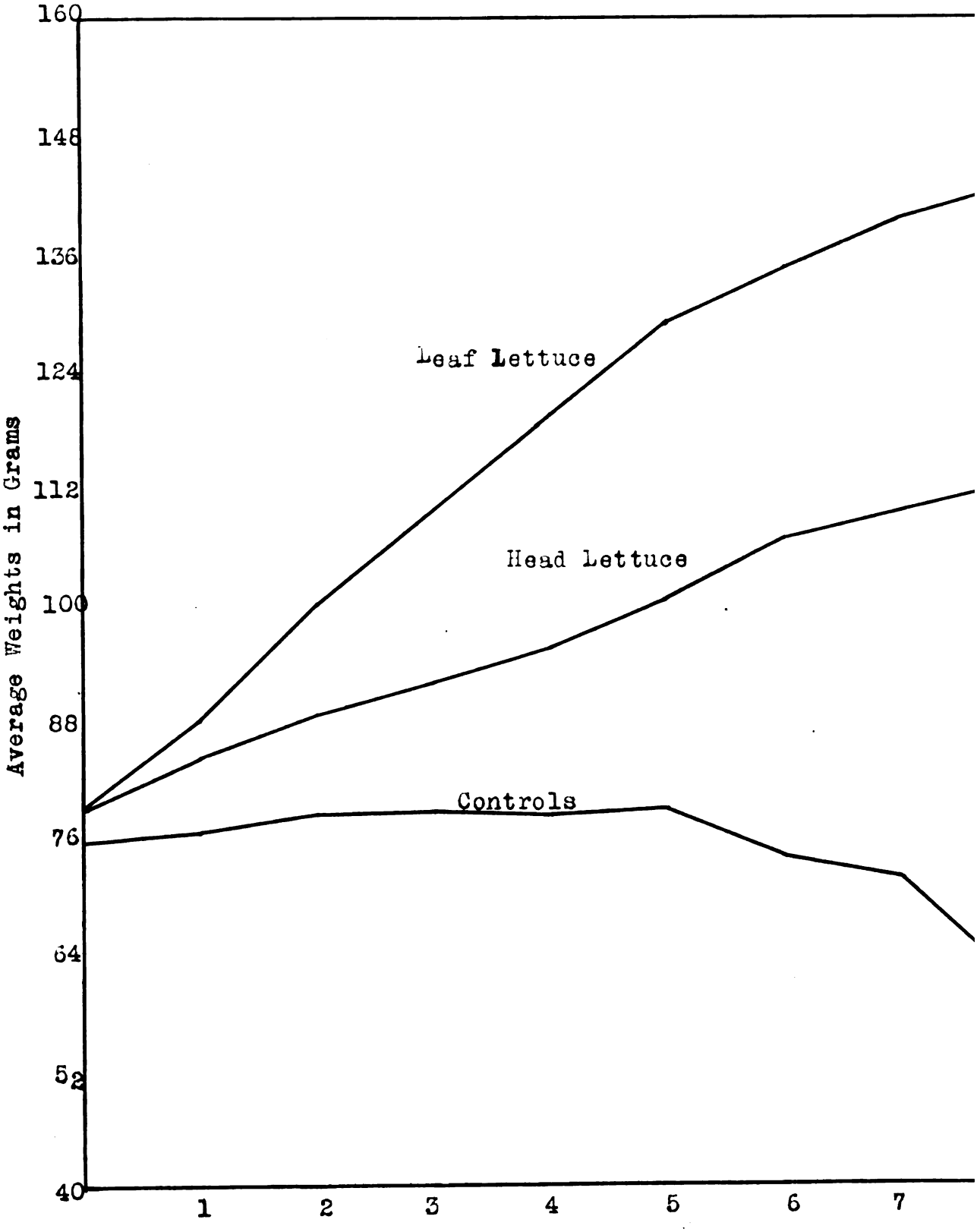

Figure 1.- Graphs of data shown in Tables 1, 2 and 3.

The rats having xerophthalmia soon recovered and all resumed growth promptly when the feeding of lettuce was started. The graph shows very clearly that leaf lettuce was much superior to head lettuce as a source of vitamin A. The animals getting 300 mg. of leaf lettuce daily had an average weekly gain of 8 grams while those receiving an equal quantity of the inside leaves of head lettuce had an average weekly gain of only 4.2 grams. The gain of the animals fed leaf lettuce was practically twice that of the animals fed head lettuce. The control animals averaged a loss in weight of 1.06 grams per week and before the end of the experiment one-half of them had died.

Experiment 2.

White rats, even when of the highest grades, show considerable individual variation under the best experimental conditions. In order to offset the influence of this variation it was deemed necessary to carry on an experiment in which the sources of vitamin A would be interchanged at the end of eight weeks and the experiment continued for eight weeks longer. Twelve animals received 300 mg. of leaf lettuce daily for eight weeks and then were given 300 mg. of head lettuce daily for the remaining eight weeks. Nine animals were fed head lettuce the first eight weeks and were likewise changed to leaf lettuce for an equal period. Eight control animals were used in this experiment. The data are given in Tables 4, 5, and 6 and are shown graphically in figure 2.

Table 4,- Record of Animals on Head Lettuce Eight Weeks,

Followed by Leaf Lettuce for Eight Weeks

Animal and Sex	Original weight (grams)	WEEKLY GAINS (in grams) 1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
217A-♂	68	6	3	3	12	12	8	3	0	7	20	16	6	3	4	9	4
217E-♀	77	20	7	7	6	7	4	2	1	4	4	2	2	-2	3	3	-3
221A-♀	75	4	5	2	4	6	2	1	-1	6	10	9	5	-1	1	0	3
222A-♂	111	4	4	2	3	1	-4	-8	13	9	9	6	0	1	1	9	3
222C-♀	107	3	1	1	-2	2	-1	-1	0	5	4	4	1	2	6	1	-6
228C-♂	78	0	-2	-2	1	-7	3	-5	-2	3	1	6	3	5	-7	Dead	---
228G-♀	71	3	2	0	-2	6	13	5	2	7	11	5	1	3	5	6	2
229A-♂	57	8	12	8	4	3	10	5	1	9	9	12	0	5	3	4	5
229C-♂	45	6	2	7	6	12	17	10	7	6	7	9	0	-2	0	5	5
Totals (grams)	689	54	34	28	32	42	54	12	21	56	75	69	18	14	16	37	13
Ave. gains (grams)	76.5	6.0	3.8	3.1	3.6	4.7	6.0	1.3	2.3	6.2	8.3	7.7	2.0	1.7	1.8	4.1	1.4

Table 5.- Record of Animals on Leaf Lettuce Eight Weeks,
Followed by Head Lettuce for Eight Weeks

Animals and Sex	Original weight (grams)	WEEKLY GAINS (in grams)															
		1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
216C-♂	72	1	22	17	17	9	12	7	12	3	-2	-3	2	-2	2	-1	1
216E-♂	88	13	9	9	14	12	11	7	5	-4	14	1	2	-3	-2	2	-3
217C-♀	68	15	11	11	8	6	6	4	3	-2	3	1	3	-3	4	0	-1
217F-♂	78	19	14	12	10	12	9	5	3	-3	15	0	2	-1	0	4	-4
221B-♂	65	17	14	10	13	10	8	8	6	-5	3	3	7	-2	1	2	0
221E-♀	61	13	11	5	6	5	3	1	-2	2	10	1	2	-1	1	2	0
222D-♀	113	10	7	11	-1	12	-1	9	-4	-1	3	2	-8	2	-3	7	-1
222E-♀	106	-4	12	11	10	6	-2	-1	2	7	-2	0	-10	5	4	-4	4
222F-♂	94	2	15	12	13	11	4	2	1	6	0	1	1	1	2	-1	-11
228E-♀	75	3	2	11	13	8	5	1	4	-9	12	-1	2	0	-1	-3	0
228F-♀	61	9	14	3	1	6	8	12	7	-2	3	4	1	o	2	4	2
229E-♀	64	11	8	4	10	12	6	7	0	1	2	1	1	1	0	1	-2
Totals (grams)	945	109	139	116	114	109	73	62	37	-7	61	10	5	-3	10	13	-15
Ave. gains (grams)	78.7	9.1	11.6	9.8	9.5	9.1	6.1	5.2	3.1	-0.6	5.1	0.8	0.4	-0.3	0.8	1.1	-1.3

Table 6.- Record of Animals Receiving No Vitamin A (Experiment 2)

Animals and Sex	Original weight (grams)	WEEKLY GAINS (in grams)											Total Gains	Ave. weekl gains
		First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Seventh week	Eighth week	Ninth week	Tenth week	Eleventh week		
228B-♀	51	1	3	2	-3	-2	-10	Dead	---	---	---	---	-9	-1.50
222B-♀	66	2	3	1	0	1	-3	-6	-12	-12	Dead	---	-26	-2.9
229H-♀	76	2	1	-1	-1	-1	20	Dead	---	---	----	---	-20	-3.33
221D-♀	66	0	0	0	-2	1	4	-2	-12	Dead	----	---	-11	-1.37
217B-♀	102	7	2	3	5	5	0	-2	-7	-12	Dead	---	1	0.11
216A-♂	88	-2	4	-3	Dead	---	---	---	---	---	----	---	-1	-0.33
216D-♂	90	0	0	0	-1	2	-1	1	-13	-5	-9	Dead	-26	-2.6
221C-♂	55	1	1	-1	Dead	---	---	---	---	---	----	---	1	0.33
Totals (grams)	600	11	14	1	-2	6	-30	-9	-44	-29	-9	----	-82	
Ave. Gains (grams)	75	1.37	1.75	0.12	-0.33	1.0	-5.0	-2.25	-11.0	-9.67	-9.0	---	-10.25	-1.52

Average Weights in Grams

Figure 2.-

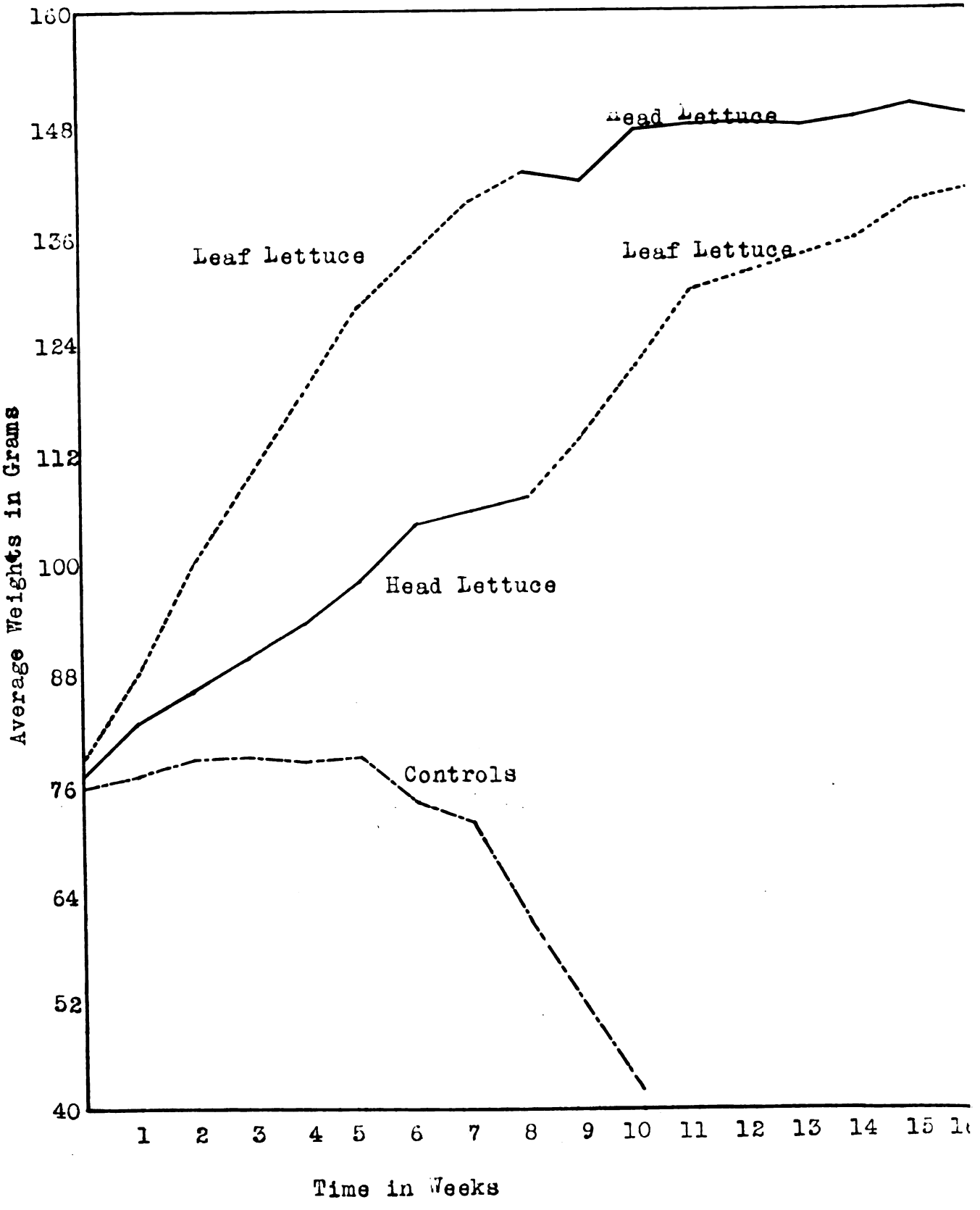

Figure 2.- Graphs of data shown in Tables 4, 5 and 6.

As in experiment one the animals fed leaf lettuce made much greater gains than those given an equal quantity of blanched head lettuce. During the first eight weeks the rats on the leaf lettuce diet gained more than twice as much as those on head lettuce, but when they were fed head lettuce the next eight weeks they gained less than one-fifth as much as the other lot which received leaf lettuce. The animals when changed from head to leaf lettuce made a very rapid gain in weight the following three weeks and a substantial, but slower, gain during the remaining five weeks. Those on the head lettuce ration made only a very slight gain during the entire eight weeks and were losing weight at the end of the experiment.

The control animals averaged a very slight gain during the first five weeks of the experiment. After that time there was a continuous and increasing loss of weight until the eleventh week when the last animal died. Half of the control animals died before the end of the seventh week. All of them lost weight and showed definite symptoms of xerophthalmia before dying.

This experiment shows very clearly that the same animals gain much more rapidly on leaf lettuce than on an equal quantity of head lettuce. As the animals grow larger the rate of gain is less, due to the fact that more vitamin A is required to maintain the larger animal, and toward the end of the experiment the head lettuce

did not furnish quite enough of this food factor to maintain the animals at a constant weight.

Experiment 3.

The object of this experiment was to determine the difference in the vitamin A content of the very inside yellowish leaves of head lettuce and the outside, green leaves of the same heads. The samples of the green lettuce were taken from the outside leaves of good commercial heads which contained much chlorophyll. Even though these leaves were quite green they were noticably less green than the leaf lettuce. The blanched leaves were taken from the hearts of the same heads.

In experiments one and two the animals had grown more rapidly than the desired rate, so it was decided that the amount of lettuce fed daily should be 200 mg. instead of 300 mg. The basal diet and method of sampling the lettuce was the same as was used in the previous experiments.

There were ten animals on each kind of lettuce and six were used as negative controls. The experiment was continued for eight weeks. The data are given in tables 7, 8, and 9 and are shown graphically in figure 3. Experiment 4 was under way at the same time, thus permitting the inclusion of a curve showing the growth of animals on leaf lettuce.

Table 7.- Record of Animals on Inside Leaves of Head Lettuce

Animals and Sex	Original weight (grams)	WEEKLY GAINS (in grams) First week	Second week	Third week	Fourth week	Fifth week	Six week	Seventh week	Eighth week	Total Gains	Average weekly gains
229B-♂	92	-2	2	0	-1	4	3	6	0	12	1.5
260D-♀	89	0	6	2	-3	-2	2	-6	-14	-15	-1.9
261C-♂	86	-1	2	1	3	2	2	2	-15	- 4	-0.5
263A-♀	89	6	-4	-2	-1	6	0	Dead	----	5	0.6
264G-♂	98	-2	8	-3	-13	0	Dead	----	----	-10	-1.2
264E-♂	112	2	8	5	4	3	-4	-7	- 7	4	0.5
260G-♀	74	-1	1	-2	3	0	0	-3	0	- 2	-0.2
261G-♀	107	-1	-5	2	-4	3	-9	-3	- 7	-24	-3.0
259A-♀	98	-3	1	-1	-5	1	0	-9	-9	-25	-3.1
262C-♂	97	-3	2	0	-4	5	-3	-6	0	- 9	-1.1
Totals (grams)	942	-5	21	2	-21	22	-9	-26	-52	-68	
Ave. Gains (grams)	94.2	-0.5	2.1	0.2	-2.1	2.2	-0.9	-2.6	-5.2	-6.8	-0.85

Table 8.- Record of Animals on Outside Leaves of Head Lettuce

Animal and Sex	Original weight (grams)	WEEKLY GAINS (in grams)								Total Gains	Average weekly gains
		First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Seventh week	Eighth week		
262F-♂	110	3	5	10	3	5	1	5	3	35	4.4
261F-♂	82	12	10	9	7	11	0	5	-1	53	6.6
260J-♂	84	6	11	7	6	4	7	3	-4	40	5.0
260B-♀	79	6	11	1	3	3	3	-1	-4	22	2.7
228H-♀	95	0	2	3	4	7	8	9	4	37	4.6
262D-♂	109	0	9	4	13	7	9	4	1	47	5.9
263G-♀	90	1	-1	6	8	6	0	3	-3	20	2.5
264B-♂	123	3	8	6	6	12	3	-3	0	35	4.4
264D-♀	114	5	1	9	4	5	4	3	0	31	3.9
259E-♀	86	4	2	5	4	5	0	1	6	27	3.4
Totals (grams)	972	40	58	60	58	65	35	29	2	347	
Av.gains (grams)	97.2	4.0	5.8	6.0	5.8	6.5	3.5	2.9	0.2	34.7	4.3

Figure 3.

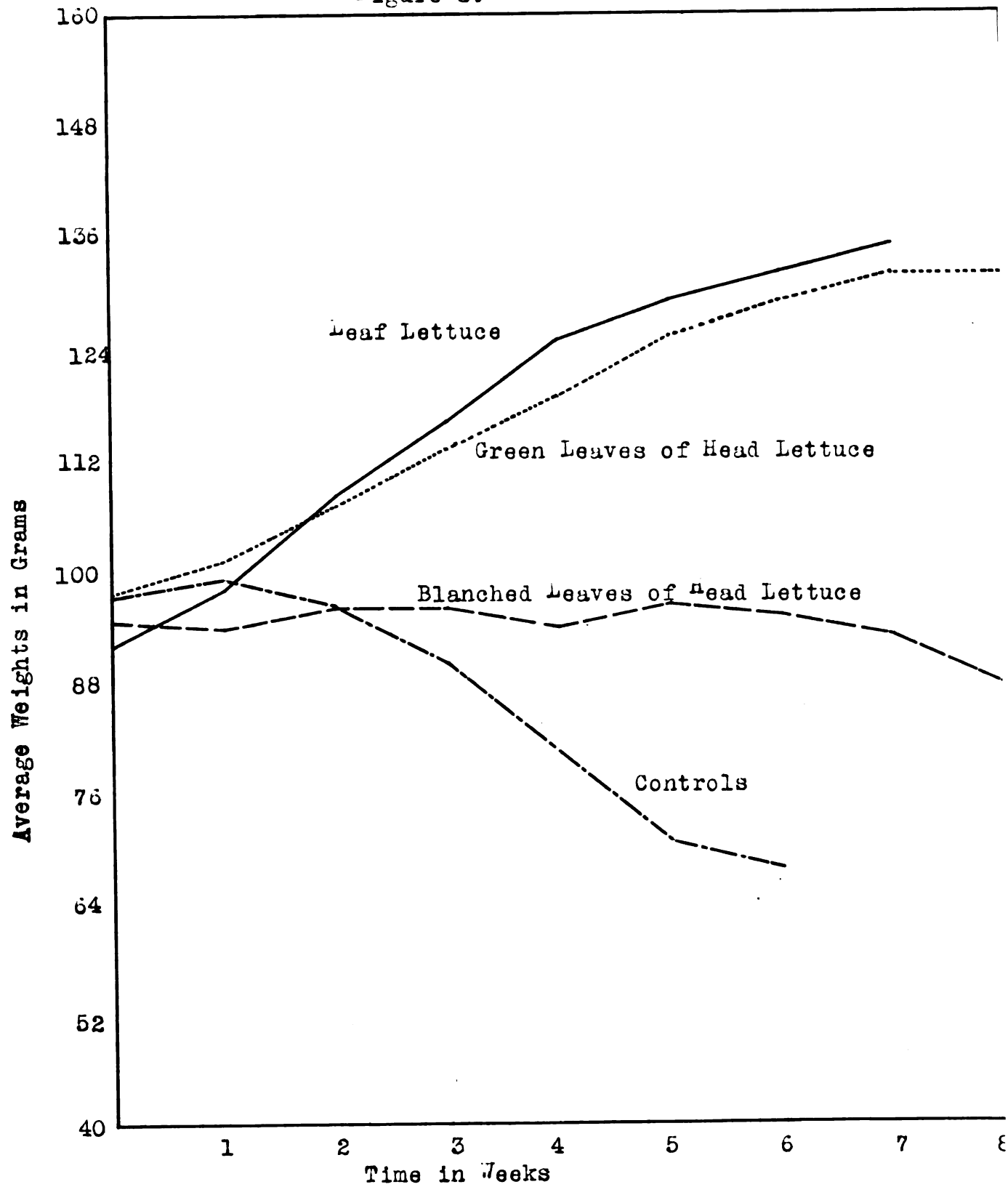

Figure 3.- Graphs of data shown in Tables 7, 8, 9, 10 and 11.

The animals fed the green outside leaves of head lettuce made an average gain in weight of over 34 grams during the eight weeks while those on the inside, yellowish leaves of the head had an average loss in weight of almost seven grams during the same length of time. The animals receiving an equal amount of leaf lettuce made an average gain of 43 grams. All of the negative control animals were dead before the seventh week of the experiment. Most of these were affected with xerophthalmia before dying and all had the appearance of animals suffering from vitamin A deficiency. Two of the animals fed on inside leaves of head lettuce died before the eighth week. Four of the animals given this part of the head lettuce had xerophthalmia when the vitamin containing food was first given them, but none of them recovered from the disease. Seven of the animals that were fed the outside leaves of the head were afflicted with xerophthalmia when they came to constant weight, but all recovered promptly when they were given lettuce.

The data show that animals receiving leaf lettuce as a source of vitamin A made better growth than those fed green leaves of head lettuce and that both of these lots made decidedly better gains than animals on the leaves of the inner part of head lettuce. In fact, the inside part of the head when fed in

quantities of 200 mg. daily did not furnish enough of this food factor to maintain the animals' constant weight.

The green leaves of head lettuce that were fed in this experiment are very seldom eaten for they are usually discarded in preparing lettuce for the table. It is very evident that head lettuce as consumed by human beings is much inferior to leaf lettuce as a source of vitamin A.

Experiment 4.

Within the last few years the question of the vitamin content of plants grown under glass has become of vital interest to vegetable forcers. Accordingly an experiment was conducted to determine if there is any difference in the vitamin A content of leaf lettuce grown in the greenhouse and that grown out in the field.

Grand Rapids Forcing lettuce was planted in the field and also in benches in the greenhouse the same day. This experiment was started in the autumn and the lettuce in the field did not grow as fast as that in the greenhouse. When there was danger of frost the outdoor grown lettuce was transplanted to a protected cold frame where it was covered with sash during the night and left uncovered during the day. The experiment was ended at the end of the seventh week because the lettuce

in the cold frame was killed by freezing. As in the previous experiment 200 mg. of lettuce was fed daily.

Eleven animals were fed the outdoor lettuce while ten were given lettuce grown in the greenhouse. The controls are the same as in Experiment 3. The data are given in tables 9, 10, and 11 and are shown graphically in figure 4.

The data show that lettuce grown under glass gives practically the same amount of growth as that grown where it is exposed to the direct rays of the sun. At least the differences are negligible for they are within the limits of experimental error. The fact that animals grew well on lettuce grown under glass shows that the untra-violet rays that are filtered out by greenhouse glass are not necessary for the synthesis of vitamin A. These results are in harmony with those obtained by Coward (2) with wheat seedlings in 1923.

Table 9,-Record of Control Animals, Experiments 3 and 4.

Animal and Sex	Original weight (grams)	Weekly Gains in Grams								Total gains	Average weekly gains
		First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Seventh week	Eighth week		
259F-♀	95	-3	-2	-2	-8	-5	Dead	---	---	-20	-4.0
260F-♀	84	-1	0	0	-9	-8	Dead	---	---	-18	-3.6
261A-♂	98	1	-1	-13	-16	-7	Dead	---	---	-36	-7.2
262A-♀	101	8	-9	-2	-4	-20	Dead	---	---	-27	-5.4
264H-♂	110	5	-4	-5	-22	Dead	----	--	---	-26	-6.5
263F-♀	94	1	-1	-16	-2	-9	-3	Dead	---	-26	-4.3
Total Gm.	582	11	-17	-38	-57	-49	-3			153	
Av.Gains in gms.	97	1.8	-2.8	-6.3	-9.5	-9.8	-3.0			- 25.5	-5.1

Table 10.- Record of Animals on Greenhouse Leaf Lettuce.

Animal and Sex	Original weight (grams)	WEEKLY GAINS (in grams) First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Seventh week	Total Gains	Average weekly gains
228D-♀	87	10	7	14	7	-1	0	8	45	6.4
260I-♀	85	2	9	4	1	5	1	-2	20	2.9
259D-♀	73	5	10	8	8	-2	4	-8	25	3.6
261D-♂	90	1	15	8	13	10	10	7	64	9.1
264C-♀	78	10	6	7	11	5	2	0	41	5.9
259C-♂	105	6	13	14	13	9	4	-7	52	7.4
263E-♀	101	7	15	11	8	7	3	6	57	8.1
259B-♀	98	5	8	5	5	8	3	4	38	5.4
262B-♂	95	2	14	10	15	8	0	9	58	8.3
260C-♀	88	0	10	2	4	-3	-1	4	16	2.3
Totals grams	900	48	107	83	85	46	26	21	416	
Av.gains in grams	90.0	4.8	10.7	8.3	8.5	4.6	2.6	2.1	41.6	5.9

Table 11.- Record of Animals on Out-of-doors Leaf Lettuce.

Animals and Sex	Original Weight (grams)	WEEKLY GAINS (in grams) First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Seventh week	Total Gains	Av. Weekly Gains
262E-♂	103	16	13	9	8	13	10	3	72	10.3
264F-♂	102	6	4	4	4	-5	6	3	22	3.1
260A-♀	78	4	9	6	1	4	1	-1	24	3.4
261E-♂	110	9	10	11	8	8	4	4	54	7.7
228J-♀	73	6	18	9	4	10	1	8	56	8.0
229G-♀	85	10	15	11	8	0	3	0	47	6.7
261B-♂	110	7	12	9	15	5	-6	1	43	6.1
262G-♂	97	2	15	14	11	5	9	10	66	9.4
260H-♀	81	5	6	3	7	4	0	0	25	3.6
264A-♂	111	1	9	-2	7	7	13	6	41	5.9
263D-♂	72	8	3	9	6	6	1	6	39	5.6
Totals in grams.	1022	74	114	83	79	57	42	40	489	
Av.Gains in grams	92.9	6.7	10.4	7.5	7.2	5.2	3.8	3.4	44.5	6.3

Figure 4.

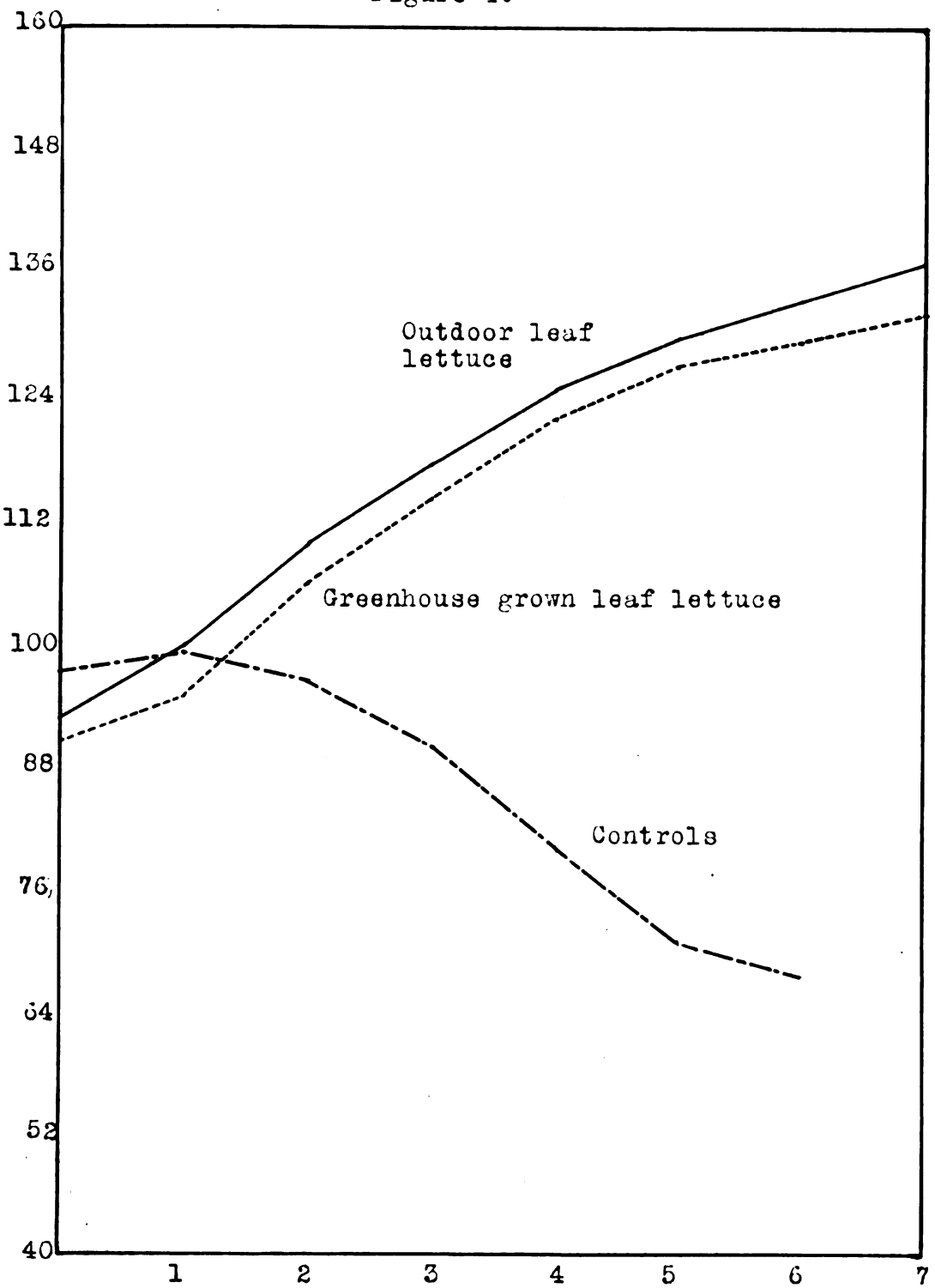

Figure 4.-Graphs of data in Tables 9, 10, and 11.

DISCUSSION

The results from the four experiments reported in this paper clearly indicate that leaf lettuce contains more vitamin A than head lettuce. It is superior, in this respect, to even the outside, green leaves of the head lettuce which contain much larger amounts of this vitamin than the inside yellowish portion of the head. That the blanched portion, or hearts of head lettuce, contains some vitamin A is also shown by the data. When fed in amounts of 200 mg. daily this kind of lettuce just about enabled the animals weighing around 90 grams to maintain their weight for eight weeks. There was a very slight average loss in weight by the animals fed on that amount in this experiment.

The data submitted show that there is a correlation between the amount of vitamin A present and the greenness of lettuce leaves. However, it would be unsafe to infer either a causal relationship on an indissoluble association of these two things. Available data clearly show that etiolated seedlings and white or yellow "hearts" of lettuce and cabbage contain some vitamin A, but this amount is very small in proportion to the amount contained in the green parts of the same, or similar plants.

The investigations of Monteverde and Lubimenko

(8) some years ago led them to state that a pigment called "chlorophyllogen" is formed, independently of light, in the chromatophores of all potentially green plants. This pigment or "mother substance" of chlorophyll is immediately converted into chlorophyll on exposure to light, but in some species, (ferns and evergreen seedlings (Larix, Pinus, Picea) , it becomes chlorophyll in darkness, but according to Lubimenko(9) conifer seedlings form less chlorophyll in darkness than in light. Issachenko,and Liro (8) showed that oxygen and carbohydrates were not necessary for the transformation of chlorophyllogen into chlorophyll under favorable temperature conditions. Liro (8) claimed to have killed etiolated seedlings in a careful manner so as to save some of the chlorophyllogen, when some formation of chlorophyll was still observed on exposure to light.

In 1859 Sachs (8) mentioned a colorless chromogen called leucophyll from which chlorophyllogen is said to arise. In the June and July numbers of "Revue Générale de Botanique" for 1926 Lubimenko, M.V.(9) has summarized his extensive investigations and also the present situation regarding the pigments of the plastids and photosynthesis. He strongly reaffirms his position concerning chlorophyllogen and certain other phenomena of the plastids and pigments. Most embryos

develop a green color during the first stages of their development after fertilization and green color was found to be present in a few of them even after full maturity of the seed. In the mature cucurbits a rich store of chlorophyllogen is found in what was the inner integument of the ovule. In speaking of chlorophyllogen in seedlings of angiosperms grown in the dark Lubimenko makes the following statements: "The seedlings of angiosperms produced in the dark accumulate the yellow pigments and a very small quantity of pigment called chlorophyllogen". "As a general rule, the quantity of chlorophyllogen in seedlings is so small in proportion to the quantity of yellow pigments that this pigment has no sensible influence on the color of the seedlings". "With angiosperms the chlorophyll is replaced in the dark by the chlorophyllogen of which the accumulation, as in case of the chlorophyll, depends primarily on the organic nutrition of the plastid."

In his entire summation of the evidence he endeavors to establish the proposition that the development of the plastids as well as the various pigments in these structures is more dependent on the organic nutrition of the cells than any other factor, light included. In light of these considerations it may be possible that in apparently chlorophyll free tissues there are substances that are closely related to chloro-

phyll, that is substances from which chlorophyll is formed, such as chlorophyllogen and leucophyll, which could be related to vitamin A in a very significant manner.

SUMMARY

1. Leaf lettuce contains more vitamin A than head lettuce.

2. The green, outside leaves of head lettuce contain larger amounts of this vitamin than the yellow, or blanched, portion of the head sometimes called "the hearts of lettuce."

3. The blanched head lettuce contains some vitamin A but this occurs in relatively small amounts.

4. Leaf lettuce grown in the greenhouse contains practically the same amount of this growth promoting vitamin as that grown in the field where it gets the direct sun rays.

5. Evidence points toward a close relationship between chlorophyll, or some of its primary phases and vitamin A in lettuce tissue, but this relationship has not been definitely proved.

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