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THE RELATION OF THE CAROTENOID PIGMENTS OF THE DIET TO THE GROWTH OF YOUNG CHICKS AND TO THE STORAGE IN THEIR TISSUES

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The Relation of the Carotenoid Pigments of the Diet to the Growth of Young Chicks and to the Storage in their Tissues

ADELIA E. WEIS* AND BERTHA BISBEY

REVIEW OF LITERATURE

Yellow to deep violet-red pigments, known as carotenoids, which are widely and abundantly distributed in nature, were recognized at an early date. Wachenroder (1826) prepared a crystalline product of the yellow pigments from carrot roots, which he named "carotin". In 1837 Berzelius designated the yellow pigment which he extracted from autumn leaves with alcohol, as "xanthophyll".

That xanthophyll and carotin could be separated by the difference in their solubilities in alcohol and petroleum ether, was discovered by Borodin (1833). Later, Willstätter and Stoll (1913) developed a quantitative method for extracting and measuring carotenoid pigments. It is upon this method that many of the present day studies of these chemically related compounds are based.

Stokes (1864) using the spectrographic method of analysis concluded that there were at least two xanthophylls present in green leaves.

Tswett (1906) showed that a mixture of yellow pigments, in petroleum ether, when passed through a tightly packed column of calcium carbonate, formed layers due to the differences in affinity of the carotenoids for the calcium carbonate. By means of this ingenious device, which he named a "chromatogram", he was able to demonstrate the presence of carotin and several xanthophylls in the material extracted from nettle leaves.

Tswett (1911) proposed the class name "carotinoid" for the yellow pigments discovered up to that time. The two groups included in the classification were: (1) the hydrocarbons, carotins, and (2) the oxygenated pigments of which xanthophyll is an example. This classification is still in use. Since then, however, the spelling of the original word "carotin", in order to conform to international rules for naming organic compounds, has been changed to carotene and the class name for these compounds is now spelled carotenoid. In 1939 Peterson, Hughes and Payne proposed the name "carotenols" for oxygenated carotenoid pigments.

At the present time a combination of the partition method and the chromatographic method together with the physical and chemical methods

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*The data contained in this bulletin were submitted by Adelia E. Weis in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the University of Missouri, 1942.

of identification are resulting in a rapid accumulation of new, heretofore undiscovered, carotenoids. Not only have the physical and chemical properties of this interesting class of compounds been investigated, but also many studies have been made to determine their biological importance.

The presence of a fat-soluble factor necessary for the growth and well being of experimental animals was simultaneously and independently demonstrated by two groups of workers, Osborne and Mendel (1913), (1913a), and McCollum and Davis (1913). These investigators found that rats fed a purified diet, containing all of the then known factors necessary for good nutrition, developed xerophthalmia, became generally emaciated and finally died when lard, olive oil or almond oil was the sole source of fat. If, however, these fats were replaced by butterfat, egg yolk fat, or cod liver oil the animals were healthy, grew well and were normal.

Steenbock (1919) noted the close correlation between the amount of yellow plant pigment present in the experimental ration and its nutritive quality; for this reason he advanced the theory that the fat-soluble vitamin A is one of the yellow pigments. The correctness of his theory was doubted, however, because it was shown by various investigators that animals could grow and be normal in all respects even though no yellow plant pigment was present in the ration. As an example of such investigations, the work of Palmer and Kempster (1919) may be cited. Their work showed that chicks raised to maturity on a carotenoid-free diet were healthy and normal except for the lack of color in their skin, body fat and egg yolks, when pork liver was fed as a source of vitamin A. Chicks hatched from the non-pigmented eggs developed into hens normal in all respects except that no yellow color was present in their tissues. These authors agreed with Steenbock that the yellow pigments and vitamin A are closely related but concluded that they are not the same thing.

Euler, Euler and Hellström (1928) and Moore (1929) showed that carotene and vitamin A were similar in their growth promoting properties but differed in physical and chemical properties.

Moore (1930) demonstrated conclusively that carotene was a precursor of vitamin A. Livers of rats depleted of vitamin A and those of rats which had received carotene after depletion were analyzed. Results for the former group were negative for vitamin A, but in the latter group a marked absorption band appeared at 6100-6300 Å when the solution was tested with antimony trichloride, and at 3280 Å in the untreated sample. Both of these absorption maxima are characteristic of vitamin A under the given conditions. Traces of carotene were also observed in the livers of the animals which had received the carotene.

The work of Capper, McKibben and Prentice (1931) showed that chickens as well as rats were able to convert carotene into vitamin A.

Of the carotenoids which have been discovered, a larger number belong to the xanthophyll group than to the carotene group. Strain (1936)

using the chromatographic method of separating the alcohol-soluble fraction from leaves reported that there were at least 12 of these compounds present and that xanthophyll (lutein), a specific carotenoid of the group, made up about one-half of the pigments recovered.

Xanthophyll (lutein) and zeaxanthin are known to occur abundantly in nature. The structural formula of the former, established by Karrer, Zubrys and Morf (1933) showed it to be a dihydroxy-alpha-carotene, while the latter according to Karrer (1933) is a dihydroxy-beta-carotene.

Structural formulae have been established for the following carotenoid pigments known to possess vitamin A activity: Alpha-carotene (Karrer, Morf, and Walker, 1933), beta-carotene (Karrer, Helfenstein, Wehrli and Wettstein, 1930), gamma-carotene (Kuhn and Brockmann, 1933) and cryptoxanthin (Kuhn and Grundmann, 1933). They all, in common with vitamin A, contain the beta-ionone ring which Palmer (1938) believed to be important for biological activity. Just how it functions is not known. The primary alcohol structure indicates that esterification is possible, hence the vitamin is capable of uniting with fat acids, bile acids and proteins.

Xanthophylls are transferred from the feed to the tissues and eggs of the avian organism to a far greater extent than are the carotenes (Palmer, 1915; Palmer and Kempster, 1919a; Sjollema and Donath, 1940).

Hughes and Payne (1937) observed that a larger percentage of xanthophyll pigments were stored in egg yolks when yellow corn was fed to hens than when either alfalfa or barley was fed. They concluded that the greater deposition of the alcohol-soluble fraction from the corn is due to the type of xanthophyll present.

Willstätter and Escher (1911) prepared crystalline xanthophyll from egg yolks which aside from its higher melting point seemed identical to plant xanthophyll (lutein). In addition to this xanthophyll, Gillam and Heilbron (1935) have reported that egg yolks contain cryptoxanthin, a small amount of carotene and vitamin A. The amounts of these constituents present depend upon the kind of feed consumed by the hen. Kuhn, Winterstein and Lederer (1931) observed that xanthophyll from egg yolks when chromotographed on a calcium carbonate column could be resolved into two fractions: xanthophyll (lutein) identical to plant lutein, and zeaxanthin.

Numerous studies have been made on the biological activity of xanthophyll. The work of Rydbom (1933) indicated that xanthophyll functioned as a precursor of vitamin A for guinea pigs but not for rats. Virgin and Klussmann (1932) reported that xanthophyll could be converted into vitamin A in the avian organism. However, results obtained by Karrer, Euler and Rydbom (1930), and Fraps and Kemmerer (1941) showed that xanthophyll was not a precursor of vitamin A either for chickens or for rats. Kline, Schultze and Hart (1932) fed chicks a xanthophyll supplement which they had extracted from green spinach leaves and recrystallized 5 times. This supplement fed at a level of 0.1

and 0.25 milligrams per chick per day was ineffective as a source of vitamin A. More recently Braude and others (1941) have tested the biological activity of zeaxanthin and found it to be inactive as provitamin A for both pigs and rats. Hence the weight of evidence shows that xanthophyll cannot function as vitamin A.

Results obtained by Jacob (1939) in this laboratory indicated that some growth promoting factor was present in the xanthophyll fraction prepared from egg yolks when fed to rats as the sole source of vitamin A. As far as could be determined, without the aid of spectrographic equipment, the xanthophyll fraction appeared to be free of vitamin A, carotene and cryptoxanthin. The growth results, however, were unexpected and not in agreement with results reported in the literature.

The purpose of this investigation was: (1) to note the effect upon the growth and storage in young chicks receiving the vitamin A-free basal diet, when carotene-cryptoxanthin or xanthophyll extracted from yellow corn was fed separately or in combination in amounts in which they appeared in yellow corn and; (2) to compare the results of these experiments with those obtained when yellow corn, in an amount equivalent to the extracted pigments was fed.

Since the results showed conclusively that the xanthophyll from yellow corn possessed no vitamin A activity, a xanthophyll fraction from egg yolks was prepared for further study.

EXPERIMENTAL PROCEDURE

Description of the Solvents

All solvents used in the analyses were analytical reagent grade with the exception of the ethyl alcohol. This was an industrial product, consisting of approximately 93 per cent ethyl alcohol by weight. It was made as nearly absolute as possible by refluxing over unslaked lime. In order to prevent the alcohol from absorbing moisture from the air during the distillation process, the air which entered the system was dried as it passed through a tube of calcium chloride. Wilkie (1940) reported that alcohol subjected to contact with rubber contained substances which absorbed light strongly in the lower wave lengths of the spectrum and made it undesirable for use as a solvent in spectrographic analyses where measurements in the ultra violet region were desired. Hence, in order to minimize this undesirable effect, the rubber stoppers used in the distillation system were covered with tinfoil. Aldehydes were removed by redistilling the alcohol over potassium hydroxide and zinc dust.

Even though the ethyl ether was free of peroxides, as indicated on the label, it was treated to rid it of any peroxides which might have formed in it on standing. A saturated solution of ferrous ammonium sulfate, 18 N sulfuric acid and iron filings were added to several liters of ethyl ether and the mixture thoroughly shaken. It was allowed to stand until the two layers separated. The ether was decanted off, dried over Hydralo (a drying agent), and distilled over a water bath. An ice condenser was used to condense the ether vapor. The purified ether was kept in amber glass bottles containing Hydralo until it was used.

Construction of Reference Curves

Commercial carotene* (85 per cent beta and 15 per cent alpha) was weighed on a microbalance and made up to volume at 25° centigrade with absolute ethyl alcohol. By means of a constant temperature water bath it was possible to prepare all dilutions at the same temperature $\pm 0.5°$ centigrade. These solutions containing known amounts of carotene were measured in an Aminco Type "V" electrophotometer. This instrument was equipped with a pyrex absorption cell (inside length 49.839 ± 0.001 millimeters), a mercury vapor lamp and two pairs of filters (one pair in each of two balanced paths of light). The filters were made up of numbers 038 Novial Shade "A" and 585 Blue Purple Ultra, a combination designed to isolate the mercury line 4358 Å which closely approximates the absorption maxima of carotene (Strain, 1934) and of xanthophyll (Kuhn and Winterstein, 1931) in ethyl alcohol.

Likewise, commercial xanthophyll** was weighed on a microbalance, made up to volume at 25° centigrade with absolute ethyl alcohol and known dilutions measured in the electrophotometer. The average dial reading of two samples at each dilution was used to construct the ref-

*Purchased from S.M.A. Corporation, Cleveland, Ohio.
**Purchased from the American Chlorophyll Company, Alexandria, Va.

erence curve for the xanthophyll. Values for the construction of the carotene reference curve were obtained similarly.

In order to establish a base of reference for vitamin A determinations, reference cod liver oil containing 1700 International Units of vitamin A per gram was prepared for spectrographic analysis according to the technique used in the laboratory of the United States Food and Drug Administration, Washington D. C. (Wilkie, 1940).

Absorption spectra measurements* of these samples were made with a Hilger Medium Quartz Spectrograph and a Hilger Spekker Spectrophotometer. The slit width of the instrument was 0.03 millimeters. Photographs were taken at density steps of 0.05 intervals (0, 0.05, 0.10, 0.15, 0.20, 0.25, to 1.75). The time of exposure was gradually lengthened from 0.5 to 14 seconds.

Points of equal intensity in each of the two cell images in the photograph (one image obtained through the solvent cell and the other through the analysis cell) when marked off on the plate showed the form of the absorption curve of the solution, and made it possible to read off density values at the corresponding wave lengths.

The relationship of density to other factors in the spectrographic analysis is shown in the following equation:

$$\text{Density (extinction) } E = \log_{10} \frac{I_o}{I} = Ecl,$$

where E is the absorption coefficient, I_o is the incident intensity, I is the transmitted intensity, c is the concentration and l is the cell length in centimeters. Thus the concentration of the solution is proportional to the extinction (density). This equation is obtained from Beer's Law which holds at or near the absorption peak,

$$I = I_o\, 10^{-Ecl}, \quad \frac{I_o}{I} = 10^{Ecl}, \quad \log \frac{I_o}{I} = Ecl.$$

Hence, it is possible to calculate either the concentration of vitamin A needed to cause absorption equivalent to 0.01 density unit or the absorption density produced by a known unitage of vitamin A, and to use the values to calculate the vitamin A concentrations of unknown solutions from their absorption density (extinction) values.

In some of the first analyses, absolute ethyl alcohol was used in the comparison cell of the spectrophotometer. However, due to extraneous materials in the cod liver oil samples, which absorbed highly near the 3280 Å wave length, it was found desirable to use a blank in the comparison cell in place of ethyl alcohol.

*The spectrographic measurements were made and interpreted by Dr. Victor R. Ells, Department of Agricultural Chemistry, University of Missouri.

Analysis of Yellow Corn for Carotene-Cryptoxanthin and Xanthophyll

The corn used in this experiment was Midland Yellow Dent, a very dark yellow variety, harvested in the fall of 1941*. It was stored in the laboratory at room temperature for about a month before it was ground. After it was freed of white grains, bad grains, and any foreign matter, it was ground until fine enough to pass a 50 mesh sieve. The finely ground corn was stored, in closely stoppered amber glass bottles, at 4° centigrade until used.

Carotene-cryptoxanthin and xanthophyll determinations were run in duplicate on approximately 15-gram samples of the finely ground room-dried corn. Three milliliters of freshly prepared potassium hydroxide (10 per cent KOH in aldehyde free ethyl alcohol) were added per gram of sample. The contents of the flask were shaken to mix thoroughly the alcoholic KOH with the corn. It was refluxed over a hot water bath one hour, cooled and filtered through a Buechner funnel. The corn residue remaining in the funnel was washed with 93 per cent ethyl alcohol and ethyl ether until no more color was removed by the solvents. Judging by the appearance of the corn residue, the yellow pigments were satisfactorily extracted by this procedure.

Beginning here, the procedure is essentially the same as the one used by Jacob (1939) in this laboratory for the quantitative separation and measurement of pigments extracted from egg yolks and is based on the method of Schertz for the quantitative measurement of carotene (1923, 1925 a) and of xanthophyll (1925, 1925 b).

The ethyl alcohol and ethyl ether extractions from the yellow corn were diluted with distilled water, cooled, placed in a separatory funnel and the two layers allowed to separate. The aqueous alcoholic layer was further extracted with ethyl ether until the latter came off colorless. The combined ethyl ether extractions were washed with distilled water until the latter was free of alkali as shown by the phenolphthalein test.** The ethyl ether was then dried by filtering through anhydrous sodium sulfate. The separatory funnel and the sodium sulfate were washed with ethyl ether. The ether was distilled off, in a vacuum system flooded with carbon dioxide at the beginning and termination of the distillation. When only a few milliliters of the ether solution remained, twenty-five milliliters of 90 per cent methyl alcohol were added. The distillation process was continued slightly longer in order to rid the solution of any traces of ethyl ether which, if left in the solution, would have interfered with the partition of the pigments between 90 per cent methyl alcohol and petroleum ether.

The methyl alcohol solution containing all of the carotenoid pigments was transferred to a separatory funnel and the carotene-cryptoxanthin separated from the xanthophyll by partitioning between 90 per cent

*Grown by Mr. Thomas R. Douglass, Columbia, Missouri.

**This method has been used throughout the study to test for the presence of alkali.

methyl alcohol and petroleum ether. The combined petroleum ether fractions were washed with distilled water and dried by filtering through anhydrous sodium sulfate. The sodium sulfate was washed with petroleum ether but some of the pigment was strongly adsorbed and could not be removed with this solvent. It was easily removed with ethyl ether and added to the ethyl ether extractions of the methyl alcohol fraction. The dried petroleum ether fraction was evaporated down to an oily residue. This was taken up in absolute ethyl alcohol, filtered into a volumetric flask through a small amount of anhydrous sodium sulfate, made up to volume and measured in the electrophotometer. No attempt was made to separate the carotene from the cryptoxanthin. Kuhn and Grundmann (1933) state that spectrographically cryptoxanthin is indistinguishable from beta-carotene. Hence, the carotene concentration at the given electrophotometer dial reading was considered equivalent to the carotene-cryptoxanthin content of the solution.

The 90 per cent methyl alcohol extractions, diluted with distilled water, were extracted with ethyl ether until the latter came off colorless. The combined ether extractions were washed with distilled water, dried and evaporated. The residue was taken up in absolute ethyl alcohol, filtered into a volumetric flask through anhydrous sodium sulfate, made up to volume and measured in the electrophotometer. Likewise, no attempt was made to separate the xanthophyll into its possible components; the total xanthophyll in the sample was measured and the concentration of the solution was determined by referring the dial reading to the xanthophyll reference curve.

Preparation of the Pigments for Feeding

Series I.—Carotenoids extracted from yellow corn. The procedure for the preparation of carotene-cryptoxanthin and xanthophyll for feeding was the same as that used for the quantitative measurement of these pigments in yellow corn, with the following exceptions. After evaporation of the final traces of ethyl ether from the 90 per cent methyl alcohol solution containing all of the pigments, the sterols which crystallized out of the solution were removed. In order to accomplish this the solution was placed in the refrigerator at 4° centigrade until the next day. Then the sterol crystals were filtered off and the carotene-cryptoxanthin was separated from the xanthophyll by the difference in solubility of these pigments in 90 per cent methyl alcohol and petroleum ether.

The combined petroleum ether extractions containing the carotene-cryptoxanthin were treated the same as described for the quantitative measurement of these pigments in yellow corn. After the concentration of carotene in the absolute ethyl alcohol solution was determined by means of the electrophotometer, it was added to Wesson oil and the ethyl alcohol was distilled off. More Wesson oil was then added until the final product contained 200 micrograms of carotene-cryptoxanthin per milliliter of solution.

The 90 per cent methyl alcohol containing the xanthophyll was condensed under vacuum to approximately one-half of its original volume. It was diluted with distilled water and then extracted with ethyl ether until the latter came off colorless. The combined ethyl ether extractions, approximately 600 milliliters, were washed with distilled water, dried and distilled to an oily residue. The residue containing the xanthophyll was taken up in absolute ethyl alcohol, filtered into a volumetric flask, made up to volume, measured in the electrophotometer, and added to Wesson oil as described above for carotene-cryptoxanthin extracted from yellow corn. The final concentration of xanthophyll was 450 micrograms per milliliter of Wesson oil solution.

Similarly, commercial crystalline xanthophyll and commercial crystalline carotene were measured and added to Wesson oil. The concentration of the former pigment was 450 micrograms and of the latter 200 micrograms per milliliter of Wesson oil.

Series II.—Carotenoid pigments extracted from egg yolks. The extraction of the pigments and the separation of the carotene-cryptoxanthin from the xanthophyll by means of the difference in solubility in 90 per cent methyl alcohol and petroleum ether, was the same as previously described. The alcohol-soluble fraction was further separated on an adsorption column.

In this series only the alcohol-soluble fraction was prepared for feeding. Since this fraction was to be passed through an adsorption column, a method differing from the one described in Series I for extracting xanthophyll from the 90 per cent methyl alcohol was necessary. The 90 per cent methyl alcohol solution, was diluted with distilled water and extracted repeatedly with petroleum ether. The diluted alcoholic solution was further extracted with ethyl ether. Alcohol dissolved in the petroleum ether and ethyl ether extractions was washed out with distilled water. When the petroleum ether solution was dried an appreciable amount of xanthophyll was adsorbed on the anhydrous sodium sulfate. This adsorbed xanthophyll was easily removed when ethyl ether (containing xanthophyll) was filtered through the anhydrous sodium sulfate. The dried ethyl ether extractions were evaporated to an oily residue, to which the petroleum ether solution, containing xanthophyll, was added. The distillation was continued until only about 15 milliliters of petroleum ether remained.

Magnesium oxide* and Hyflo Super-Cel (1:1 by weight) were thoroughly mixed, heated at 100° centigrade overnight and placed in closely stoppered bottles until used. The adsorption column was prepared according to the method used by Jacob (1939) which closely followed the method of Strain (1934). The speed of flow of the xanthophyll solution onto the column was controlled by means of a small separatory funnel arranged above the column. Pigments were protected from oxidation with

*Magnesium oxide, Micron brand #2640, California Chemical Company, Newark, California.

carbon dioxide during the adsorption process. After the adsorbent was moistened with petroleum ether, the highly concentrated petroleum ether solution containing xanthophyll was added. In the process of developing the column with petroleum ether only a small amount of pigment was washed out into the filtrate, the remainder was adsorbed in two distinct zones—an orange layer at the very top and a light violet-red layer about one-third of the way down on the column. The lower two-thirds of the adsorbent in the column presented a yellow-gray appearance. The pigments and the material adsorbed on the entire column were eluted collectively with absolute ethyl alcohol. They were placed in a volumetric flask, made up to volume, and the concentration of the solution determined. This alcoholic solution was added to Wesson oil as described for the xanthophyll extracted from yellow corn, and made up to a concentration of 450 micrograms xanthophyll per milliliter of Wesson oil.

Series III.—Carotenoid pigments extracted from egg yolks. The procedure for the extraction, separation and adsorption of the carotenoid pigments extracted from egg yolks was the same as that described in Series II. In Series III, however, the pigments adsorbed on the magnesium oxide Hyflo Super-Cel adsorption column were divided into two parts, namely: Fraction I, the top orange layer; and Fraction II, the light violet-red layer plus any material adsorbed on the remainder of the column. A diagram of the column is shown in Figure 1. Each of the two fractions was carefully removed from the column, eluted with ethyl alcohol, measured in the electrophotometer and added to Wesson oil to give the concentration desired for the supplement for feeding. Even though the alcoholic solution of Fraction II was light violet-red in color and possessed no perceptible yellow color it was thought best to measure the light absorption of this solution at the 4358 Å wave length. The dial reading, when referred to the reference curve for carotene, showed that this alcoholic solution contained material equivalent to only 0.6 micrograms of carotene per milliliter. Since there was a limited quantity of the solution available and since rats require less supplement than chicks, it was decided to test the biological activity of this material by feeding it to rats. It was added to Wesson oil so that each milliliter contained the unknown in an amount equivalent to 1.6 micrograms of carotene per milliliter.

The petroleum ether washings of the adsorption column labeled Fraction III were evaporated to dryness, taken up in absolute ethyl alcohol and measured on the electrophotometer. The amount of light absorbed by this material was equivalent to 1.15 micrograms of carotene per milliliter. It was added to Wesson oil and treated so that the final concentration was equivalent to 3 micrograms of carotene per milliliter. Rats were used as the experimental animals in testing the biological activity of this material.

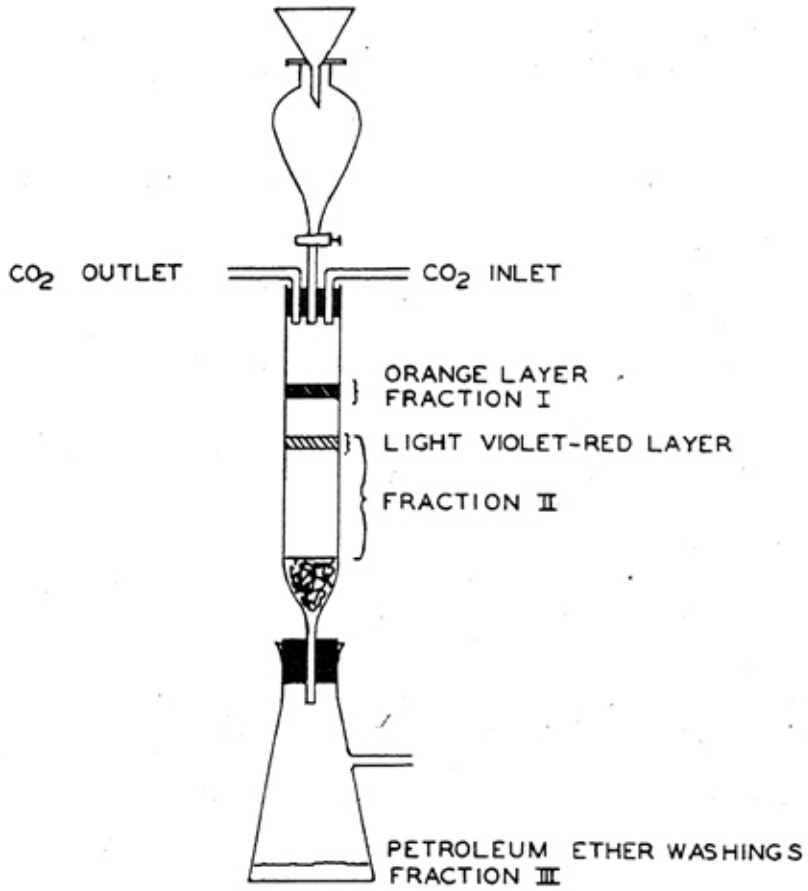


Figure 1.—Diagram showing the construction of the magnesium oxide adsorption column and the location of the three fractions into which the alcohol-soluble substances from egg yolks were divided for biological assay.

Spectrographic Analyses of Materials Used in This Investigation

Reference Cod Liver Oil.—Absorption measurements were made of (1) reference cod liver oil with ethyl alcohol in the comparison cell, (2) reference cod liver oil with the blank in the comparison cell and (3) the blank with ethyl alcohol in the comparison cell.

Carotenoid Supplements.—Samples of each of the pigments fed as supplements to the vitamin A-free basal diet were removed for spectrographic analysis while still in solution in absolute ethyl alcohol. Solutions of the following pigments were analyzed:

Series I—commercial crystalline carotene, commercial crystalline xanthophyll, xanthophyll extracted from yellow corn; Series II—the alcohol-soluble fraction from egg yolks, adsorbed on magnesium oxide adsorption column (1) the orange layer (2) the light violet-red layer and all below it and (3) the petroleum ether washings of the column. The light violet-red layer alone, from the alcohol-soluble fraction from egg yolks, chromatographed on magnesium oxide was analyzed but not fed.

Biological Assay of the Pigments

Experimental Animals.—Day-old White Leghorn female chicks* were purchased for this experiment by the Department of Poultry Husbandry, University of Missouri.

The chicks were wing-banded, weighed and placed in brooders in the laboratory. By means of a thermostatically controlled heating unit in the brooder a temperature of 36-37° centigrade was maintained for the first few days, after which it was gradually reduced until at the end of three weeks it was approximately 29° centigrade (Hogan and Boucher, 1933). Distilled water and the basal diet were available at all times. Records were kept of the weekly weight and food consumption. On the seventeenth day, or near that time, symptoms of vitamin A deficiency appeared in some of the chicks. Beginning at that time and continuing until they were depleted, they were weighed daily. When a chick reached constant weight or lost a few grams a day, it was placed on supplement. Care was taken to have the number of grams of chick in each of the supplement groups as nearly equal as possible.

In Series III rats were used as experimental animals in addition to chicks. These young rats were reared by mothers on a stock diet consisting of two-thirds whole wheat, one-third whole milk powder and sodium chloride two percent of the weight of the wheat. When the rats were two weeks old the mother and young were fed the vitamin A-free diet in place of the regular stock diet. The young rats weighing 35-40 grams each were weaned at 21 days of age. The vitamin A-free basal diet and distilled water were fed ad libitum. They were weighed weekly until symptoms of vitamin A deficiency began to appear in some rats of the litter; after this they were weighed daily until depleted. Criteria for judging depletion were a slight loss in weight or stationary weight for one to two days. When depleted, they were divided into groups, care being taken to distribute them as uniformly as possible in regard to sex and weight. They were fed the vitamin A-free basal diet alone or the vitamin A-free basal diet plus the supplement to be tested. At the end of the 5-week experimental period the animals were chloroformed and autopsied.

Basal Diet.—The composition of the basal diet was as follows:

	Per cent
White corn	55.0
Wheat middlings	25.0
Casein	12.0
Yeast, dried brewer's, non-irradiated	3.5
Yeast, dried brewer's, irradiated	1.5
Limestone	1.0
Bonemeal	1.0
Sodium chloride, iodized	1.0
Manganese	0.05

*From the Edwards Hatchery, Springfield, Missouri.

Vitamin A was removed from the casein by extracting it 4 times with boiling 95 per cent alcohol.

The dried brewer's yeast was irradiated with a Hanovia Alpine Sun Lamp using the method of Steenbock, Hart, Hanning and Humphrey (1930).

Supplements to the Basal Diet.—After the chicks were depleted of their stores of vitamin A, several were chloroformed and their tissues prepared for analysis. The rest were fed supplements to the basal diet 3 times per week, as indicated for Series I, Table 5, page 31. The carotenoid pigments incorporated in Wesson oil were kept in amber bottles in a refrigerator at 4° centigrade. Enough of each of the solutions for one day's feeding was transferred to a small amber bottle and allowed to come to room temperature before it was fed. The solution was introduced directly into the crop of the chick by means of a two-milliliter hypodermic syringe, graduated in 0.1 milliliters and having a special blunt tipped needle 3 inches in length. In order to avoid danger of contamination of one kind of carotenoid with another, a separate hypodermic syringe and needle was used for each of the solutions fed.

The chicks on the yellow corn supplement were kept in individual cages so that it was possible to keep an accurate record of their food consumption. Each chick was fed 100 grams of a diet in which yellow corn replaced the white corn and to which 2.74 per cent of Wesson oil was added. As soon as it had consumed the yellow corn diet it was fed the basal diet for the remainder of the week. In this way each chick received (1) 55 grams of yellow corn per week and (2) the same amount of Wesson oil as each of the chicks in the pigment-Wesson oil supplemented groups.

The xanthophyll and the carotene-cryptoxanthin extracted from yellow corn were fed in the same quantities in which they occurred in the 55 grams of yellow corn. The amount of each of the pigments required to accomplish this was indicated by the results of the quantitative analysis of the yellow corn for carotene-cryptoxanthin and for xanthophyll.

Commercial carotene and xanthophyll were fed in order that a comparison could be made of the biological activity of these products with the carotene-cryptoxanthin and xanthophyll prepared in this laboratory from yellow corn and from egg yolks.

Negative Control Group. Several chicks were kept on the basal diet alone and fed 3 milliliters of Wesson oil per chick per week.

Positive Control Group. Each chick in this group was fed the basal diet plus 3 milliliters per week of reference cod liver oil-Wesson oil mixture, containing 150 International Units of vitamin A per milliliter.

At the close of the experimental period of 5 weeks the chicks were chloroformed and autopsied. The livers were placed in a container separate from the rest of the tissues. The composite sample of liver and of tissue was cooked at 10 pounds pressure for 30 minutes and then cooled. The liver was ground, mixed with the juice and kept frozen until analyzed

for vitamin A and for carotenoid pigments. After removing the bones, the tissues were prepared in the same manner as described for the liver.

The general feeding procedure for the chicks in Series II and III was the same as for Series I. The kind and amount of supplements fed in Series II are given in Table 6, page 31, for Series III in Table 7, page 33. Supplements fed to the rats in Series III are shown in Table 8, page 33.

Analyses of the Tissues of the Chicks for Carotene-Cryptoxanthin, Xanthophyll, and Vitamin A

All tissues were kept in closely covered glass jars in a refrigerator at 0° centigrade until they were analyzed. They were removed from the refrigerator, allowed to come to room temperature and samples weighed for the analyses.

Carotene-cryptoxanthin and xanthophyll determinations were made: (1) on the liver and (2) on a composite of all edible tissues except the liver, from each of the groups as indicated in Series I, Tables 9, page 47, and 10, page 47; and Series II, Tables 11, page 48, and 12, page 48. The quantity of material used for each analysis was approximately 30 grams for the muscle tissue and 15 grams for liver tissue. Aldehyde free ethyl alcohol and 100 per cent aqueous potassium hydroxide were added. The mixture was saponified on a hot water bath, diluted with distilled water and cooled. The extraction method was the same as described for the quantitative analysis of these pigments in yellow corn. The amounts of each of the pigments present in the solution were determined by means of the electrophotometer.

Vitamin A determinations were made on liver and on a composite of all edible tissue except liver, for all groups in Series I and Series II. The sample was prepared for the spectrographic analysis according to the method described by Wilkie (1940). Here again, as with the cod liver oil samples, a blank was prepared to be used in the comparison cell of the spectrophotometer in place of the absolute ethyl alcohol. Even with the blank in the comparison cell the amount of extraneous material was so large that in most cases it minimized or obliterated the vitamin A peak. It was therefore necessary to estimate the amount of absorption due to the extraneous material and to subtract this from the total density. The estimation was based on a careful study and comparison of the shape of standard reference cod liver oil curves, protein curves, sample curves and sample plus cod liver oil curves, taking into consideration the concentration of each of these solutions.

Moisture determinations were made in duplicate on all of the tissues analyzed for carotenoids and for vitamin A.

RESULTS AND DISCUSSION

Reference Curves

Carotene and Xanthophyll.—Solutions of commercial crystalline carotene and of commercial crystalline xanthophyll, when measured in the electrophotometer gave dial readings as shown in Tables 1 and 2.

TABLE 1.—DIAL READINGS FOR KNOWN CONCENTRATIONS OF THE CAROTENE SOLUTION

Carotene in absolute ethyl alcohol Micrograms per liter	Dial Readings		
	Sample Number		
	1	2	Average
1594	182.7	182.7	182.7
1435	178.8	178.8	178.8
1275	174.1	174.1	174.1
1116	167.1	167.2	167.2
956	157.4	157.3	157.4
797	145.4	145.5	145.5
638	128.3	128.3	128.3
478	105.7	105.6	105.7
319	75.3	75.3	75.3
159	38.9	39.0	39.0

TABLE 2.—DIAL READIN.GS FOR KNOWN CONCENTRATIONS OF THE XANTHOPHYLL SOLUTION

Xanthophyll in absolute ethyl alcohol Micrograms per liter	Dial Readings		
	Sample Number		
	1	2	Average
1480	178.4	178.4	178.4
1332	173.7	173.7	173.7
1184	167.7	167.5	167.6
1036	159.5	159.5	159.5
888	148.4	148.4	148.4
740	135.1	134.3	134.7
592	117.1	116.8	117.0
444	92.8	92.8	92.8
296	64.7	64.4	64.6
148	30.5	30.3	30.4

The concentration of the carotene and of the xanthophyll solutions together with their corresponding average dial readings are plotted in Figure 2 and form the carotene and the xanthophyll reference curves from which it is possible to read off the concentration of these pigments in an unknown solution when the dial reading is known.

Vitamin A.—Absorption spectra measurements made on samples of reference cod liver oil prepared for the vitamin A analysis are shown in Table 3.

Results of these measurements made in a 10 centimeter cell showed that 0.022 I. U. of vitamin A per milliliter of solution were required to give 0.01 density unit. By referring the observed density value of the

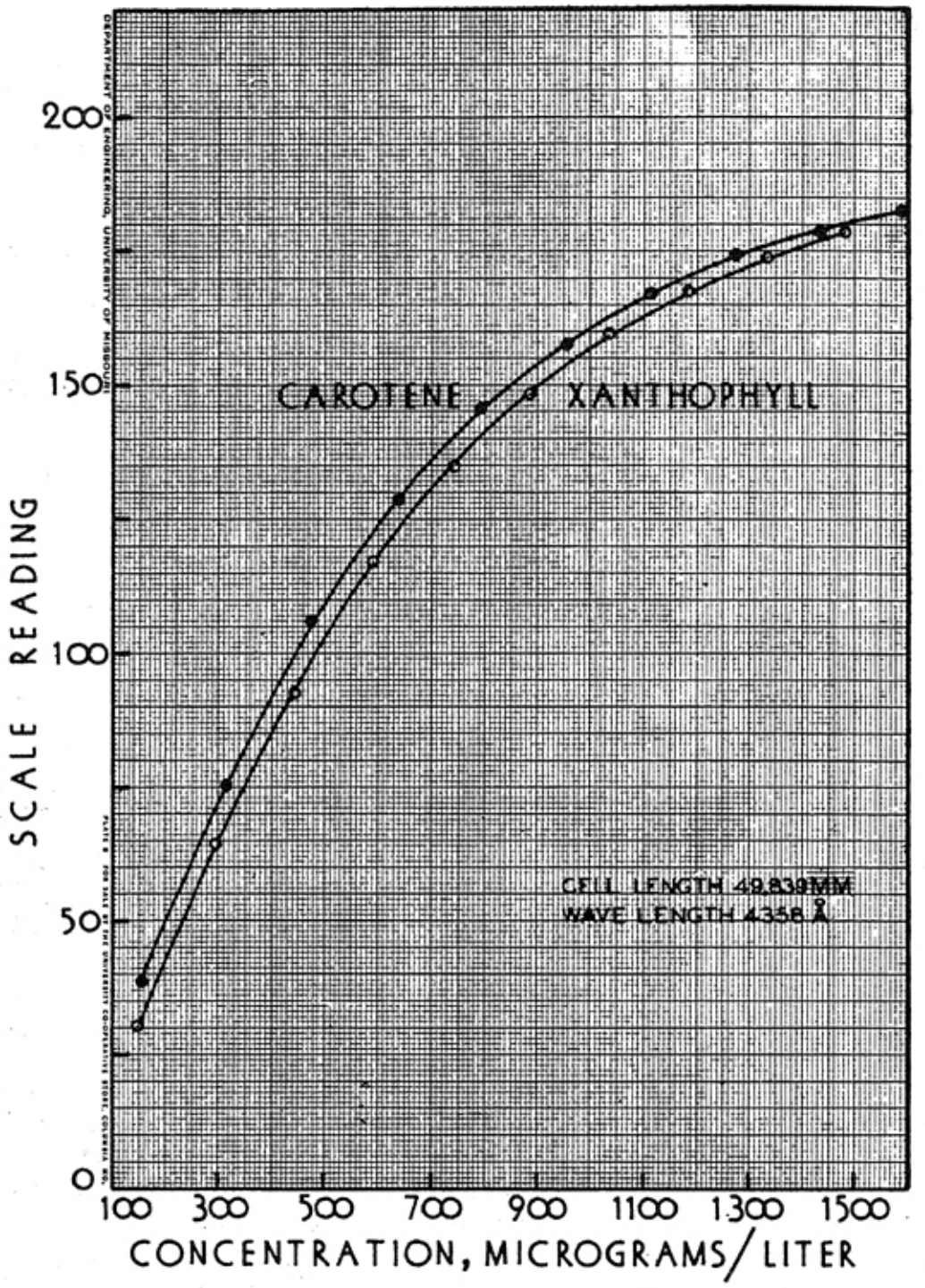


Figure 2.—Reference curves for carotene and xanthophyll. Measurements were made on the electrophotometer.

unknown sample at 3300 Å to the nearest density value given in Table 3 it is possible to calculate the amount of vitamin A present in the sample

being analyzed. For example, if 5 grams of liver prepared for analysis and made up to a volume of 25 milliliters give a density value of 0.69 when measured, undiluted in a 10 centimeter cell the units of vitamin A per gram of liver may be calculated as follows:

$$\frac{1.616 - (.10 \times .022 \times 100) \times 25}{5} = 6.98 \text{ I. U./gram}$$

TABLE 3.—DENSITY VALUES AT 3300 Å FOR KNOWN CONCENTRATIONS OF VITAMIN A FROM REFERENCE COD LIVER OIL

Cell length cm.	I.U. of vitamin A per ml. of sample	Density at 3300 Å
10	3.086	1.50
10	1.616	0.79
10	0.828	0.43

Analysis of Yellow Corn for Carotene-Cryptoxanthin and Xanthophyll

Results of the quantitative measurements made on the amount of carotene-cryptoxanthin and of xanthophyll present in the room-dried yellow corn used in this study are reported in Table 4. The average amount of moisture present in the corn was 6.74 per cent.

The average value of each of these carotenoid fractions per gram of sample was found to be 11.17 micrograms for carotene-cryptoxanthin and 24.70 micrograms for xanthophyll—a ratio of carotene-cryptoxanthin to xanthophyll of 1:2.24. It is interesting to note that the ratios of carotene-cryptoxanthin to xanthophyll, 1:2.29, reported by other investigators (Kuhn and Grundmann, 1934 and Randolph and Hand, 1940) are in close agreement with the results obtained in this study.

TABLE 4.—CAROTENE-CRYPTOXANTHIN AND XANTHOPHYLL IN YELLOW CORN (ROOM-DRIED)

Sample No.	Weight of sample grams	Carotene-cryptoxanthin Micrograms per gram	Xanthophyll Micrograms per gram	Ratio of Car-crypto. to Xan.
I[a]	14.8600	12.69	24.33	1 : 1.92
II[a]	15.0034	13.18	24.43	1 : 1.85
I[b]	14.9951	9.77	24.84	1 : 2.54
II[b]	14.5810	10.68	26.20	1 : 2.45
I[c]	15.0460	10.37	23.93	1 : 2.31
II[c]	14.9202	10.30	24.46	1 : 2.37
Average		11.17	24.70	1 : 2.24

Spectrographic Analyses of Vitamin A and Carotenoid Solutions

Reference Cod Liver Oil.—The reference cod liver oil, used to establish the base of reference for the vitamin A determinations in samples of tissues, was saponified and prepared for analysis as previously described. In the course of these analyses it was found that the absorption maxima instead of appearing at 3280 Å specific for vitamin A, appeared at 3180 Å

when the cod liver oil sample was measured with absolute ethyl alcohol in the comparison cell, Figure 3. The use of a blank in the comparison cell in place of the absolute ethyl alcohol caused the maximum absorption to shift to 3300 Å Figure 4. This is more nearly the wave length, 3280 Å, specific for maximum absorption of vitamin A. It will be observed, however, that the shape of the absorption curve, Figure 4, is different

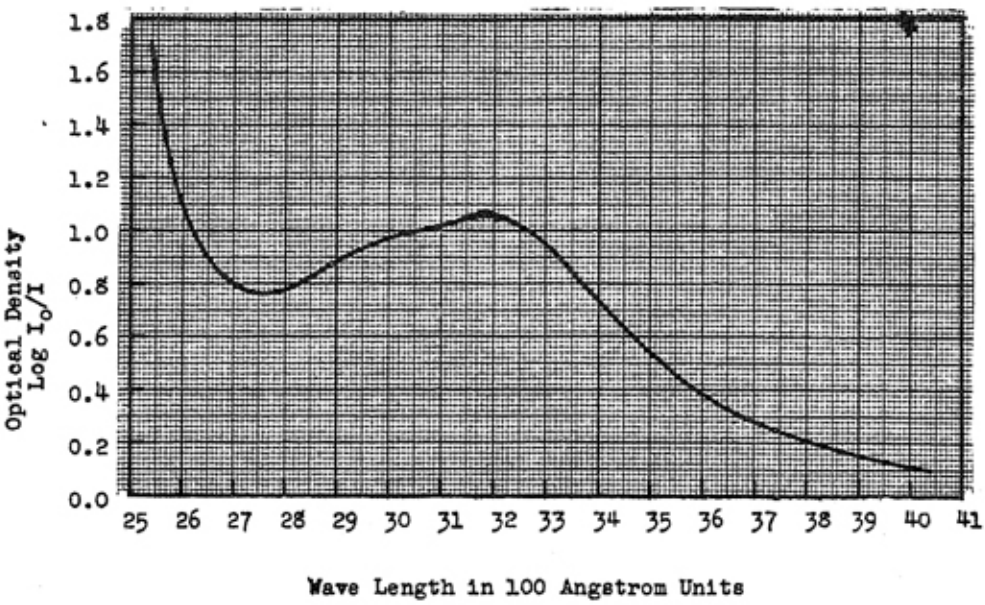


Figure 3.—Absorption spectrum of reference cod liver oil (19.067 I.U. per ml.) in ethyl alcohol. Ethyl alcohol in the comparison cell.

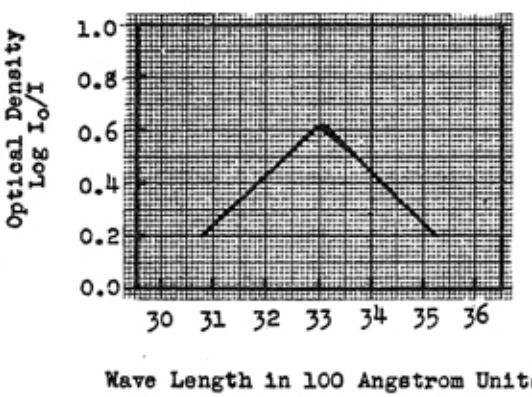


Figure 4.—Absorption spectrum of reference cod liver oil, the same as in Figure 3, with the blank in the comparison cell.

from typical vitamin A absorption curves reported in the literature. The amount of absorption in the blank itself when measured against absolute ethyl alcohol is shown in Figure 5. The sharp rise in the amount of absorption beginning at 3450 Å and increasing steadily at the lower wave lengths, shows that some material is present in the blank which causes an appreciable amount of light absorption in this part of

the spectrum. The presence of this extraneous material in the blank itself which might have been present in the solvents or might have developed in the blank as it was prepared for analysis probably accounts for maximum absorption at 3180 Å, Figure 3. Similar extraneous material would also be present in the cod liver oil sample prepared for analysis and cause a shift in the point of maximum absorption to the lower wave length. In order to overcome the difficulty, a blank was prepared for all vitamin A determinations. The use of this blank in the comparison cell of the spectrophotometer in place of absolute ethyl alcohol automatically subtracts a part of the absorption due to extraneous material in the sample.

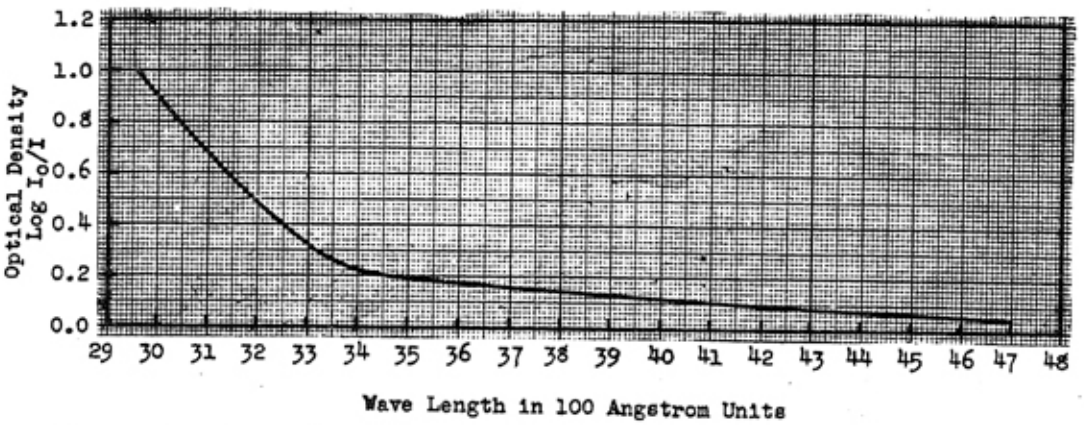


Figure 5.—Absorption spectrum of the blank in ethyl alcohol. Ethyl alcohol in the comparison cell.

Supplements Fed in Series I.—Absorption spectra measurements with absolute ethyl alcohol in the comparison cell of the spectrophotometer, were made on alcoholic solutions of the following: (1) commercial crystalline carotene, (2) commercial crystalline xanthophyll, (3) alcohol-soluble fraction from yellow corn, and (4) petroleum ether-soluble fraction from yellow corn.

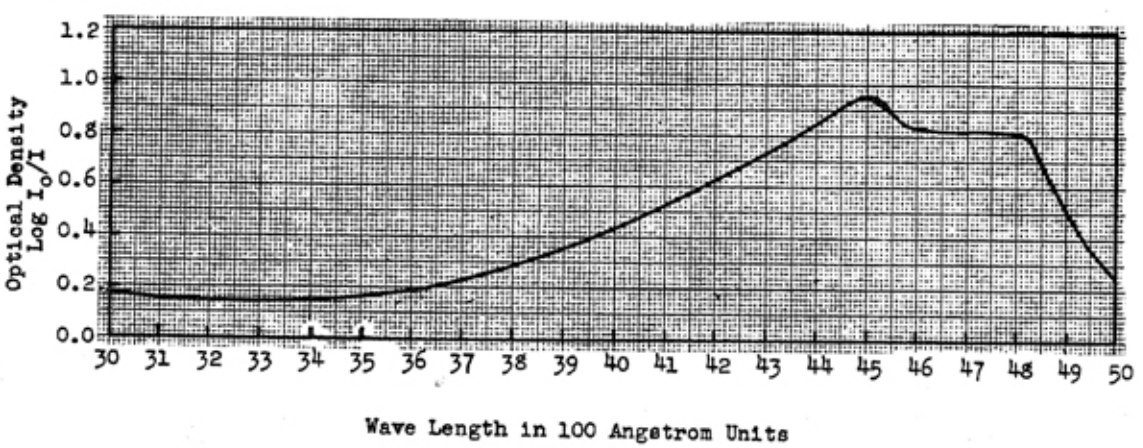


Figure 6.—Absorption spectrum of commercial crystalline carotene in ethyl alcohol.

Maximum absorption from the commercial carotene, Figure 6, appeared at 4500 Å but there was no maximum at 4818 Å. Instead of this the absorption of light remained approximately the same from 4600 to 4820 Å. Considering the fact that this carotene was a commercial product not recrystallized before these measurements were made, and also that it was a mixture of the alpha and beta-carotene, the results obtained here agree fairly well with those reported by Strain (1934) who obtained absorption maxima at 4520 and 4818 Å for beta-carotene in ethyl alcohol and 4457 and 4760 Å for alpha-carotene in ethyl alcohol.

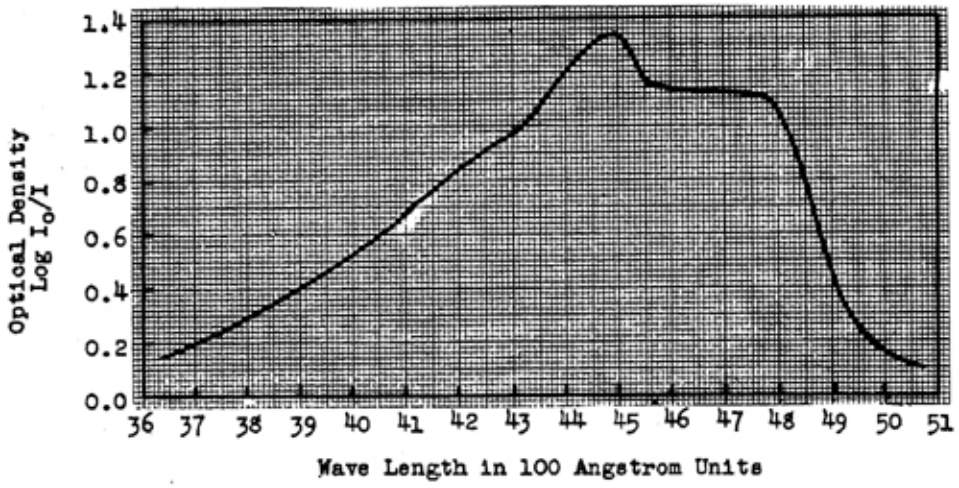


Figure 7.—Absorption spectrum of commercial crystalline xanthophyll in ethyl alcohol.

The absorption curve for commercial xanthophyll Figure 7, shows a slight inflection near 4200 Å, a maximum at 4495 Å and a plateau at 4600 to 4770 Å. Absorption maxima for xanthophyll (lutein) in ethyl alcohol have been reported as 4200, 4465 and 4760 Å (Karrer, Zubrys, and Morf, 1933) and for zeaxanthin in ethyl alcohol at 4235, 4515 and 4830 Å (Kuhn and Winterstein, 1931). Even though the alcohol-soluble fraction from yellow corn was not separated into its components, it probably consisted of a mixture of carotenoids which according to Karrer,

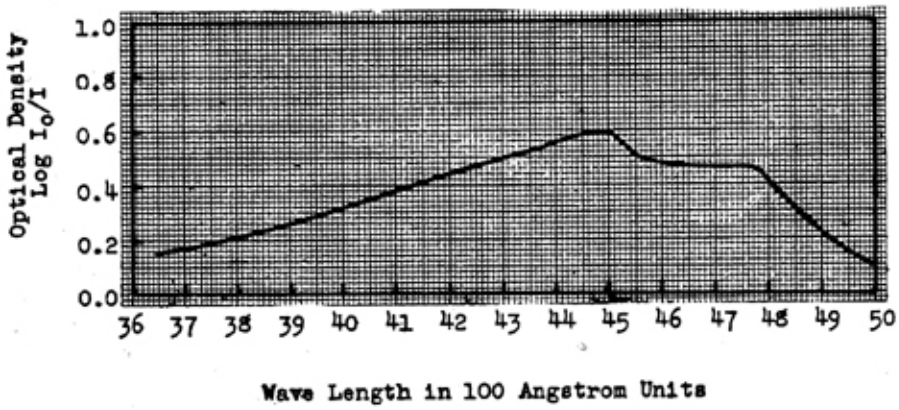


Figure 8.—Absorption spectrum of xanthophyll extracted from yellow corn in ethyl alcohol.

Salomon and Wehrli (1929) is chiefly zeaxanthin. For this fraction, Figure 8, an absorption maximum was obtained at 4495 Å and the location of a plateau similar to that obtained with commercial xanthophyll was observed. Strain (1938) obtained absorption maxima for cryptoxanthin in ethyl alcohol at 4200, 4520 and 4860 Å. The petroleum ether-

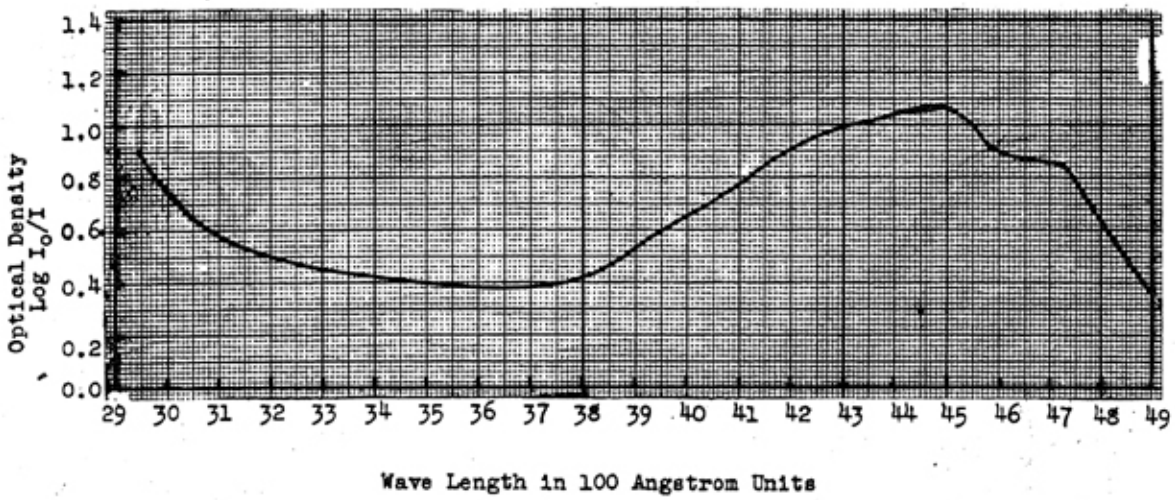


Figure 9.—Absorption spectrum of carotene-cryptoxanthin extracted from yellow corn in ethyl alcohol.

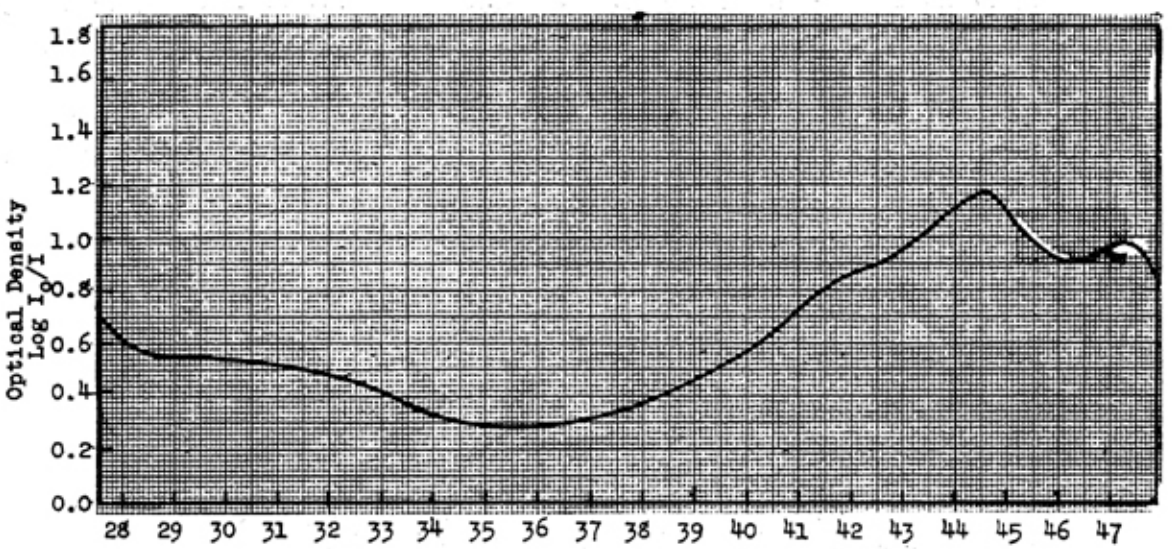


Figure 10.—Absorption spectrum for the alcohol-soluble fraction from egg yolks, eluted with ethyl alcohol, from a magnesium oxide adsorption column.

soluble fraction of yellow corn in ethyl alcohol, Figure 9, showed a point of maximum absorption at 4495 Å, and a leveling off of the curve beginning at 4650 Å and ending at 4725 Å.

Since the absorption spectrum of cryptoxanthin is practically the same as that of beta-carotene and zeaxanthin it is difficult to distinguish these pigments spectrographically (Kuhn and Grundmann, 1933). Hence

one would not expect closer agreement between the results of these experiments and those reported in the literature.

Supplements Fed in Series II.—The alcohol-soluble fraction from egg yolks was chromatographed on a magnesium oxide adsorption column. The material adsorbed on the entire column was eluted with ethyl alcohol. Spectrographic analysis of this material, with ethyl alcohol in the comparison cell gave the absorption curve shown in Figure 10. In addition to the absorption maxima at 4460 Å and 4725 Å, and the slight inflection centered near 4160 Å, there was a broad inflection centered at 3100 Å.

Supplements Fed in Series III.—In a manner similar to that described for the pigments fed in Series II, the alcohol-soluble fraction from egg yolks was chromatographed on the adsorption column. However, for

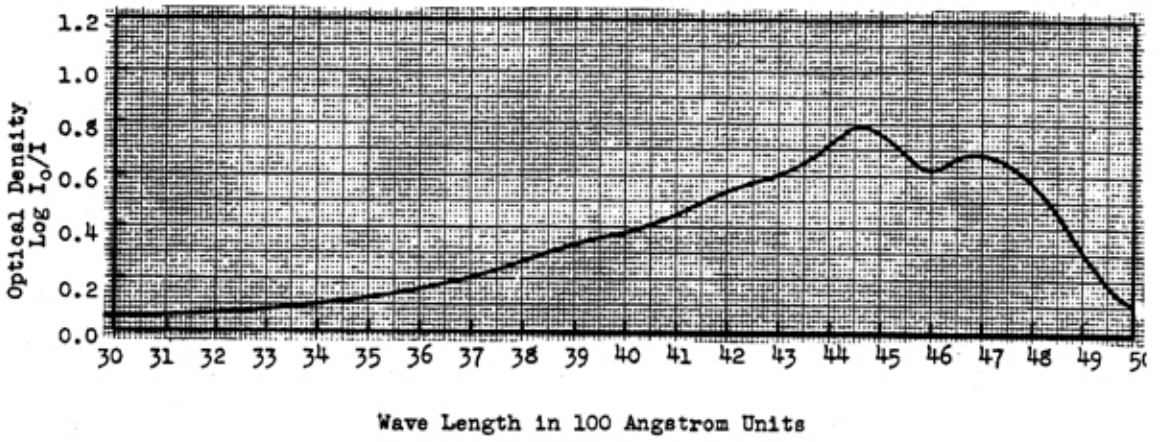


Figure 11.—Absorption spectrum of the orange layer, eluted with ethyl alcohol, from a magnesium oxide adsorption column through which the alcohol soluble fraction from egg yolks had been passed. (Figure 1, page 15.)

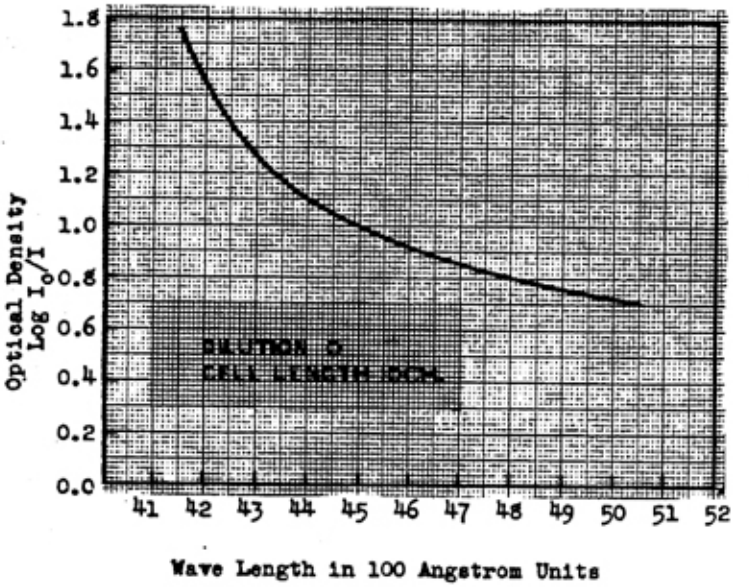


Figure 12.—Absorption spectrum of the light violet-red layer and all below it, eluted with ethyl alcohol, from a magnesium oxide adsorption column through which the alcohol-soluble fraction from egg yolks had been passed. Figure 13 shows two additional absorption spectra of this solution.

the supplements prepared for Series III the column was divided into parts as illustrated in Figure 1, page 15. The top orange layer was removed from the column and the pigment was eluted with ethyl alcohol. Likewise the light violet-red layer and all below it was removed from the column and eluted with alcohol. Absorption maxima, typical of xanthophyll appear at 4465 and 4695 Å for the orange layer, Figure 11. It should be noted also that there is no inflection near the 3100 Å wave length in this fraction. Absorption values for the light violet-red layer, and all below it, are just the opposite of those for the orange layer. The light violet-red solution diluted 1-9 and measured in a 1 centimeter cell showed a band of maximum absorption at 3075 Å. When measured un-

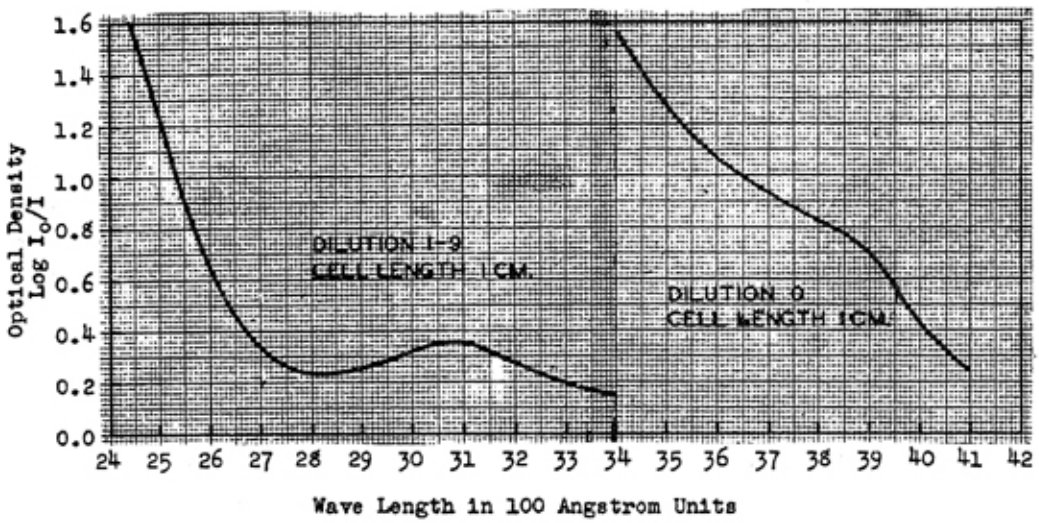


Figure 13.—Absorption spectra of the solution described in Figure 12.

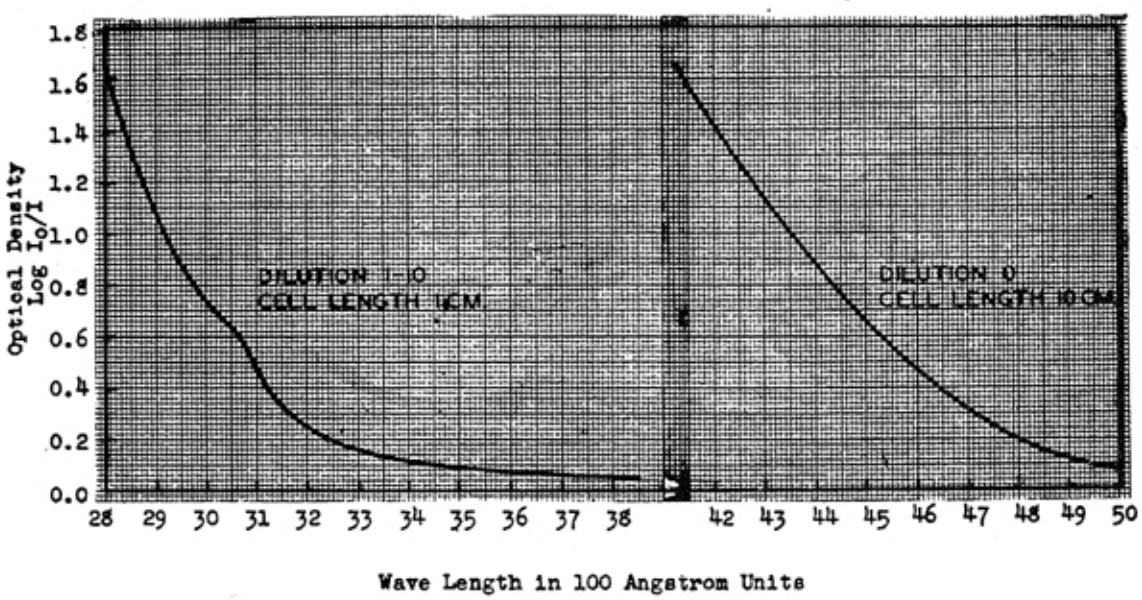


Figure 14.—Absorption spectra of that part of the alcohol-soluble fraction from egg yolks washed through a magnesium oxide adsorption column with petroleum ether.

diluted in a 1 centimeter cell a slight inflection or hump appeared between 3800 and 3950 Å, and the undiluted solution measured in a 10 centimeter cell showed definitely that no carotene-cryptoxanthin or xanthophyll was present, Figures 12 and 13.

Fraction III, the petroleum ether washings of the alcohol-soluble fraction, adsorbed on magnesium oxide, when measured undiluted in a 10 centimeter cell, gave no maxima characteristic for carotenoids. The same solution diluted 1 to 10 and measured in a 1 centimeter cell, showed an inflection between 3000 and 3100 Å, Figure 14.

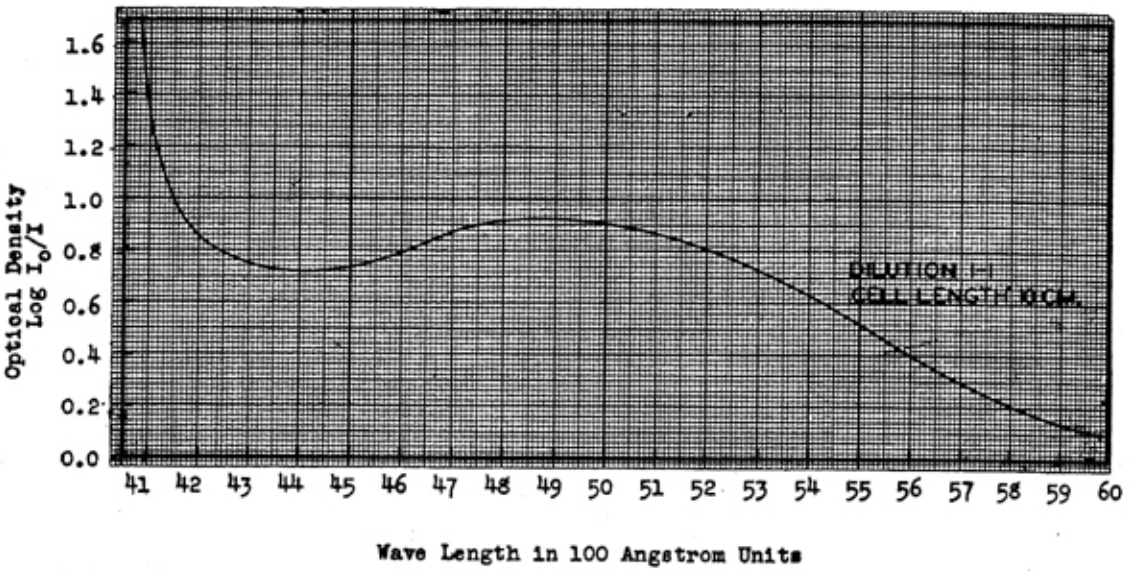


Figure 15.—Absorption spectrum of the light violet-red layer only. This layer was removed from the magnseium oxide adsorption column through which the alcohol-soluble fraction from egg yolks had been passed. The adsorbed material was eluted with ethyl alcohol. Figure 16 shows another absorption spectrum of this solution.

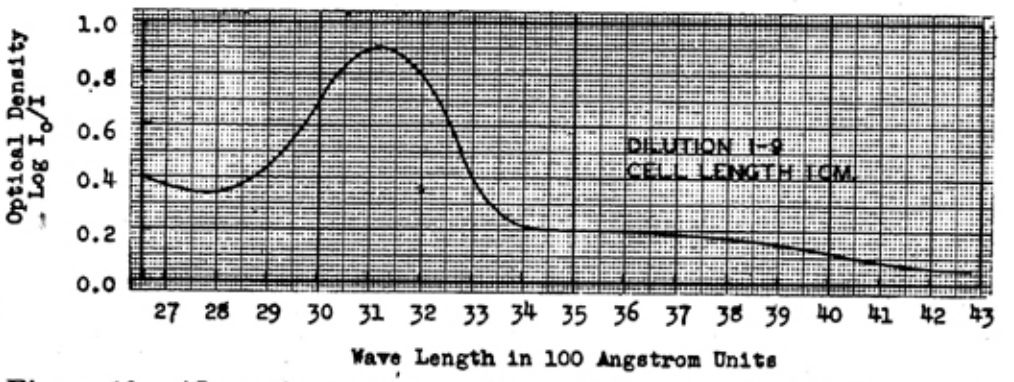


Figure 16.—Absorption spectrum of the solution described in Figure 15.

Solution Analyzed Spectrographically but not Fed.—The light violet-red layer adsorbed on magnesium oxide was eluted with ethyl alcohol and the absorption spectrum of the solution measured. In a dilution of 1 to 1 measured in a 10 centmieter cell, this solution gave a broad inflection centered at 4900 Å. Absorption maxima typical for carotene-

cryptoxanthin and xanthophyll were absent, Figure 15. The same solution from the light violet-red layer (Figure 16) diluted 1-9 and measured in a 1 centimeter cell showed a definite absorption maximum at 3115 Å which is similar to the point of maximum absorption for the light violet-red layer plus all below it on the magnesium oxide column (Figure 13).

Biological Assay

The average length of time required to deplete the chicks of their body-stores of vitamin A was 22 days. Those chicks kept on the vitamin A-free diet after depletion lived only an average of 7 days beyond the depletion period. This agrees closely with the work of Frohring and Wyeno (1934) who reported that the 232 negative controls, in their investigation of the carotene and vitamin A requirements of White Leghorn chicks, lived an average of 27.2 days. Kline, Schultze and Hart (1932) in a similar study reported that the chicks were depleted in 28 days and that the negative controls died 4-6 days after the depletion period.

Symptoms of vitamin A deficiency in the chicks as described by Elvehjem and Neu (1932) and Record, Bethke and Wilder (1937) were observed in this study. Poor equilibrium was the first symptom to appear and was the most prevalent. On further depletion the condition described as ruffled feathers became pronounced. Less prevalent symptoms were ophthalmia and a swelling of the glands in the larynx. These symptoms of vitamin A deficiency were overcome in the 5-week experimental period when commercial carotene, carotene-cryptoxanthin from yellow corn, yellow corn or cod liver oil supplemnets were fed. In those chicks kept on the vitamin A-free diet after depletion or in those fed the xanthophyll supplements in addition to the basal diet, the symptoms became progressively worse. At autopsy, urates in the kidneys and ureters were observed in all the negative controls and in those fed the xanthophyll supplements. The difference in appearance of the chicks is shown in photographs, Figures 21-29.

Series 1.—The results summarized in Table 5 show that neither the xanthophyll from yellow corn nor the commercial crystalline xanthophyll possessed vitamin A activity. The chicks in these groups lost weight and exhibited symptoms of vitamin A deficiency similar to the negative controls. Mortality in the xanthophyll groups and in the negative control group was 100 per cent, while none of the chicks died in those groups receiving either commercial carotene, carotene-cryptoxanthin from yellow corn, yellow corn or cod liver oil.

The carotene-cryptoxanthin from yellow corn fed at a level of 600 micrograms per chick per week induced an average gain of 431 grams in 5 weeks. The same amount of carotene-cryptoxanthin plus 1350 micrograms of xanthophyll from yellow corn per chick per week produced an average gain of 375 grams in 5 weeks. Gains similar to the latter group were made when 55 grams of yellow corn, or 600 micrograms of com-

mercial carotene were fed. The slightly higher gains made by th chicks receiving 450 International Units of vitamin A from cod liver o were not significantly higher than gains of the other groups in th series exclusive of the negative controls and those receiving xanthophyl

The average gains of the chicks in Series I are plotted in Figure 1'

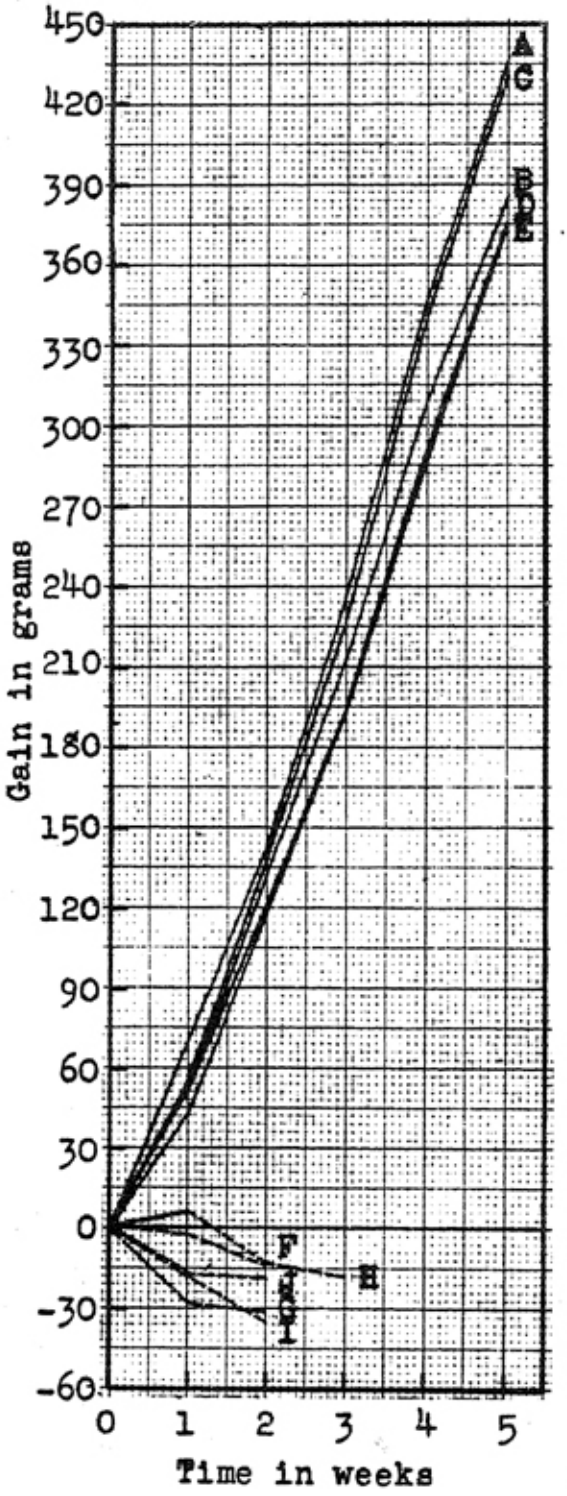


Figure 17

Average gain curves of chicks in Series I fed a vitamin A-free basal diet plus supplements expressed in International Units for the cod liver oil and in micrograms for the carotenoid pigments. The amount of supplement per chick per week was as follows:

A - Reference cod liver oil ---------- 450
B - Commercial crystalline carotene --------- 600
C - Carotene-cryptoxanthin extracted from yellow corn ---------- 600
D - Carotene-cryptoxanthin extracted from yellow corn ---------- 600 plus xanthophyll extracted from yellow corn ---------- 1350
E - Yellow corn ------ 55*
F - Xanthophyll extracted from yellow corn 1350
G - Xanthophyll extracted from yellow corn 2700
H - Commercial crystalline xanthophyll ---- 1350
I - Commercial crystalline xanthophyll ---- 2700
J - Negative controls. A-free diet alone.

Broken lines indicate the point at which one or more of the chicks died.

* Yellow corn--55 grams per chick per week.

Table 5.-Summary of Growth Records of Chicks Receiving the Vitamin A-free Diet Alone or Plus Supplements per Chick per Week as Indicated - Series I

Group Number	Supplement		Number of Chicks	Weight after depletion	Total gain	Total food intake	Survival	Mortality
	Kind	Amt. per chick per wk. micrograms		grams	grams	grams	days	%
1	Negative controls	--	5	129.6	-18.6	45	6	100
2	Reference cod liver oil	450*	7	163.3	437.0	1050	35	0
3	Commercial crystalline carotene	600	5	165.4	386.4	1136	35	0
4	Carotene-cryptoxanthin from yellow corn	600	5	165.4	431.4	1053	35	0
5	Carotene-cryptoxanthin plus xanthophyll from yellow corn	600+ 1350	6	152.0	374.7	939	35	0
6	Yellow corn	55**	5	151.0	374.2	925†	35	0
7	Xanthophyll from yellow corn	1350	7	144.6	-14.4	86	9	100
8	Xanthophyll from yellow corn	2700	2	177.0	-31.5	99	7	100
9	Commercial crystalline xanthophyll	1350	5	150.6	-18.2	106	12	100
10	Commercial crystalline xanthophyll	2700	2	199.5	-35.5	69	11	100

*International units.
**Grams; † of this amount 275 grams was yellow corn.

Series II.—The results summarized in Table 6 show that all of the chicks in Series II survived the 5-week experimental period except the negative controls.

Commercial crystalline carotene fed at a level of 45 micrograms per chick per week, induced a gain of 409 grams in 5 weeks. The alcohol-soluble fraction from egg yolks, which had been chromatographed on magnesium oxide and the adsorbed material eluted from the entire column, when fed at a level of 675 micrograms in addition to 45 micrograms of commercial crystalline carotene per chick per week caused an average gain of 456 grams in 5 weeks. When fed alone at a level of 1350 or 2700

Table 6.-Summary of Growth Records of Chicks Receiving the Vitamin A-free Diet Alone or Plus Supplements per Chick per Week as Indicated - Series II

Group Number	Supplement		Number of Chicks	Weight after depletion	Total gain	Total food intake	Survival	Mortality
	Kind	Amt. per chick per wk. micrograms		grams	grams	grams	days	%
1	Negative controls	--	7	166.7	-41.0	47	10	100
2	Commercial crystalline carotene	45	7	165.1	409.1	1049	35	0
3	Commercial crystalline carotene plus alcohol-soluble fraction from egg yolks	45 + 675	6	170.5	456.3	1098	35	0
4	Alcohol-soluble fraction from egg yolks	1350	4	157.0	324.7	1141	35	0
5	Alcohol-soluble fraction from egg yolks	2700	3	185.0	388.3	1153	35	0

micrograms per chick per week the alcohol-soluble fraction induced a gain of 325 and 388 grams respectively in 5 weeks. However, some signs of vitamin A deficiency such as poor equilibrium and a weakened condition of the muscles of the third eyelid (nicitating membrane) were present in both of these groups. Average gains for the chicks in this series are plotted in Figure 18.

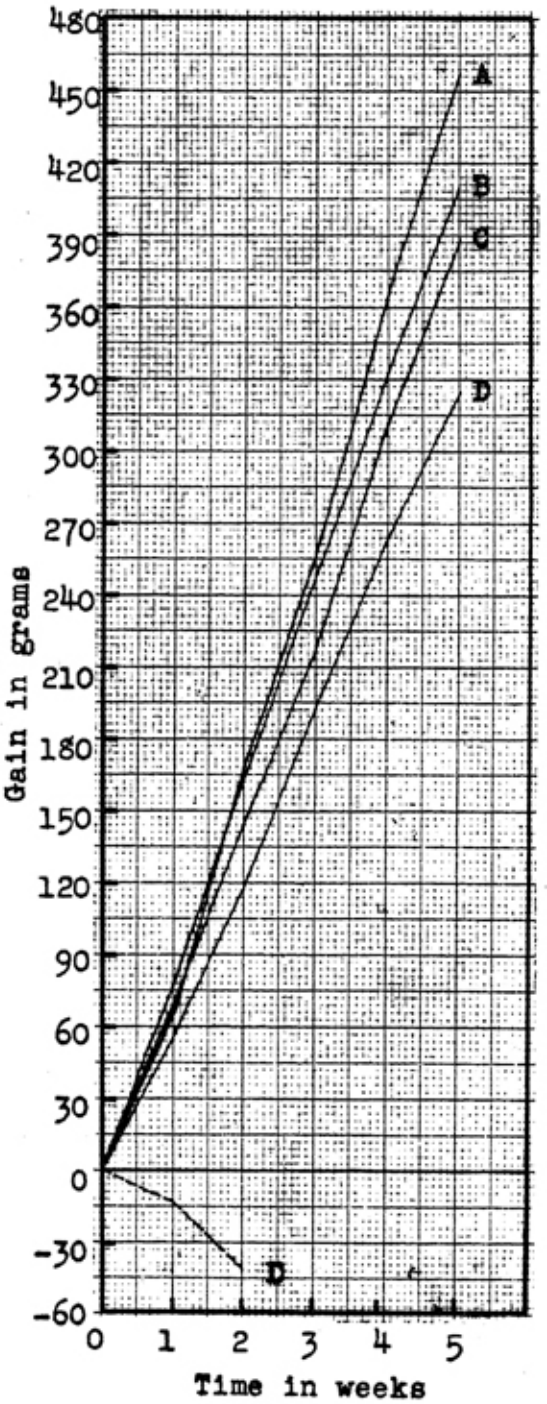


Figure 18

Average gain curves of chicks in Series II fed the vitamin A-free diet plus supplements expressed in micrograms per chick per week as follows:

A - Commercial crystalline carotene ---------- 45
plus
alcohol soluble fraction from egg yolks ---- 675

B - Commercial crystalline carotene ---------- 45

C - Alcohol soluble fraction from egg yolks --- 2700

D - Alcohol soluble fraction from egg yolks --- 1350

E - Negative controls - vitamin A-free diet alone.

Broken line indicates the point at which one or more of the chicks died.

It is recognized that the light violet-red layer, Fraction II, on the magnesium oxide column possessed vitamin A activity in sufficient amounts to enable the chicks to live during the entire experimental period when the alcohol-soluble fraction was fed at 1350 and 2700 micrograms (with respect to xanthophyll) per chick per week. Had the chicks been fed the orange layer alone they would not have lived long enough to allow for a study of the storage of the carotenoid pigments in the tissues. Similarly, the commercial carotene fed in addition to the 675 micrograms of the alcohol-soluble fraction from egg yolks, made it possible to study storage at this level of xanthophyll feeding.

Series III.—It will be observed (Table 7) that 225 micrograms of commercial carotene per chick per week were as effective for growth as 600 micrograms of commercial carotene. In this series, only the orange layer (Fraction I, Figure 1, page 15) of the alcohol-soluble fraction from egg yolks was fed at a level of 1350 micrograms per chick per week. Results showed that the pigment was devoid of vitamin A activity. The

Table 7.-Summary of Growth Records of Chicks Receiving the Vitamin A-free Diet Alone or Plus Supplements per Chick per Week as Indicated - Series III

Group Number	Supplement		Number of Chicks	Weight after depletion	Total gain	Total food intake	Survival	Mortality
	Kind	Amt. per chick per wk. micrograms		grams	grams	grams	days	%
1	Negative controls	--	4	142.0	-17.8	29	5	100
2	Alcohol-soluble fraction from egg yolk. I Orange layer.	1350	6	160.3	-22.3	77	9	100
3	Commercial crystalline carotene	225	5	174.4	401.6	1025	35	0
4	Commercial crystalline carotene	600	6	161.5	386.5	967	35	0

Table 8.-Summary of Growth Records of Rats Receiving the Vitamin A-free Diet Alone or Plus Supplements per Rat per Week as Indicated - Series III

Group Number	Supplement		Number of rats	Weight after depletion	Gain in 4 weeks	Gain in 5 weeks	Survival	Mortality
	Kind	Amt. per rat per wk. micrograms		grams	grams	grams	days	%
1	Negative controls	--	4	92.8	-16.6	-18.8	19	100
2	Commercial crystalline carotene	6	5	98.2	49.6	55.8	35	0
3	Petroleum ether-soluble fraction from egg yolks, not chromatographed	12	4	93.0	70.9	75.8	35	0
4	Alcohol-soluble fraction from egg yolks, chromatographed I Orange Layer	144	6	95.0	-11.0	-14.3	26	100
5	I Orange Layer	288	6	94.2	7.0	- 6.0	30	100
6*	II Light violet-red layer	2.4	3	100.3	63.0	--	28	0
7*	II Light violet-red layer	4.8	3	102.0	73.0	--	28	0
8*	III Petroleum ether washings of the adsorption column	9	2	101.5	22.0	--	28	0

*Groups 6, 7 and 8 kept on experiment for 4 weeks only.

chicks in this group were comparable to the negative controls in survival, weight loss and mortality. Average gains for this series are plotted in Figure 19. A chick representative of the group fed Fraction I is shown in Figure 29, page 45. The general appearance of the chicks receiving commercial xanthophyll, xanthophyll from yellow corn or xanthophyll from egg yolks is strikingly similar (Figures 24, 26 and 29, paes 42, 43, and 45.

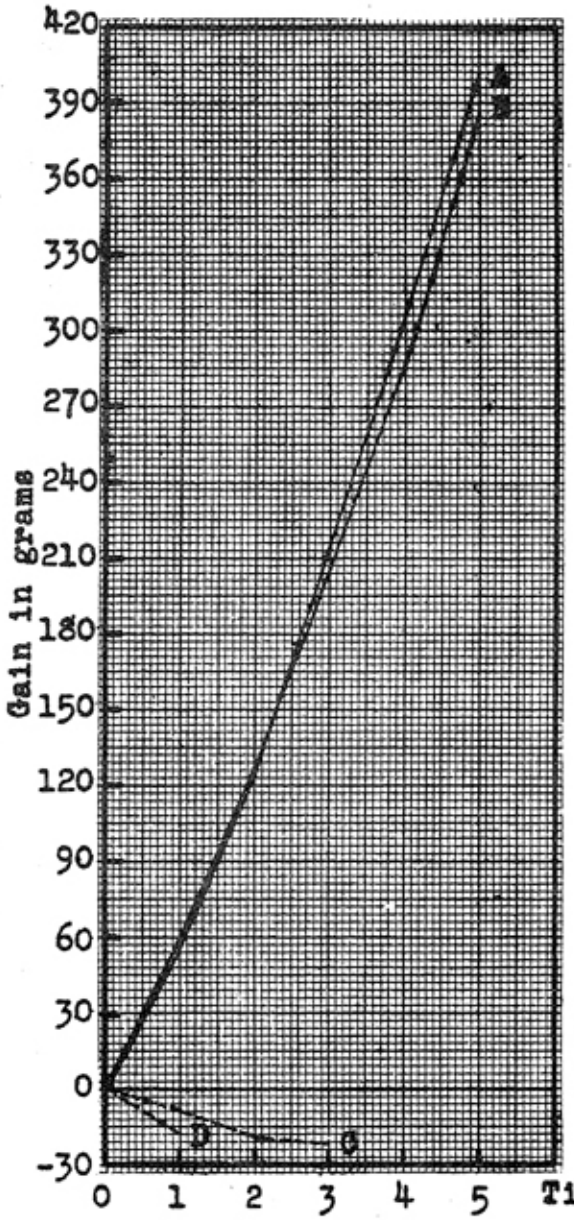


Figure 19

Average gain curves of chicks in Series III fed the vitamin A-free diet plus supplements expressed in micrograms per chick per week as follows:

A - Commercial crystalline carotene ---------- 225

B - Commercial crystalline carotene ---------- 600

C - Alcohol soluble fraction from egg yolks, chromatographed (Figure 1 page 15)

I Orange layer -- 1350

D - Negative controls - vitamin A-free diet alone.

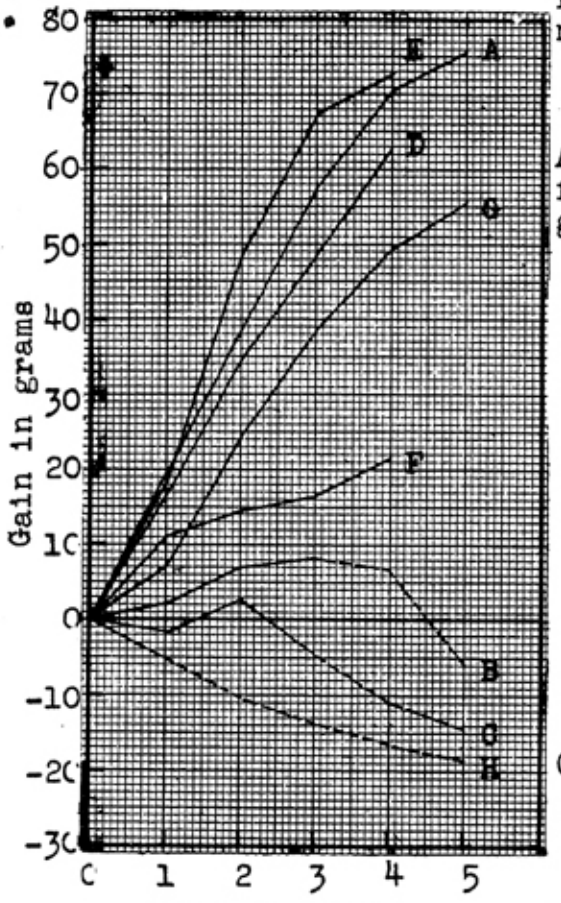
Broken lines indicate the point at which one or more of the chicks died.

As previously indicated the biological activity of Fraction II (the light violet-red layer of the alcohol-soluble fraction from egg yolks) was determined by feeding it to rats. Other fractions assayed by the rat growth method are shown in Table 8.

It will be observed that the orange layer, Fraction I, possessed no vitamin A activity when fed to rats at levels of 144 or 288 micrograms per rat per week. The light violet-red layer, Fraction II. was highly

active in promoting growth. The petroleum ether washings of the column, Fraction III, also possessed some growth promoting activity. The petroleum ether-soluble fraction from egg yolks, not chromatographed, was comparable to commercial carotene in its effect on growth. Average gain curves for these animals are shown in Figure 20. The photographs, Figures 30 and 31, page 46, show the difference in the appearance of the rats fed the light violet-red layer (Fraction II) and the orange layer (Fraction I).

Figure 20. - Average gain curves of rats in Series III fed the vitamin A-free diet plus supplements expressed in micrograms per rat per week as follows:



Petroleum ether soluble fraction from egg yolks, not chromatographed

A - Entire fraction 12.0

Alcohol soluble fraction from egg yolks, chromatographed (Figure 1 page 15)

B - I Orange layer 288.0

C - I Orange layer 144.0

D - II Light violet-red layer 2.4

E - II Light violet-red layer 4.8

F - III Petroleum ether washings of the alcohol soluble fraction on magnesium oxide 9.0

Commercial product

G - Crystalline carotene 6.0

Negative controls

H - Vitamin A-free diet alone

Broken lines indicate the point at which one or more of the animals died.

The experimental period for Groups D, E and F was 4 weeks only.

Analyses of the Tissues of the Chicks for Carotene-Cryptoxanthin, and Xanthophyll

The amount of storage of carotene-cryptoxanthin and xanthophyll in the livers and in a composite of all edible tissues except the livers was determined by means of the electrophotometer as previously described for the quantitative analysis of yellow corn. The average number of micrograms of carotene-cryptoxanthin or of xanthophyll per gram of moist sample was multiplied by the moisture factor in order that the units could be expressed on a dry basis. This was done to eliminate differences due to variations in the moisture content of the samples. The amount of storage of the carotenoid pigments during the 5-week experimental period was based upon the number of micrograms per gram present in the tissues of the chicks killed at the end of the depletion period. The ratio of carotene-cryptoxanthin to xanthophyll was calculated from the values obtained for the dry tissue.

Series I.—Tables 9 and 10 show that in most cases as the amount of carotenoid pigments was increased in the ration of the chick, there was a fairly consistent increase in the amount of carotene-cryptoxanthin and xanthophyll stored in the tissues. This increase was observed to be slightly greater in the liver than in the composite of the other edible tissues. For each 55 grams of yellow corn fed to one group of chicks an equivalent amount of carotenoid pigments was fed to another group. In all cases the storage of pigments in the tissues was greater when the yellow corn was fed.

The ratio of carotene-cryptoxanthin to xanthophyll in the livers of the chicks at the end of the 5-week experimental period was approximately 1:1 in the following groups: negative control; commercial crystalline carotene; and carotene-cryptoxanthin plus xanthophyll from yellow corn. In the remainder of the edible tissues the ratio was about 1:2 for these groups. When yellow corn was fed as a source of the pigments, more xanthophyll than carotene was stored both in the liver and in all other edible tissues, the ratios being 1:1.5, and 1:3.9 respectively.

Series II.—Here again as in Series I the amounts of the carotene-cryptoxanthin and of the xanthophyll stored were fairly consistently increased when the amount of the carotenoid supplements was increased in the ration of the chick. There was a greater increase of both carotene-cryptoxanthin and xanthophyll in the liver than in the remainder of the tissues. The ratio of carotene-cryptoxanthin to xanthophyll in the livers of the chicks fed the alcohol-soluble fraction from egg yolks (1350 micrograms per chick per week) was 1:1.9. The same supplement fed at a 2700 microgram level gave a ratio of 1:2.6 (Table 11). In all edible tissues exclusive of liver, the former amount of supplement produced a storage ratio of 1:2.8, while for the latter the ratio was 1:4.2 (Table 12).

Analyses of the Tissues of the Chicks for Vitamin A

The amount of vtiamin A present in liver tissues and in all edible tissues, other than liver, was determined spectrographically. The Inter-

national Units of vitamin A per gram of tissue averaged from duplicate samples were multiplied by the moisture factor in order to place the measurements on an equal basis. The amount of storage of vitamin A during the 5-week experimental period was based upon the number of International Units (per gram) present in the tissues of the chicks killed at the end of the depletion period.

Series I.—The materials fed in the various groups, per chick per week, were: commercial crystalline carotene 600 micrograms; carotene-cryptoxanthin from yellow corn 600 micrograms; carotene-cryptoxanthin plus xanthophyll from yellow corn 600 and 1350 micrograms; yellow corn 55 grams; and cod liver oil 450 International Units. As a result of feeding these materials, the storage of vitamin A in the liver tissues per gram dry weight was: 127, 7, 11, 31, and 30 I. U., respectively (Table 13). In the composite of the edible tissues exclusive of liver, a similar trend of vitamin A storage was observed. Results also showed that more vitamin A was stored per gram of liver than per gram of other edible tissue (Table 14).

Series II.—Supplements fed in the various groups, per chick per week, were: commercial crystalline carotene 45 micrograms; commercial crystalline carotene plus alcohol-soluble fraction from egg yolks 45 and 675 micrograms; alcohol-soluble fraction from egg yolks 1350 micrograms; and alcohol-soluble fraction from egg yolks 2700 micrograms. International Units of vitamin A stored in the liver per gram of dry tissue when these supplements were fed were: 3, 7, 6, and 7 (Table 15) and in all other edible tissues, 3, 3, 1, and 0, respectively (Table 16). The slight differences in the number of International Units per gram of dry liver and per gram of all edible tissue except liver are not significant.

SUMMARY AND CONCLUSIONS

A study has been made of the effect of the diet upon the growth of young chicks and the storage of vitamin A and carotenoid pigments in their tissues. The basal diet was supplemented with carotene-cryptoxanthin or xanthophyll, extracted from yellow corn, fed separately or in combination, in the amounts in which they appeared in the yellow corn. The results were compared with those obtained when an amount of yellow corn, equivalent to the extracted pigments was fed.

The carotenoid pigments were extracted from yellow corn and divided into (1) a petroleum ether-soluble phase, containing carotene and cryptoxanthin; and (2) a 90 per cent methyl alcohol-soluble phase, containing xanthophyll. These pigments when fed separately or in combination showed (1) that the fraction containing xanthophyll was devoid of vitamin A activity; and (2) that the fraction containing carotene-cryptoxanthin was comparable in growth promoting properties to commercial carotene, to vitamin A in cod liver oil, and to yellow corn.

The carotenoid pigments extracted from egg yolks were separated as described above. The alcohol-soluble fraction was chromatographed on magnesium oxide. Two distinct layers formed on the column—an orange layer, and a light violet-red layer. In Series II, the adsorbed material, from the entire column, was eluted collectively, and fed to chicks. The chicks showed mild symptoms of vitamin A deficiency but lived during the entire experimental period of 5 weeks. This made it possible to study the amount of storage in the tissues.

The liver and all edible tissues except liver were analyzed for carotene and cryptoxanthin, for xanthophyll and for vitamin A. The results showed (1) that as the amount of carotenoid pigments was increased in the diet of the chick there was a consistent increase in the amount of these pigments stored in the tissues; (2) that the pigments and also vitamin A were stored to a less extent when pigments extracted from yellow corn were fed than when yellow corn supplying an equivalent amount of pigment was fed; and (3) that more pigments and vitamin A (per gram of weight) were stored in the liver than in the remaining edible tissues.

Xanthophyll from yellow corn, xanthophyll from egg yolks, and commercial xanthophyll were all devoid of vitamin A activity as tested under the conditions of these experiments.

Spectrographic analyses of the two layers of the alcohol-soluble fraction from egg yolks showed absorption maxima at 3100 Å for the light violet-red layer and at 4465 and 4695 Å for the orange layer. These maxima parallel the results of the biological assays in which young growing rats were used as the experimental animals.

Appendix

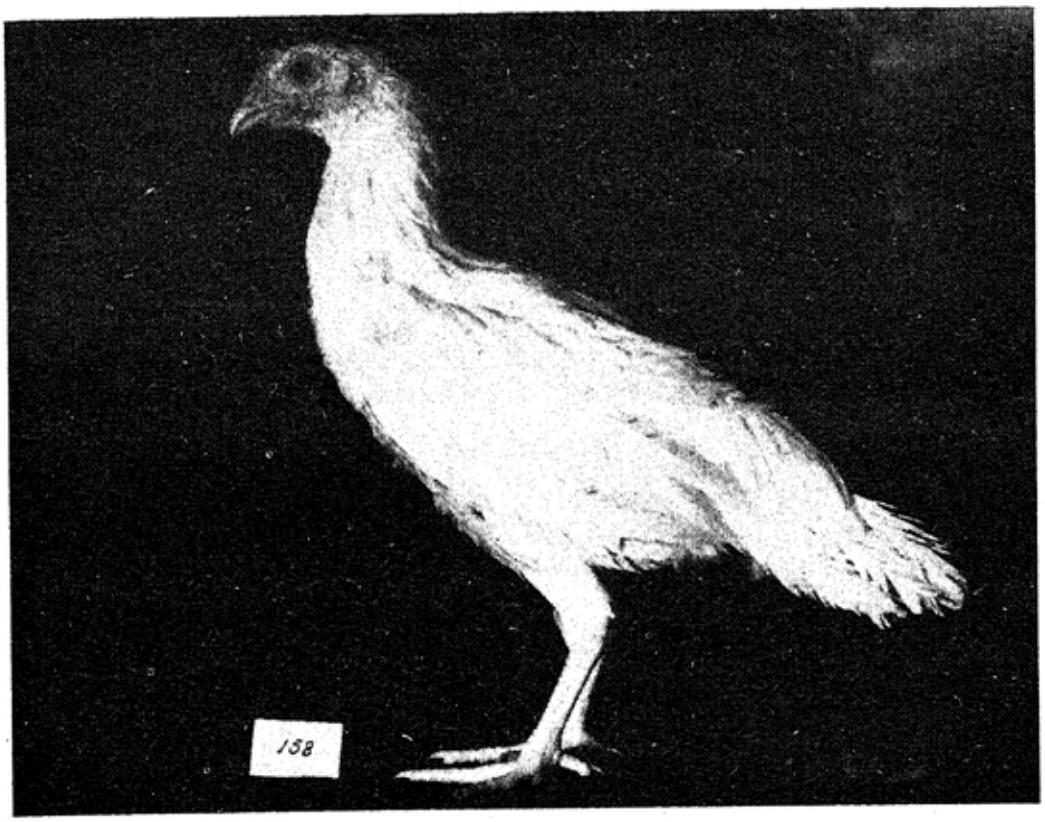


Figure 21.—Positive control, 450 International Units per week from cod liver oil.

Figure 22.—Negative control. Basal diet alone.

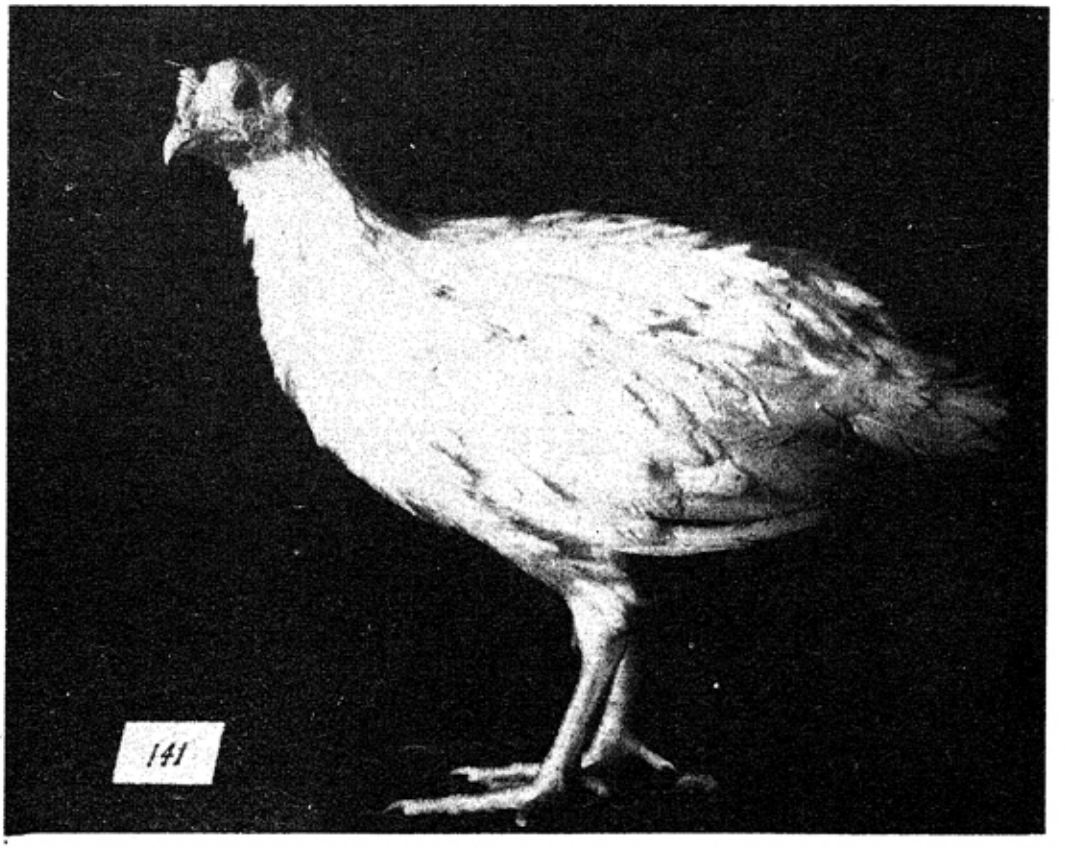


Figure 23.—Commercial carotene 600 micrograms per week.

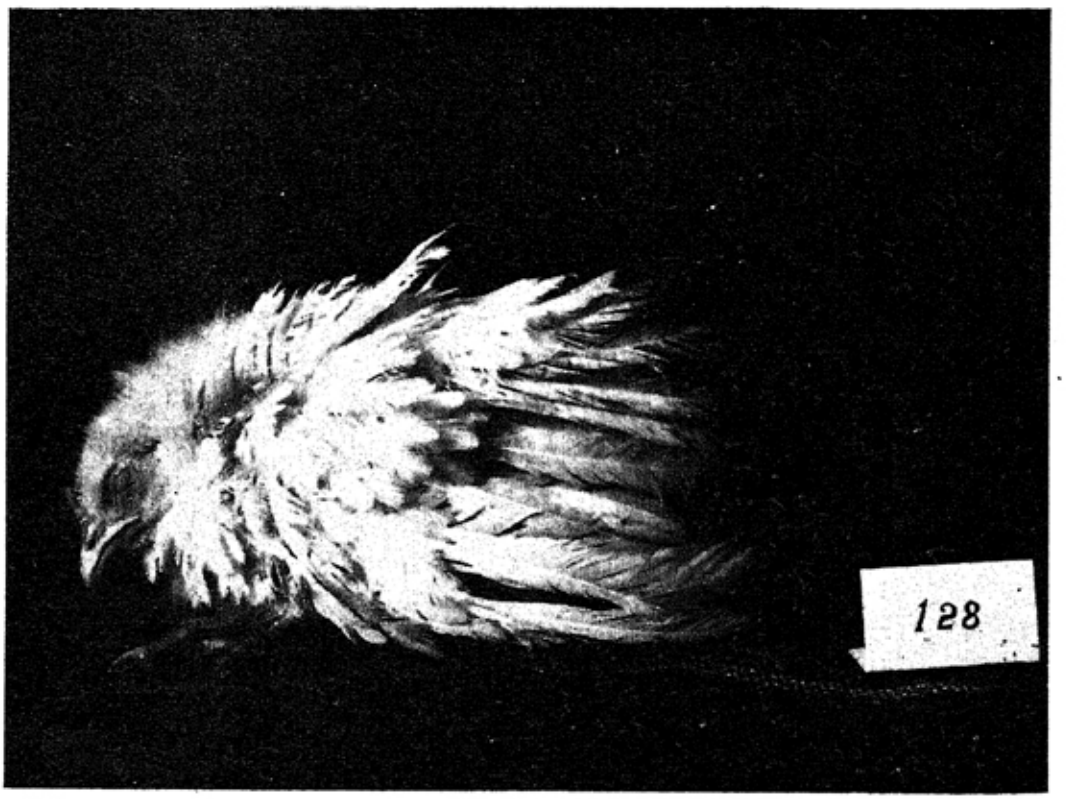


Figure 24.—Commercial xanthophyll 1350 micrograms per week. The chick died the 7th day of the experimental period.

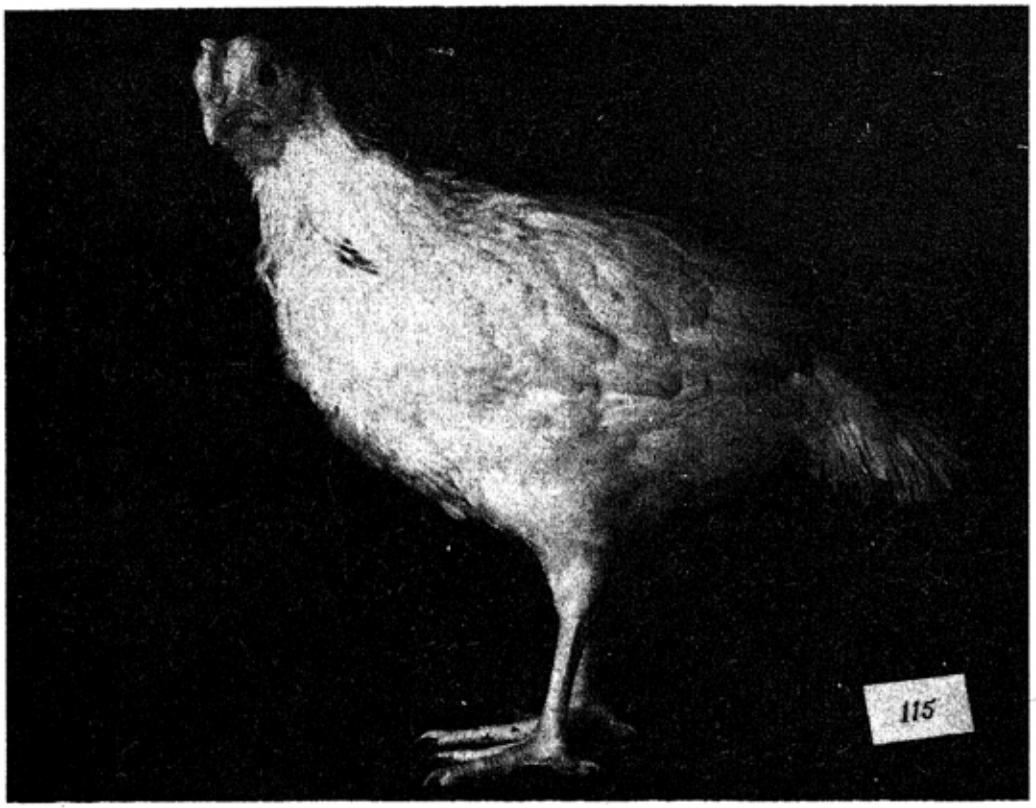


Figure 25.—Carotene-cryptoxanthin extracted from yellow corn 600 micrograms per week.

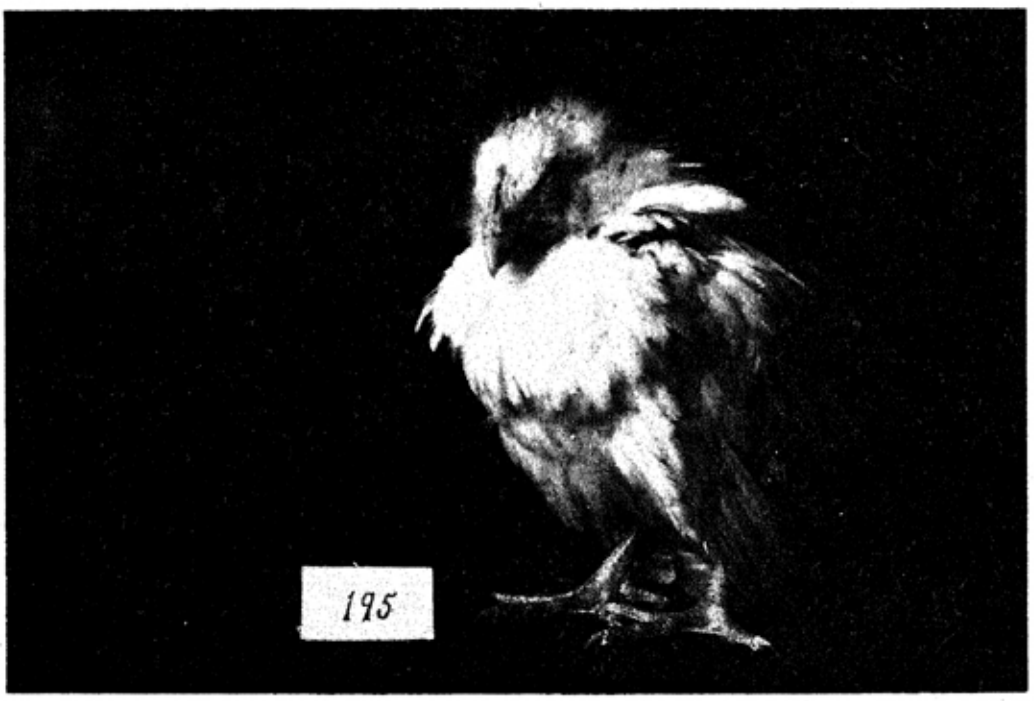


Figure 26.—Xanthophyll extracted from yellow corn 1350 micrograms per week. The chick lived 12 days after the feeding of the supplement was begun.

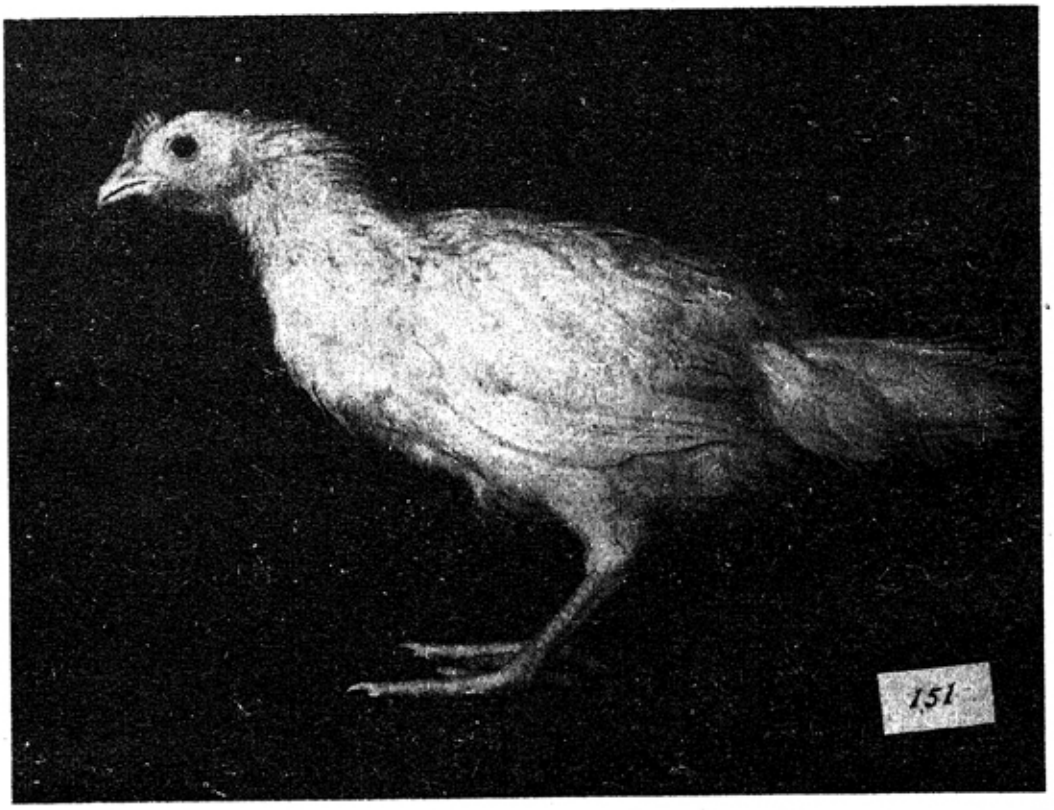


Figure 27.—Carotene-cryptoxanthin 600 micrograms plus xanthophyll 1350 micrograms (from yellow corn) per week.

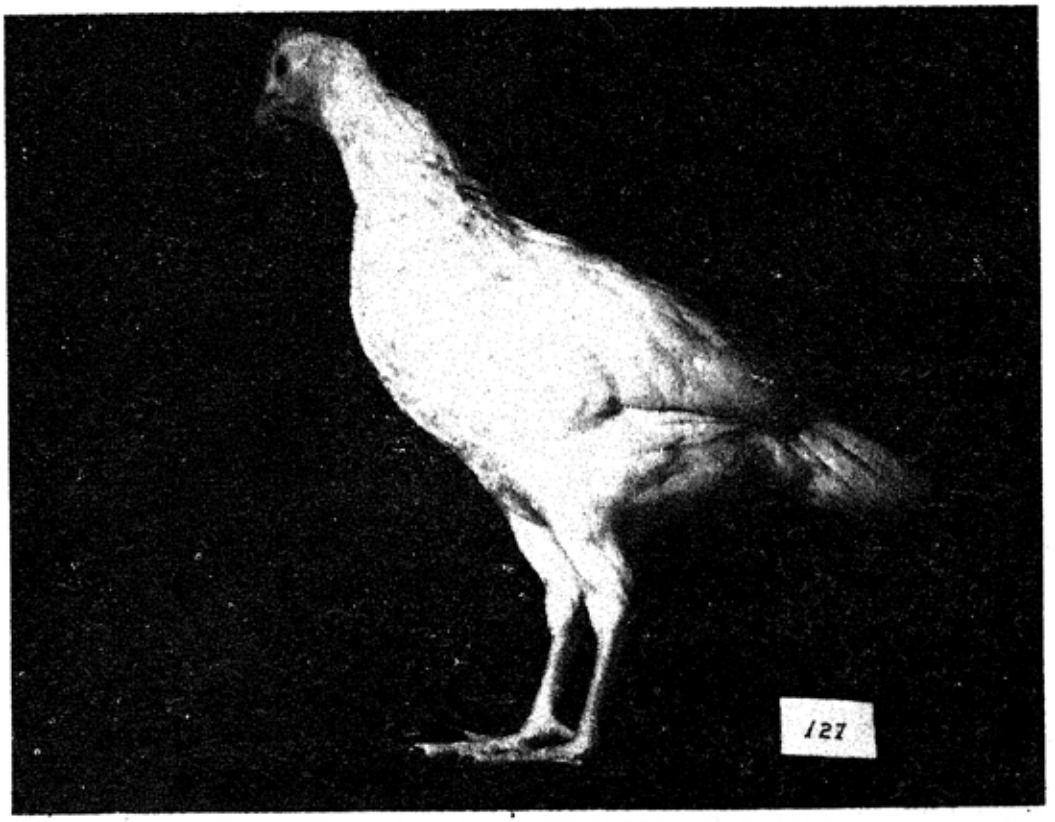


Figure 28.—Yellow corn 55 grams per week.

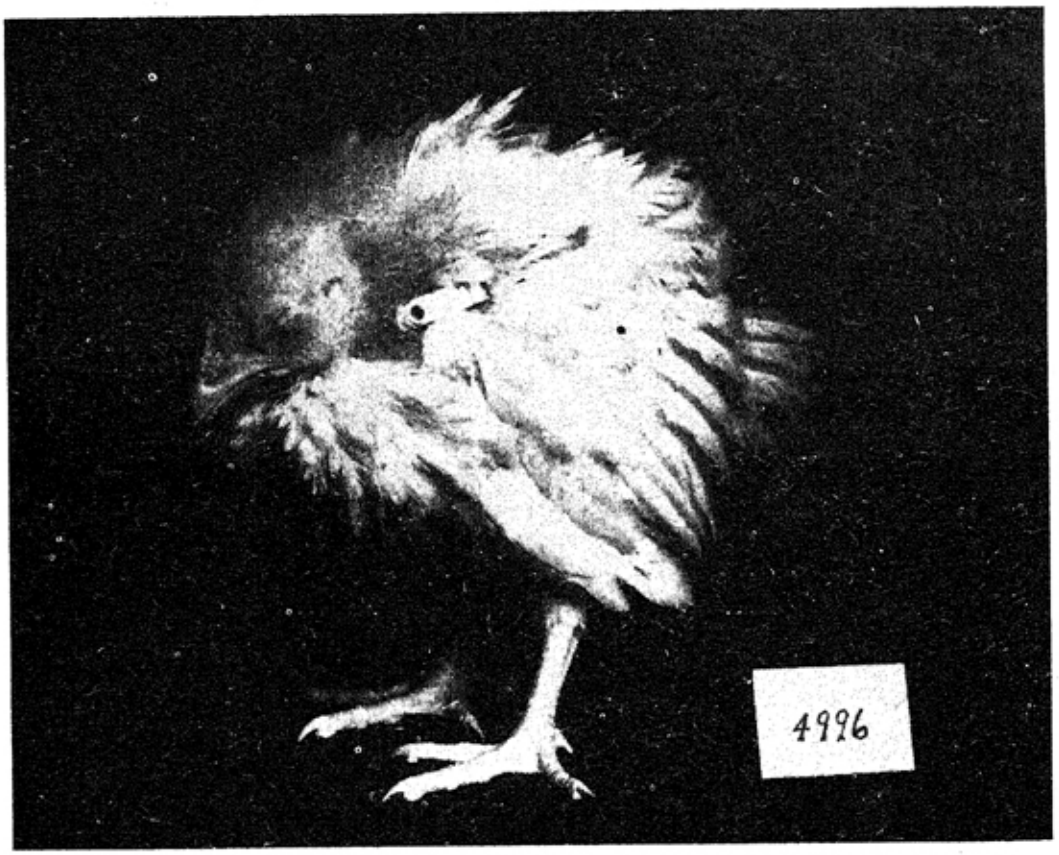


Figure 29.—Orange Layer, Fraction I, of the alcohol-soluble phase from egg yolks 1350 micrograms per week.

Figure 30.—Orange layer, Fraction I, of the alcohol-soluble phase from egg yolks 288 micrograms per week.

Figure 31.—Light violet-red layer, Fraction II, of the alcohol-soluble phase from egg yolks. Supplement equivalent to 2.4 micrograms of carotene per week.

Table 9.-Carotene-Cryptoxanthin and Xanthophyll Content of Chick Liver as Affected by the Kind and Amount of Supplement Fed - Series I

Supplement		Moisture factor	Micrograms per gram of tissue								Ratio carotene-cryptoxanthin: xanthophyll
			Carotene-cryptoxanthin				Xanthophyll				
			Moist		Dry	±Amount present at end of depletion (dry)	Moist		Dry	±Amount present at end of depletion (dry)	
Kind	Amt. fed per chick per week		Individual sample	Average	Average		Individual sample	Average	Average		
Killed at end of depletion	0	3.2	0.69	0.69	2.21	±0	1.23	1.23	3.94	±0	1:1.8
Negative controls	0	2.3	1.35	1.35	3.11	+0.90	1.56	1.56	3.59	-0.35	1:1.2
Commercial crystalline carotene	600γ	4.2	0.63 0.65	0.64	2.69	+0.48	0.77 0.50	0.64	2.69	-1.25	1:1.0
Carotene-cryptoxanthin from yellow corn	600γ	2.8	1.07	1.07	3.00	+0.79	0.48	0.48	1.34	-2.60	1:0.4
Carotene-cryptoxanthin plus xanthophyll from yellow corn	600γ + 1350γ	3.2	1.21 1.27	1.24	3.97	+1.76	1.27 1.27	1.27	4.06	+0.12	1:1.0
Yellow corn	55 gms.	4.2	1.26 1.24	1.25	5.25	+3.04	1.82 1.81	1.82	7.64	+3.70	1:1.5

The number of chicks analyzed in each group was the same as in Table 13.

Table 10.-Carotene-Cryptoxanthin and Xanthophyll Content of All Edible Chick Tissue, Except Liver, as Affected by the Kind and Amount of Supplement Fed - Series I

Supplement		Moisture factor	Micrograms per gram of tissue								Ratio Carotene-cryptoxanthin: xanthophyll
			Carotene-cryptoxanthin				Xanthophyll				
			Moist		Dry	±Amount present at end of depletion (dry)	Moist		Dry	±Amount present at end of depletion (dry)	
Kind	Amt. fed per chick per week		Individual sample	Average	Average		Individual sample	Average	Average		
Killed at end of depletion	0	3.1	0.03 0.03	0.03	0.09	±0	0.14 0.13	0.14	0.43	±0	1:4.8
Negative controls	0	3.5	0.05 0.05	0.05	0.18	+0.09	0.13	0.13	0.46	+0.03	1:2.6
Commercial crystalline carotene	600γ	3.0	0.05 0.04	0.05	0.15	+0.06	0.11 0.08	0.10	0.30	-0.13	1:2.0
Carotene-cryptoxanthin from yellow corn	600γ	3.2	0.07 0.06	0.07	0.22	+0.13	0.08 0.11	0.10	0.32	-0.11	1:1.5
Carotene-cryptoxanthin plus xanthophyll from yellow corn	600γ + 1350γ	3.3	0.06 0.06	0.06	0.20	+0.11	0.12 0.12	0.12	0.40	-0.03	1:2.0
Yellow corn	55 gms.	3.7	0.16 0.15	0.16	0.59	+0.50	0.59 0.67	0.63	2.33	+1.90	1:3.9

The number of chicks analyzed in each group was the same as in Table 13.

Table 11.-Carotene-Cryptoxanthin and Xanthophyll Content of Chick Liver as Affected by the Kind and Amount of Supplement Fed - Series II

Supplement		Moisture factor	Micrograms per gram of tissue								Ratio carotene-cryptoxanthin: xanthophyll
			Carotene-cryptoxanthin				Xanthophyll				
			Moist		Dry	±Amount	Moist		Dry	±Amount	
Kind	Amt. fed per chick per week		Individual sample	Average	Average	present at end of depletion (dry)	Individual sample	Average	Average	present at end of depletion (dry)	
Killed at end of depletion	0	4.4	0.54 0.53	0.54	2.38	±0	0.64 0.68	0.66	2.90	±0	1:1.2
Negative controls	0	4.8	0.36	0.36	1.73	-0.65	0.64	0.64	3.07	+0.17	1:1.8
Commercial crystalline carotene	45γ	4.0	0.33 0.34	0.34	1.36	-1.02	0.20 0.18	0.19	0.76	-2.14	1:0.6
Commercial crystalline carotene plus alcohol soluble fraction from egg yolks	45γ + 675γ	4.0	0.17 0.19	0.18	0.72	-1.66	0.24 0.23	0.24	0.96	-1.94	1:1.3
Alcohol soluble fraction from egg yolks	1350γ	4.3	0.16 0.16	0.16	0.69	-1.69	0.34 0.25	0.30	1.29	-1.61	1:1.9
Alcohol soluble fraction from egg yolks	2700γ	4.0	0.47 0.56	0.52	2.08	-0.30	1.41 1.33	1.37	5.48	+2.58	1:2.6

The number of chicks analyzed in each group was the same as in Table 15.

Table 12.-Carotene-Cryptoxanthin and Xanthophyll Content of All Edible Chick Tissue, Except Liver, as Affected by the Kind and Amount of Supplement Fed - Series II

Supplement		Moisture factor	Micrograms per gram of tissue								Ratio carotene-cryptoxanthin: xanthophyll
			Carotene-cryptoxanthin				Xanthophyll				
			Moist		Dry	±Amount	Moist		Dry	±Amount	
Kind	Amt. fed per chick per week		Individual sample	Average	Average	present at end of depletion (dry)	Individual sample	Average	Average	present at end of depletion (dry)	
Killed at end of depletion	0	3.7	0.04 0.03	0.04	0.15	±0	0.16 0.13	0.15	0.56	±0	1:3.7
Negative controls	0	4.3	0.05 0.04	0.05	0.22	+0.07	0.18 0.21	0.20	0.86	+0.30	1:3.9
Commercial crystalline carotene	45γ	3.6	0.05 0.04	0.05	0.18	+0.03	0.06 0.06	0.06	0.22	-0.34	1:1.2
Commercial crystalline carotene plus alcohol soluble fraction from egg yolk	45γ + 675γ	3.8	0.03 0.04	0.04	0.15	±0	0.09 0.10	0.10	0.38	-0.18	1:2.5
Alcohol soluble fraction from egg yolks	1350γ	3.6	0.05 0.04	0.05	0.18	+0.03	0.15 0.13	0.14	0.50	-0.06	1:2.8
Alcohol soluble fraction from egg yolks	2700γ	3.3	0.06 0.06	0.06	0.20	+0.05	0.24 0.25	0.25	0.83	+0.27	1:4.2

The number of chicks analyzed in each group was the same as in Table 13.

Table 13.-Vitamin A Content of Chick Liver as Affected by the Kind and Amount of Supplement Fed - Series I

Supplement		No. of chicks	Sample weight	Cell length	Dilution	Corrected Density $3300\ \text{Å}^{0}$	Moisture factor	I.U. per gram of tissue			
								Moist		Dry	±Amt. present at end of depletion (dry)
Kind	Amt. fed per chick per week		gms.	cm.				Individual sample	Average	Average	
Killed at end of depletion	0	6	5.2968 5.1764	10 10	1-1 1-1	.36 .38	3.2	6.36 6.94	6.65	21.28	± 0
Negative controls	0	5	4.7168 3.9953	10 10	1-1 1-1	.24 .18	2.3	4.35 3.48	3.92	9.02	- 12.26
Reference cod liver oil	450 I.U.	6	5.0845 5.0368	10 10	0 0	1.19 1.23	4.2	11.82 12.37	12.10	50.82	+ 29.54
Commercial crystalline carotene	600γ	5	5.2368 5.4337	1 1	0 0	.39 .40	4.2	35.33 35.06	35.20	147.84	+126.56
Carotene-cryptoxanthin from yellow corn	600γ	4	4.3044 4.6459	10 10	1-1 1-1	.47 .47	2.8	10.64 9.86	10.25	28.70	+ 7.42
Carotene-cryptoxanthin plus xanthophyll from yellow corn	600γ + 1350γ	5	5.0071 5.0045	10 10	1-1 1-1	.50 .53	3.2	9.81 10.47	10.14	32.45	+ 11.17
Yellow Corn	55 gms.	5	5.5280 5.3265	10 10	0 0	1.31 1.35	4.2	12.07 12.94	12.51	52.54	+ 31.26

Table 14.-Vitamin A Content of All Edible Chick Tissues, Except Liver, as Affected by the Kind and Amount of Supplement Fed - Series I

Supplement		No. of chicks	Sample weight	Cell length	Dilution	Corrected density $3300\ \text{Å}^{0}$	Moisture factor	I.U. per gram of tissue			
								Moist		Dry	±Amt. present at end of depletion (dry)
Kind	Amt. fed per chick per wk.		gms.	cm.				Individual sample	Average	Average	
Killed at end of depletion	0	6	5.1761 5.0091	10 10	0 0	.25 .21	3.1	2.09 1.72	1.91	5.92	± 0
Negative controls	0	5	5.1913 5.2332	10 10	0 0	.19 .20	3.5	1.44 1.54	1.49	5.22	- 0.70
Reference cod liver oil	450 I.U.	6	5.2274 5.2639	10 10	0 0	.45 .45	3.7	4.17 4.14	4.16	15.39	+ 9.47
Commercial crystalline carotene	600γ	5	5.3852 5.2537	10 10	1-1 1-1	.43 .45	3.0	7.69 8.30	8.00	24.00	+18.08
Carotene-cryptoxanthin from yellow corn	600γ	4	5.1288 5.1910	10 10	0 0	.30 .33	3.2	2.64 2.93	2.79	8.93	+ 3.01
Carotene-cryptoxanthin plus xanthophyll from yellow corn	600γ + 1350γ	5	5.2184 5.0834	10 10	0 0	.38 .36	3.3	3.44 3.31	3.38	11.15	+ 5.23
Yellow corn	55 gms.	5	5.1611 5.0314	10 10	0 0	.37 .39	3.7	3.37 3.68	3.53	13.06	+ 7.14

Table 15.-Vitamin A Content of Chick Liver as Affected by the Kind and Amount of Supplement Fed - Series II

Supplement		No. of chicks	Sample weight	Cell length	Dilution	Corrected density at 3300 Å	Moisture factor	I.U. per gram of tissue			
								Moist		Dry	±Amt. present at end of depletion (dry)
Kind	Amt. fed per chick per wk.		gms.	cm.				Individual sample	Average	Average	
Killed at end of depletion	0	11	5.0666 5.1200	10 10	1-1 1-1	.27 .28	4.4	4.70 4.86	4.78	21.03	±0
Negative controls	0	7	4.0575 3.9685	10 10	1-1 1-1	.23 .21	4.8	4.78 4.33	4.56	21.89	+0.86
Commercial crystalline carotene	45 γ	7	4.9025 4.9173	10 10	1-1 1-1	.32 .32	4.0	5.98 5.96	5.97	23.88	+2.85
Commercial crystalline carotene plus alcohol soluble fraction from egg yolks	45 γ + 675 γ	6	5.0101 4.9310	10 10	1-1 1-1	.39 .36	4.0	7.39 6.83	7.11	28.44	+7.41
Alcohol soluble fraction from egg yolks	1350 γ	4	5.0499 4.5294	10 10	1-1 1-1	.32 .34	4.3	5.80 6.95	6.38	27.43	+6.40
Alcohol soluble fraction from egg yolks	2700 γ	2	3.8300	10	1-1	.30	4.0	7.08	7.08	28.32	+7.29

Table 16.-Vitamin A Content of All Edible Chick Tissues, Except Liver, as Affected by the Kind and Amount of Supplement Fed - Series II

Supplement		No. of chicks	Sample weight	Cell length	Dilution	Corrected density at 3300 Å	Moisture factor	I.U. per gram of tissue			
								Moist		Dry	±Amt. present at end of depletion (dry)
Kind	Amt. fed per chick per wk.		gms.	cm.				Individual sample	Average	Average	
Killed at end of depletion	0	11	5.0551 5.2701	10 10	0 0	.26 .25	3.7	2.25 2.05	2.15	7.96	±0
Negative controls	0	7	5.5704 5.5011	10 10	0 0	.21 .20	4.3	1.54 1.46	1.50	6.45	-1.51
Commercial crystalline carotene	45 γ	7	5.0766 5.0188	10 10	0 0	.33 .33	3.6	2.99 3.03	3.01	10.84	+2.88
Commercial crystalline carotene plus alcohol soluble fraction from egg yolks	45 γ + 675 γ	7	4.9111 4.8401	10 10	0 0	.33 .30	3.8	3.10 2.80	2.95	11.21	+3.25
Alcohol soluble fraction from egg yolks	1350 γ	6	5.1615 5.1713	10 10	0 0	.30 .28	3.6	2.63 2.41	2.52	9.07	+1.11
Alcohol soluble fraction from egg yolks	2700 γ	2	4.8759 5.0464	10 10	0 0	.26 .27	3.3	2.33 2.36	2.35	7.76	-0.20

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