DETERMINATION OF CAROTENE

IN

CHICKEN BLOOD

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By

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DETERMINATION OF CAROTENE IN CHICKEN BLOOD

Vitamins are necessary in the proper nutrition of chickens. Only small quantities are required, but they are absolutely essential for the maintenance of health, growth, and reproduction. (12).

Vitamin A is necessary for growth, reproduction, and hatchability in poultry. It is of value in preventing infections of the eyes and respiratory tract. A condition known as nutritional roup is caused by its deficiency. The symptoms of this disease are lameness or a staggering gait; discharge from the nostrils; swelling beneath the eyes; and a discharge from the eyes. In severe cases of nutritional roup, blindness and finally death occur. Growth ceases in young chickens. (13).

Taylor and Chichester (11) proved that the quantity of vitamin A in egg yolk may be made to vary over a wide range by varying the quantity of the vitamin in the diet. They showed that the output of vitamin A in eggs may be as much as 32% of the quantity consumed in the feed. These workers proved that dark yellow eggs are richer in vitamin A than light-colored eggs.

Record, Bethke, and Wilder (7) conducted experiments with chickens to compare prophylactic and curative types of feeding. It required a minimum of 50 to 100 micrograms of carotene per 100 grams of ration for normal growth and the prevention of external and internal symptoms of vitamin A deficiency in chicks to about 8 weeks of age.

According to Kemmerer and Fraps (3), chickens digested 29 percent of the carotene in alfalfa leaf meal. Russel (8) reported that the presence

of fat in the diet improved the absorption of carotene. He also reported that there appeared in the excreta a yellow pigment which had the solubility properties of carotene; but which, according to spectrophotometric determinations, was not a member of the carotene group of pigments. The findings of Kemmerer et al. (3) agreed with Russel in that they also found yellow pigments in the excrements which could not be separated from the carotene.

Several investigations have proved carotene to be a precursor of vitamin A. Steenback and Boutwell (10), using white and yellow maize in feeding experiments with rats, first demonstrated the correlation between the yellow pigment and vitamin A. Two years later Steenback (9) confirmed these findings and definitely proved the relationship between carotene and vitamin A.

In 1925 Moore (5) discovered that carotene is changed into vitamin A in the liver and that the liver is the chief storage place for vitamin A and carotene.

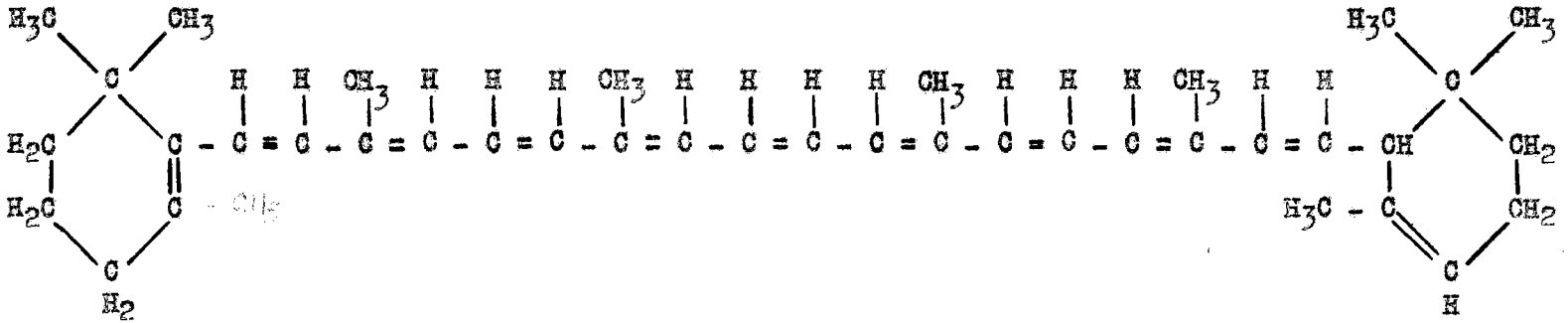
Olcott and McCan (6) showed the change of carotene to vitamin A by incubation with fresh liver tissue. The agent responsible for the transformation appeared to be an enzyme carotenase. The conversion of carotene to vitamin A in vivo was confirmed by these workers.

These experiments showed that carotene is not vitamin A, but that it is converted into the vitamin in the animal body.

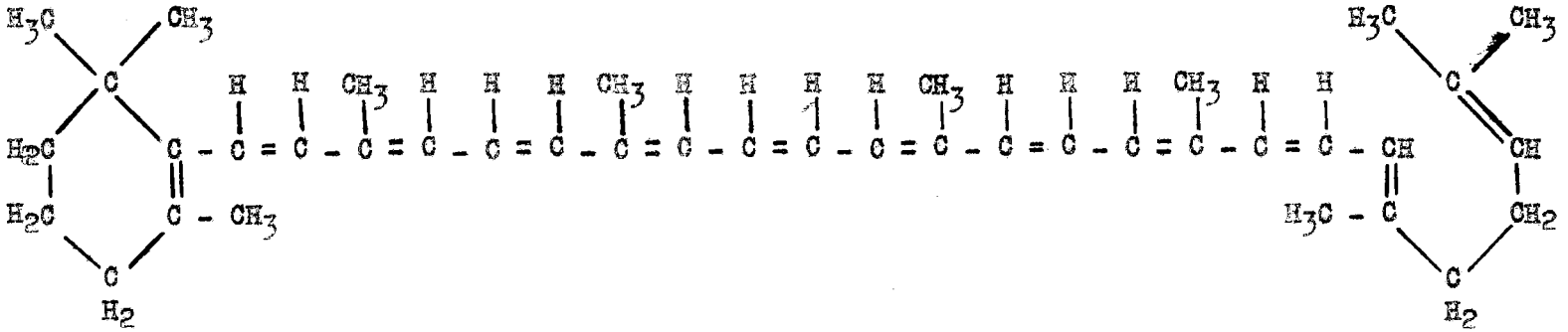
Carotene exists in three isomeric forms--alpha-, beta-, and gamma-carotene. Beta-carotene is optically active, and alpha-carotene is optically inactive. One molecule of beta-carotene yields two molecules of vitamin A. Alpha- and gamma-carotene are capable of forming vitamin A molecule for molecule. The beta-carotene is symmetrical, and the point

of hydrolysis is a double bond at the center of the molecule, each half taking on both the H and OH of a molecule of water. The alpha- and gamma-carotene are not symmetrical; and, although they hydrolyze at the double bond, they each yield only one molecule of vitamin A. Following are the structural formulae for the carotenes. The reaction for the conversion of carotene to vitamin A is also shown.

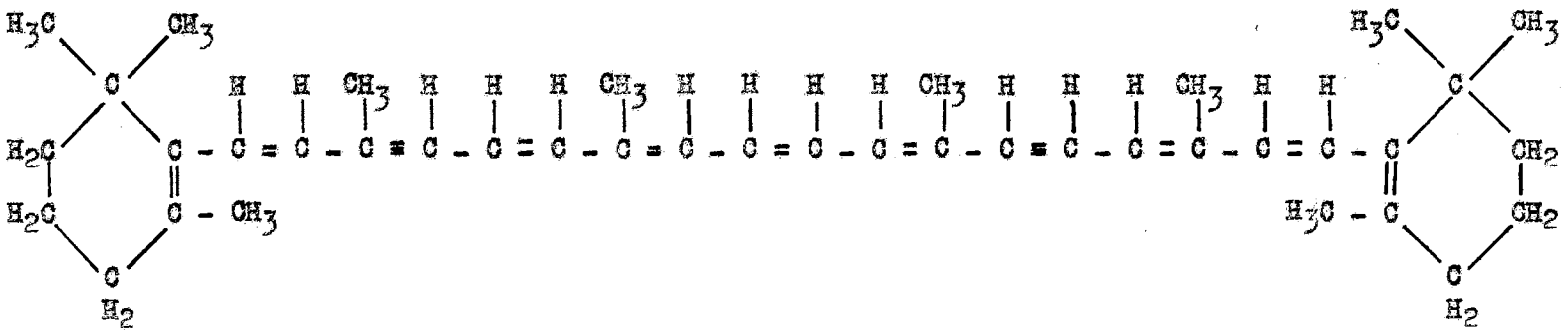
Alpha-carotene ($C_{40}H_{56}$)



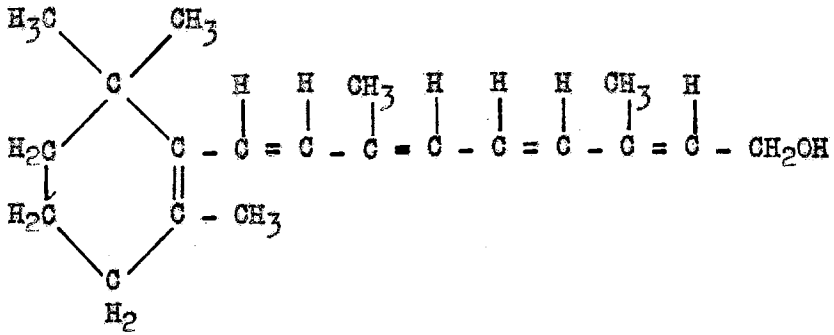
Gamma-carotene ($C_{40}H_{56}$)



Beta-carotene ($C_{40}H_{56}$)



$+ 2H_2O$ (Carotenase)



Vitamin A ($C_{20}H_{30}O$)

The carotene content of the blood was determined by the method of Kimble (4).

The blood for the analysis was drawn from the chickens by a heart puncture; then transferred to a 50-ml. flask, which had been previously prepared by adding 3 ml. of 10% lithium citrate and evaporating it to dryness. The blood was transferred to a centrifuge tube and centrifuged 10 minutes at 1,700 r.p.m. to separate the cells and plasma. Five ml. of the blood plasma were then transferred to a test tube containing 5 ml. of 95% alcohol and 12 ml. of petroleum ether. The test tube was securely stoppered, and the sample was mixed by hand shaking for ten minutes. The sample was then centrifuged 10 minutes to layer.

Ten ml. of the supernatent petroleum ether extract was used to determine the optical density, which was read on a Coleman-Universal spectrophotometer. The concentration was calculated from a carotene-optical-absorption curve which had been determined upon a highly purified sample of beta-carotene.

EXPERIMENTAL

The carotene content was determined in the bloods of different lots of birds.

STUDY A

Lot 1 -- Control Group of Young Birds

These birds were cockerels, 10 weeks old, and fed the following ration, with no green feed supplement.

	lbs.		lbs.
Yellow corn meal	508.5	Soybean meal	30
Wheat Bran	30.	Meat and bone scrap	30
Dehydrated Alfalfa meal	18.8	Salt	4.5
Dried Buttermilk	90.	Delsterol	1.5
Cottonseed meal	30.		

Results:

Sample	micrograms of carotene per 100 ml. of blood
1	691.2
2	518.4
3	504.
4	489.6
Average	550.8

Lot 2 -- 10-week old cockerels

Ration: same as Lot 1 with the addition of 10 mg. of stilbesterol per pound of feed.

Results:

Sample	micrograms of carotene per 100 ml. of blood
1	628.8
2	628.8
3	532.8
Average	596.8

Lot 3 -- 10-week old cockerels

Ration: same as Lot 1 with the addition of 30 mg. stilbesterol per pound of feed.

Results:

Sample	micrograms of carotene per 100 ml. of blood
Composite sample from 8 birds	592.8

Lot 4 -- 10-week old cockerels

Ration: same as Lot 1 with the addition of 40 mg. 3-4 diansythexane stilbesterol per pound of feed.

Results:

Sample	micrograms of carotene per 100 ml. of blood
1	693.2
2	460.8
3	993.6
Average	719.2

Lot 5 -- 10-week old cockerels

Ration: the same as Lot 1 with the addition of 50 mg. diansythexene stilbesterol per pound of feed.

Results:

Sample	micrograms of carotene per 100 ml. of blood
1	748.8
2	1200.
3	1136.8
4	592.8
5	403.2
Average	816.3

STUDY B

Birds: year-old laying hens.

Ration: following mash kept before the birds at all times supplemented with green feed of yard.

	lbs.		lbs.
Dried Buttermilk	25	Barley	72
Pulverized lime	50	Yellow corn	112
Soybean meal	22	Alfalfa meal	100
Wheat Middlings	72	Bone and meat scrap	100
Whole Shorts	75	Salt	10
Wheat Bran	100		

And the following grain mixture at the rate of 1 pound per bird per month:

Corn	1 bushel	Oats	10 pounds
Wheat	10 pounds	Barley	40 pounds

Results:

Sample	micrograms of carotene per 100 ml. of blood
1	120
2	200
3	96
4	112
5	84
6	120
7	144
8	96
9	96
10	152
11	84
12	112
Average	117

STUDY C

Lot 1 -- Birds: year-old chickens.

Ration: same as used in Study B, except the birds were caged and the green feed supplement was given in the form of chopped Swiss Chard. Birds as indicated were injected with stilbesterol.

Results:

Sample	micrograms of carotene per 100 ml. of blood
1 (rooster, injected with 100 ml. stilbesterol 3 wks. before.)	1186
2 (hen, " " " ")	1276
3 (normal hen)	285

The same birds were again injected with 100 ml. of stilbesterol and carotene determinations were made weekly. Blood samples were also taken from control birds.

First week after birds were injected with stilbesterol:

Sample	micrograms of carotene per 100 ml. of blood
1 (rooster, injected with stilbesterol)	902.4
2 (hen, " " " " " ")	979.2
3 (control hen)	414.

Second week after birds were injected with stilbesterol.

Sample	micrograms of carotene per 100 ml. of blood
1 (rooster, injected with stilbesterol)	1896
2 (hen, in poultry yard on green feed)	744
3 (" " " " " " ")	744
4 (" " " " " " ")	1200
5 (rooster, " " " " " ")	2040

Third week after the birds were injected with stilbesterol.

Sample	micrograms of carotene per 100 ml. of blood
1 (rooster, injected with stilbesterol)	3372
2 (control hen)	832
3 (control hen)	656

Fourth week after the birds were injected with stilbesterol.

Sample	micrograms of carotene per 100 ml. of blood
1 (rooster, injected with stilbesterol)	456
2 (rooster, " " ")	732
3 (hen, " " ")	720
4 (control hen, not on green feed)	132

STUDY D

Birds: 3-month old cockerels.

Ration: same basic ration as was fed in Study B, except birds were caged and the green feed given in the form of chopped Swiss Chard or green alfalfa.

Results: each of these samples was composites of blood from ten chickens. Sample A was taken from the group of birds receiving diansythexane stilbesterol in their feed in the proportion of 40 mg. per pound of feed. Sample B was taken from the group which was fed the addition of 40 mg. diansythexene per pound of feed. Sample N was taken from the control birds.

Sample	micrograms of carotene per 100 ml. of blood
First Week	
A	756
B	756
N	518
Second Week	
A	801.6
B	868.6
N	532.
Third Week	
A	530.4
B	547.2
N	520.

SUMMARY

The carotene content in the blood of laying hens is low. The average amount in the samples analyzed was 117 micrograms per 100 ml. of blood. This is probably due to the large amount transferred to the egg.

The addition of stilbesterol to the ration apparently increases the amount of carotene in the chickens' blood. This might be explained by the fact that the fat content of the blood is increased.

The average amount of carotene in the blood of chickens given diansythexane stilbesterol was 719.2 micrograms of carotene per 100 ml. of blood. This is to be compared with the 816.3 micrograms of carotene per 100 ml. of blood taken from the birds that were receiving diansythexene.

Stilbesterol injected into chickens increases the carotene content in the blood of these birds as compared with the carotene content in control birds. The amount of carotene increases for some time after one injecttion; then decreases unless additional stilbesterol is injected.

The determination of the carotene content in the blood of chickens is obviously a satisfactory method of studying carotene and vitamin A in relation to poultry.

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THE DETERMINATION OF CAROTENE AND VITAMIN A IN CHICKEN BLOOD

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INTRODUCTION

The importance of vitamin A in poultry feeding has long been recognized. This vitamin is necessary for growth and health, stimulates appetite and digestion and aids in tissue formation. It prevents infections, notably of the eye, sinuses, air passages and lungs; increases resistance to many infectious diseases and also maintains resistance to some parasites. It is necessary for good fertility and hatchability and also increases vitality and livability in chickens and probably extends the length of life. The vitamin A content of the egg depends on the vitamin A intake of laying birds. The vitamin A content of chicks liver at the time of hatching depends also on the content of the hatching eggs. (7).

Carotene is converted in the body to vitamin A. An enzyme has been isolated from the liver which is capable of converting carotene into the active form, vitamin A (6).

This study was undertaken to determine both the carotene and vitamin A content of chicken blood.

An investigation of recent literature gives evidence of the continued interest in vitamin A and its precursor carotene in relation to poultry.

Almquist, McKinney and Mecchi (1) conducted an experiment in which hens were fed vitamin A in the forms of shark liver oil concentrate; carotene was fed in the forms of dehydrated carrot powder, dried alfalfa, and crystalline carotene. The egg yolks obtained under these different sets of conditions were estimated for their carotene content, and the results showed that the conversion of carotene is efficient in the hen.

Wagener and Harms (21) reported that a lack of vitamin A caused decreased fertility, difficulties in egg laying and a drop in egg production.

Hammond and Bird (8) demonstrated that partial deficiency of vitamin A greatly increased the variability in the growth of chickens.

Harmes (9) found that hens, on rations low in vitamin A, were infected in a higher percentage with chicken dysentary bacilli than hens on a vitamin A rich diet. The progress of the infection was not completely prevented through the sufficient supply of vitamin A, but the intensity of the epidemics was considerably diminished as compared with that in the vitamin A deficient hens.

Rubin, Bird and DeVolt (15) basing their opinions on the study of commercial flocks, concluded that there is a possibility that vitamin A liver storage may be affected by environmental conditions other than the rations fed and by pathological conditions which as yet have been undetected. These investigations further concluded that evidence indicated that the amount of vitamin A storage is influenced by the source.

Bolin, Lampman and Berg (3) showed that carotene as supplied by dehydrated alfalfa was readily utilized by young chickens as early as the first week. There was a definite loss of vitamin A from the livers of chickens receiving 100 micrograms or less of carotene per 100 grams of ration, regardless of the initial amount present, or the carotene intake of the dam. The initial liver storage was maintained with diets containing 250 and 500 micrograms of carotene. The storage was markedly increased with diets containing more than 500 micrograms of carotene per 100 grams of ration.

Sjallema and Donath (18) determined that the amounts of vitamin A, carotene and xanthophyll in the content of the egg yolk of hens were definitely dependent on the materials in the diet.

According to Vermes and Meunier (19) yolks of eggs of well-fed hens contain up to 5,000 I. U. if vitamin A per 100 gram, whereas from hens almost completely deprived of green feed, the vitamin A is less than 2,500 I. U. per 100 gram of egg.

Harmes (10) proved that a marked dark yellow yolk color shows high carotene and vitamin A content, while a light yellow color indicates low carotene but not necessarily low vitamin A.

Ruben and Bird (14) observed that a group of chicks which were fed alfalfa leaf meal showed consistently better storage of vitamin A than a group receiving cod liver oil and another group receiving crystalline carotene. All three rations provided an intake of 450 micrograms per 100 grams of ration. These investigators further observed that the first five weeks of a chick's life appear to constitute a critical period from the standpoint of vitamin A metabolism.

According to Senior and Sheehy (17) an addition of 10% grass meal containing 30 milliligrams of carotene per 100 g of ration provided sufficient vitamin A for the requirements of laying pullets during the winter months. The vitamin A requirements of growing chickens were adequately supplied by adding 5% of the grass meal to the rations.

Poley (12) used 3% good grade alfalfa meal to supplement a basal ration consisting of 71% ground wheat, meat and bone scraps 20%, dried skim milk 5%, and 1% NaCl. This percentage of alfalfa meal was sufficient to supplement the vitamin A deficiency in the basal ration.

Almquist and Mecchi (2) estimate that 227 I. U. of vitamin A per 100 grams of ration will meet the requirements of laying hens. The estimation was based on chick assay results.

Radi and Warren (13) question the significance of vitamin A in the feathering of broilers.

Russel, Taylor, Walker and Polskin (16) determined that neither carotene nor vitamin A are eliminated from body stores of poultry by the kidney or intestines.

Dewel, Hrubetz, Mattson, Morehouse, and Richardson (5) reported that the vitamin A in egg yolk was unchanged from the average control level of 46.8 I. U. per gram, when 1000, 2000, and 15,000 I. U. of the vitamin was added per pound of feed. With higher doses of the vitamin A in the diet, significantly increased levels were found in the yolk. The vitamin A is progressively increased in the body fat with the higher intake in the diet. The vitamin A in the serum was unchanged except for a slight increase at the highest level intake.

In part I of this study only the carotene content of the chicken blood was determined. Apparently the carotene content in the blood of laying hens was low as compared to non-laying hens. The addition of stilbesterol to the poultry ration also increased the carotene content of the blood. The determination of the carotene content in the blood of chickens was apparently a satisfactory method of comparing carotene in relation to poultry of different ages, breeds, sex and on varying rations.

As far as could be ascertained, through a review of literature, blood analysis had not been previously used as a method of studying carotene and vitamin A in poultry.

METHOD

The method adopted in this investigation was the Kimble method (11).

REAGENTS:

1. 95 percent C. P. Alcohol.
2. Low boiling point petroleum ether.
3. Chloroform, redistilled reagent grade.
4. Antimony trichloride reagent: Prepared by dissolving 100 grams of dry antimony chloride C. P., in 400 ml of redistilled chloroform.
5. Spectrophotometer, Coleman-Universal, Model XI.

PROCEDURE:

The blood for the analysis was drawn from the chickens by a heart puncture, than transferred to a 50 ml flask which had been previously prepared by adding 2 ml of 3% lithium citrate and evaporating it to dryness. The blood was transferred to a centrifuge tube and centrifuged 10 minutes at 1700 r.p.m. to separate the cells from plasma. Three ml. of the blood plasma were transferred to a test tube, an equal volume of 95% alcohol, and 12 ml. of petroleum ether were added. The test tube was securely stoppered and the contents were mixed by hand shaking for ten minutes. The sample was then centrifuged 10 minutes to separate the petroleum ether from the protein, alcohol, and water.

Ten ml. of the supernatant petroleum ether extract were used to determine the optical density, which was read in a Coleman-Universal Spectrophotometer. The optical density reading was converted into the amount of carotene in the blood by the use of a standard curve.

The petroleum ether extract was then transferred to a test tube and the ether was evaporated off in a vacuum desiccator which was heated by being placed over warm water.

The outside of the tubes were rinsed and dried, and the residue in each was dissolved in 1 ml. of chloroform.

The galvanometer was set at 100 with an antimony trichloride blank intercepting the light beam of the spectrophotometer, with the wave length at 620 mu. The unknown tube with the chloroform extract was then put in the instrument, and 9 ml. of antimony trichloride reagent was quickly added from a quick delivery pipette.

The galvanometer reading was recorded, and the vitamin A content determined from a calibration table.

CALCULATION

The carotene was determined by the yellow color imparted to the ether extract and measuring the optical density by means of the spectrophotometer. The optical density was then referred to a standard curve which was prepared from a series of known carotene solutions similarly prepared.

Vitamin A has been determined biologically as a rule, but in recent years, the Carr-Price reaction has made the colorimetric adsorption determination possible. This procedure has been widely used. The reagent used is antimony trichloride in chloroform which imparts a brilliant blue color to the vitamin A solution (4).

The carotenoids also react with the antimony trichloride to produce a blue color in excess to that produced by the vitamin A; therefore, a correction for the carotenoids must be applied to the final calculation of the vitamin A (11).

In view of these facts, it was necessary to set up three standard curves and four calibration tables for the calculation of carotene and vitamin A.

1. An optical absorption curve and table for pure B-carotene.
2. A standard curve and table for vitamin A.
3. Correction curve for the blue color produced by the carotenoids.
4. Table for the equivalent of vitamin A as produced by carotenoids.

Carotene curve and calibration table.

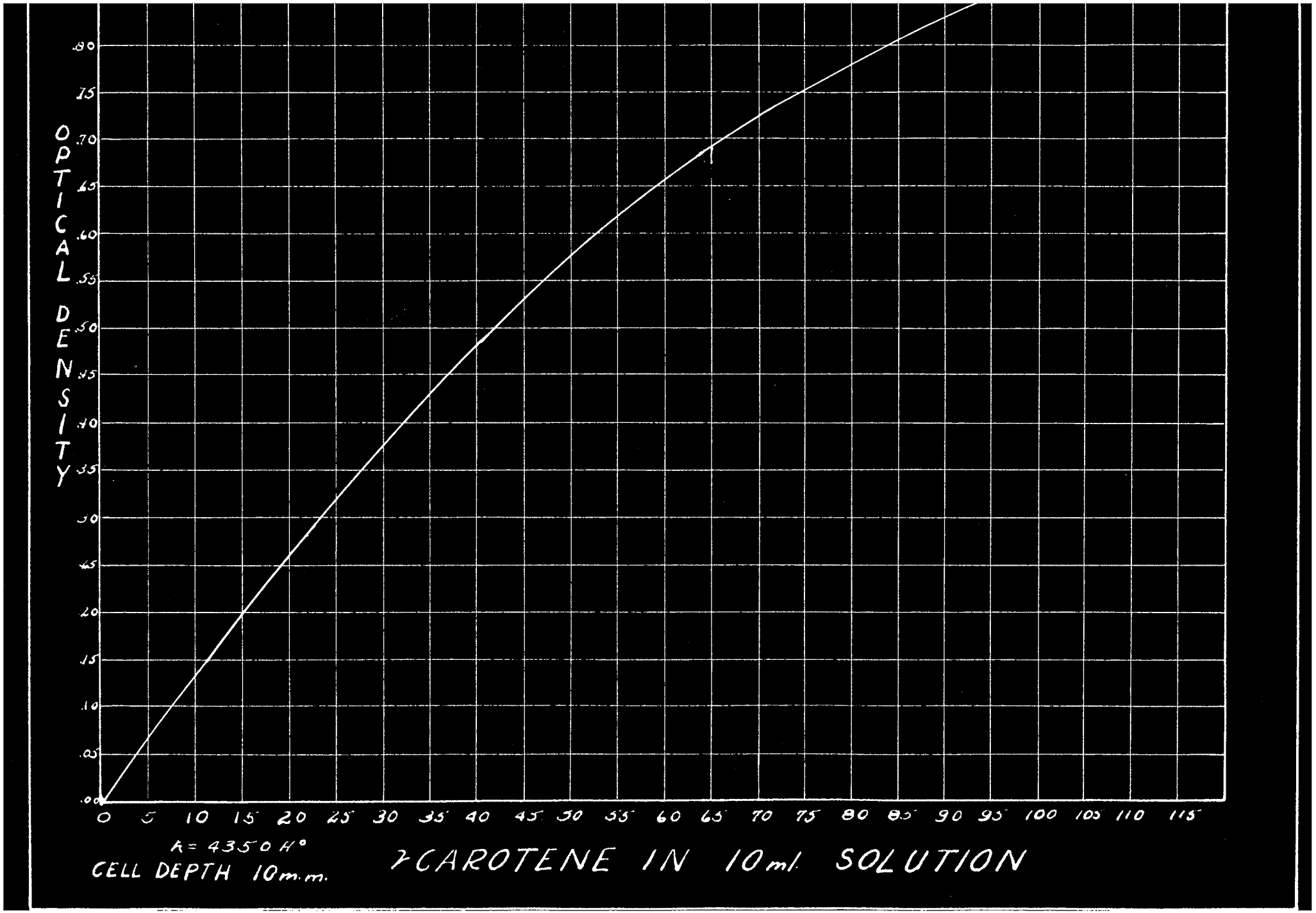
Twenty-five mg. of crystalline carotene[1] were dissolved in 250 ml of low boiling point petroleum ether. This was then diluted to a solution which contained 10 micrograms of carotene per ml. of solution and to a solution which contained 1 microgram per ml of solution. Aliquots containing between 2.5 micrograms to 100 micrograms of carotene were made up to a 10 ml. volume, and the optical density of these samples was used to construct calibration table I and curve I. These were used to determine the amounts of carotene in the chicken blood analyzed.

TABLE I

TABLE TO CONVERT OPTICAL DENSITY INTO MICROGRAMS OF CAROTENE
Carotene/10 ml. solution
Cell Depth 1 cm.
= 435 mu

	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09
.00	0.0	.8	1.6	2.5	3.2	4.0	4.9	5.5	6.2	7.0
.10	7.5	8.5	9.0	10.	10.5	11.5	12.2	13.0	13.8	14.5
.20	15.4	16.2	17.0	17.8	18.5	19.5	20.5	21.2	22.0	22.7
.30	23.5	24.2	25.1	26.0	27.0	27.7	28.7	29.7	30.5	31.5
.40	32.5	33.5	34.5	35.5	36.5	37.5	38.5	39.5	40.5	41.5
.50	42.5	43.5	44.5	45.5	46.5	47.5	48.5	49.5	50.5	51.5
.60	53.0	54.0	55.5	56.8	58.0	59.3	60.8	62.2	63.7	65.0
.70	66.8	68.5	70.0	71.5	73.5	75.0	77.0	79.0	81.0	83.0
.80	85.0	87.0	89.0	91.5	94.0	96.5	99.0	101.5	104.0	106.5

[1] Research Laboratory. S. M. A. Corporation, Chargin Falls, Ohio.


OPTICAL DENSITY
.80
.75
.70
.65
.60
.55
.50
.45
.40
.35
.30
.25
.20
.15
.10
.05
.00
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115
λ = 4350 A°
CELL DEPTH 10 m.m.
γ-CAROTENE IN 10 ml. SOLUTION

Correction curve and calibration table for the blue color produced by the cartenoids.

The aliquots containing the pure crystalline carotene, which were used for constructing the carotene curve and table, were used also in constructing the curve and table for the correction of the blue color imparted to a solution by the cartenoid, in addition to the blue color produced by the vitamin A.

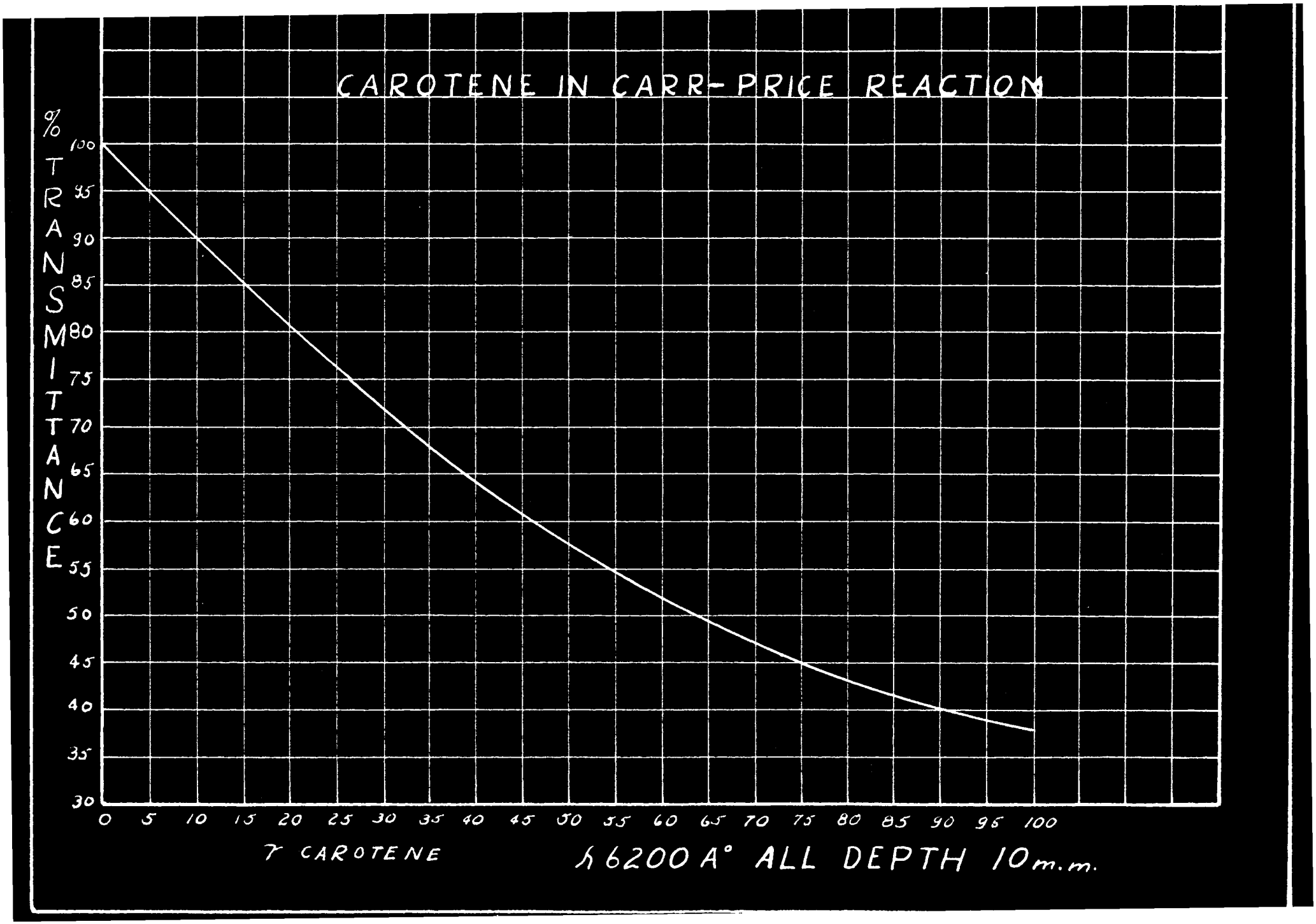
Each aliquot was evaporated to dryness, and the residue, was dissolved in 1 ml. of pure chloroform. Nine ml. of antimony trichloride reagent was added to each sample and a galvanometer deflection was recorded.

These deflections were a function of the amount of blue color produced by the various amounts of pure carotene. This amount must be subtracted from the total amount of blue color as produced by both vitamin A and carotene; thus the correction is made for blue color produced by carotene.

TABLE II

% TRANSMITTANCE FOR MICROGRAMS OF CAROTENE IN CARR PRICE-REACTION

	0	1	2	3	4	5	6	7	8	9
30									100.00	96.5
40	91.	87.	83.5	80.	77.5	75.	73.	71.	68.5	66.5
50	64.0	61.5	59.2	57.5	55.5	54.0	52.5	51.0	49.5	48.0
60	46.7	45.0	43.7	42.0	40.5	39.0	38.0	36.5	35.2	33.8
70	32.5	31.3	30.0	29.0	27.5	26.5	25.5	24.5	23.5	22.7
80	21.3	20.0	19.0	18.0	16.8	15.8	14.5	13.5	12.5	11.5
90	10.5	9.5	8.5	7.5	6.0	5.0	4.0	3.0	2.0	1.0


CAROTENE IN CARR-PRICE REACTION
% TRANSMITTANCE
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
γ CAROTENE
λ 6200 A° ALL DEPTH 10 m.m.

Vitamin A curve and calibration table.

.1 g of oil containing 5.6% vitamin A[1] was weighed and dissolved in 200 ml. of pure chloroform. 1 ml. aliquots containing from 25 to 500 micrograms of the 5.6% vitamin A were made and used for the antimony trichloride reaction. The aliquots were pipetted into test tube and the tubes were then cleaned with chloroform and polished with lens paper. Each tube was placed in the spectrophotometer and 9 ml. of antimony trichloride reagent were added.

The galvanometer of the spectrophotometer had been previously set at 100 with an antimony chloride blank intercepting the light beam of the instrument.

The galvanometer readings were recorded, and used to construct the standard curve and calibration table, by which the vitamin A content of the chicken blood was determined.

TABLE III

TABLE TO CONVERT % TRANSMITTANCE INTO VITAMIN A
620 (Round Tubes)

TRANSMITTANCE	00	1	2	3	4	5	6	7	8	9
00								28.00	27.02	26.94
10	25.20	24.08	23.24	22.40	21.70	21.14	20.58	20.02	19.60	19.18
20	18.76	18.34	17.90	17.50	17.36	16.80	16.66	15.96	15.68	15.40
30	14.98	14.56	14.22	13.86	13.24	13.16	12.88	12.60	12.38	12.10
40	11.90	11.62	11.34	11.06	10.86	10.64	10.29	10.08	9.80	9.52
50	9.24	8.96	8.74	8.50	8.26	7.98	7.70	7.45	7.28	7.00
60	6.84	6.58	6.37	6.16	5.91	5.74	5.54	5.32	5.18	4.98
70	4.76	4.62	4.48	4.26	4.06	3.92	3.78	3.58	3.44	3.22
80	3.08	2.90	2.66	2.52	2.38	2.24	2.06	1.85	1.68	1.54
90	1.40	1.19	.98	.84	.70	.56	.42	.28	.14	.07

[1] Vitamin A concentrate furnished by the Distillation Products Inc. Ridge Road, Rochester, New York.

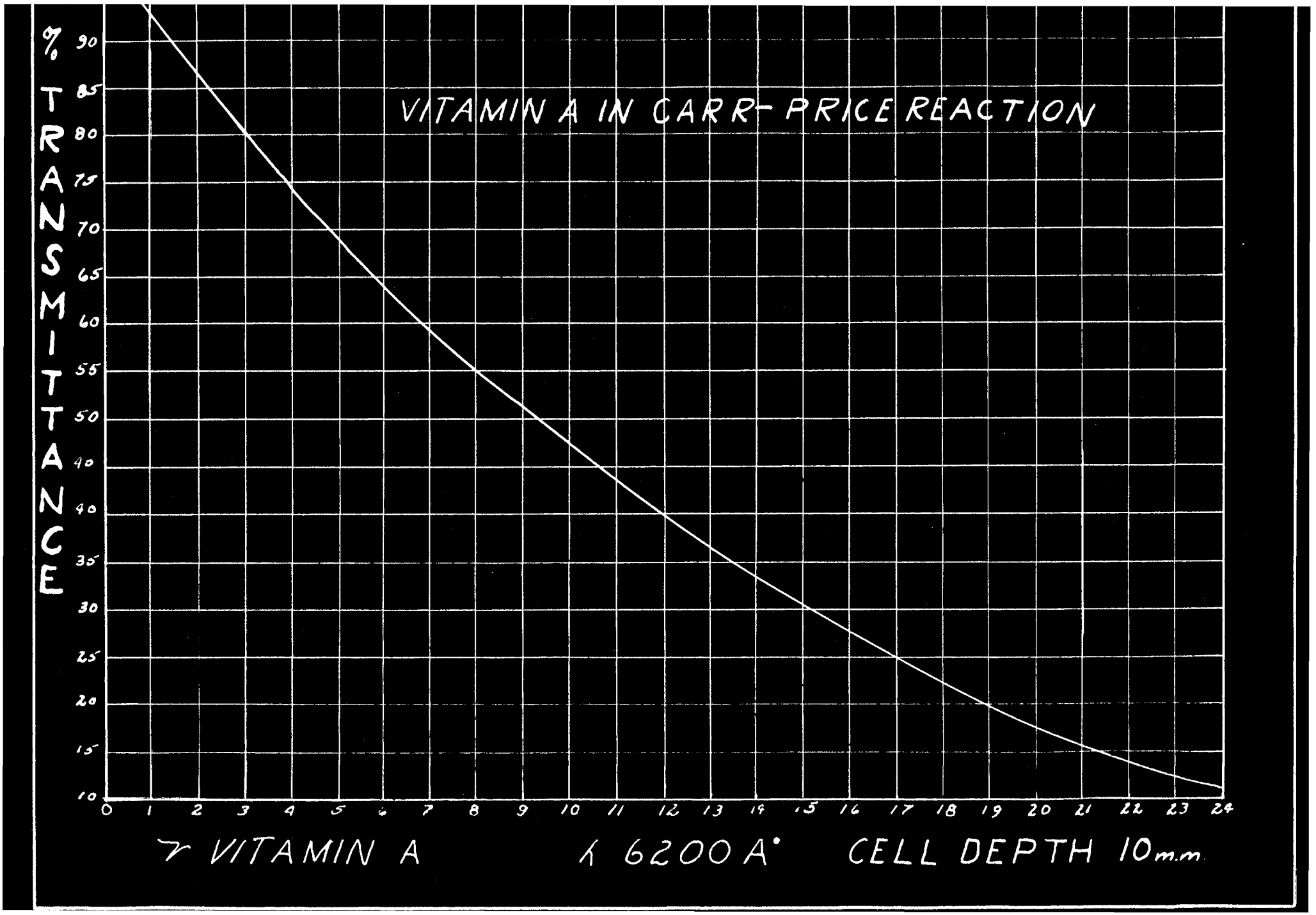

VITAMIN A IN CARR-PRICE REACTION
% TRANSMITTANCE
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
γ VITAMIN A
λ 6200 A°
CELL DEPTH 10 m.m.

Table for converting carotene into micrograms of vitamin A by Carr-Price reaction.

In order to simplify the calculations for correcting the vitamin A determination (subtracting the equivalent vitamin A produced by the carotenoids) Table IV was constructed from Table II. The percentage transmittance produced by the pure carotene and the values of percentage transmittance were converted into micrograms of vitamin A with the aid of Table III. For example the percentage transmittance of 54 micrograms of carotene gives a percentage transmittance of 55. To find its equivalent in terms of vitamin A the percentage transmittance 55 is converted into vitamin A with the aid of Table III. Percentage transmittance of 55 represents 7.98 micrograms of vitamin A. This subtracted from the total vitamin A gives the true value for vitamin A as produced by the Carr-Price reaction.

TABLE IV

TABLE TO CONVERT MICROGRAMS OF CAROTENE INTO MICROGRAMS OF VITAMIN A VIA CARR-PRICE REACTION

Carotene

	0	1	2	3	4	5	6	7	8	9
0	0	.07	.14	.28	.42	.56	.70	.78	.93	1.13
10	1.33	1.47	1.61	1.79	1.99	2.12	2.26	2.39	2.52	2.66
20	2.90	3.03	3.10	3.36	3.52	3.71	3.85	4.06	4.16	4.26
30	4.48	4.57	4.76	4.87	5.03	5.18	5.25	5.39	5.54	5.74
40	5.84	6.00	6.16	6.25	6.41	6.58	6.84	6.92	7.00	7.28
50	7.36	7.45	7.63	7.80	7.98	8.26	8.38	8.50	8.62	8.74
60	8.85	8.96	9.04	9.12	9.20	9.36	9.52	9.66	9.80	9.89
70	9.99	10.08	10.18	10.29	10.46	10.64	10.75	10.86	10.93	11.00
80	11.06	11.14	11.22	11.30	11.38	11.46	11.54	11.62	11.69	11.76
90	11.83	11.90	11.92	11.95	11.97	12.00	12.02	12.11	12.20	12.29
100	12.38									

EXPERIMENTAL

The blood from 7 lots of birds was analyzed for carotene and vitamin A content. The lots of birds differed in sex, breed, age, and rations fed.

Basal rations for laying hens consisted of:

Yellow corn	120. lbs
Pulverized oats	100. lbs
Wheat bran	100. lbs
Wheat shorts	100. lbs
Meat scraps	70. lbs
Alfalfa leaf meal	70. lbs
Cottonseed meal	56. lbs
Mung bean meal	35. lbs
Dried buttermilk	28. lbs
Oyster shell flour	14. lbs
Salt	7. lbs

This mash was kept before the hens at all times.

Basal ration for young growing chickens:

Yellow corn meal	340. lbs
Wheat bran	20. lbs
Alfalfa leaf meal	12. lbs
Dried buttermilk	60. lbs
Soy bean oil meal	20. lbs
Cottonseed meal	20. lbs
Meat scraps	20. lbs
Salt	3. lbs
Manganese solution	.8 pint
Delsterol	1/4 pound

To each 99 lbs. of this basal ration 1 lb. of crude oil meal was added. This mash was kept before the chickens at all times.

Lot I

Ten-week old cockerels receiving the basal ration for growing birds. The only green feed available was the grass and weeds in the lot.

ample	O.D.	γ Carotene per al.	% trans-mission	Vitamin A and carotene	Blue color produced by carotene	Vitamin A per al.	γ Carotene per 100 ml plasma	γ Vitamin A per 100 m blood
1	.14	10.5	51	7.45	1.40	6.05	420.	242.
2	.20	15.4	70	4.76	2.17	2.59	616.	103.6
3	.27	21.2	58	7.28	3.04	4.24	848.	169.6
4	.24	18.5	63	6.16	2.59	3.57	740.	142.8
5	.16	12.2	80	3.08	1.63	1.45	488.	58.
6	.24	10.5	61	6.58	1.40	5.18	420.	207.2
7	.25	19.5	67	5.32	2.78	2.54	780.	101.6
8	.41	33.5	55	7.98	4.95	3.03	440.	121.2
9	.16	12.2	74	4.06	1.64	2.42	488.	96.8
10	.28	22.0	69	4.98	3.10	1.88	880.	75.2
11	.22	17.0	75	3.92	2.39	1.53	680.	61.2
12	.35	27.7	65	5.74	4.12	1.62	1108.	648.
13	.36	28.7					1148.	
14	.41	33.	54	8.26	4.87	3.39	1320.	135.6
15	.23	17.8	73	4.26	2.49	1.77	712.	70.8
16	.24	18.5	56	7.70	2.59	5.11	740.	204.4
17	.21	16.2					648.	
18	.14	10.5	70	4.76	1.40	3.36	420.	134.4
19	.14	10.5	70	4.76	1.40	3.36	420.	134.4
20	.36	28.7	64	5.91	4.23	1.68	1148.	67.2
21	.26	20.	45	10.64	2.90	7.74	800.	309.6
ERAGE							727.	131.6
Average International Units							1045.[1]	565.8[2]

[1] .6 micrograms of carotene equals one International Unit (6).

[2] 1 microgram of vitamin A equals 4.3 International Unit (20).

Lot II

Brown leghorn laying hens that had received fresh green clover for one week in addition to the basal ration for laying hens.

ple	O.D.	γCarotene per al.	% trans-mission	Vitamin A and carotene	Blue color produced by carotene	Vitamin A per al.	γCarotene per 100 ml plasma	γVitamin A per 100 ml blood
	.22	17.	73.	4.26	2.39	1.87	680.	74.8
	.14	10.3	86.5	1.95	1.38	.61	412.	24.
	.08	6.2	79.	3.22	.72	2.5	248.	100.
	.13	9.5	86.	2.06	1.23	.83	380.	33.2
	.07	5.5	91.	1.19	.63	.56	220.	22.4
	.14	10.3	87.5	1.76	1.79			
	.15	11.	69.	4.98	1.47	3.51	440.	140.4
	.16	12.2	90.	1.40	1.63			
	.13	9.5						
	.16	9.5	92.	.98	1.23			
	.18	13.4	81.	2.90	1.90	1.	536.	40.
	.07	5.5	90.	1.40	.61	.79	220.	31.6
	.07	5.2	86.	2.06	.59	1.47	208.	58.8
	.24	18.5	75.	3.92	2.61	1.31	740.	52.4
	.27	21.2	79.	3.22	3.04	.18	848.	.72
	.17	13.	89.	1.54	1.79			
ERAGE							448.4	52.5
erage International Units							747.	225.8

Lot III

White Rock laying hens on basal ration

le	O.D.	γ Carotene per al.	% trans-mission	Vitamin A and carotene	Blue color produced by carotene	Vitamin A per al.	γ Carotene per 100 ml plasma	γ Vitamin A per 100 ml blood
	.11	8.5	80.	3.08	1.03	2.05	340.	82.
	.06	4.9	87.	1.85	.55	1.30	196.	52.
	.95	7.3	83.	2.52	.79	1.73	292.	69.2
	.04	3.2	94.	.70	.30	.40	128.	58.8
	.06	4.9	83.	2.52	.55	1.47	196.	58.8
	.03	2.5					100.	
	.07	5.5					220.	
	.17	13.	76.	3.78	1.78	2.00	520.	80.
	.12	9.	77.5	3.51	1.13	2.38	360.	95.2
	.04	3.2					128.	
RAGE							248.	64.7
rage International Units							413.	278.2

Lot IV

White Rock hens in laying house on basal ration,
no green feed except few weeds in yard

le	O.D.	γ Carotene per al.	% trans-mission	Vitamin A and carotene	Blue color produced by carotene	Vitamin A per al.	γ Carotene per 100 ml plasma	γ Vitamin A per 100 ml blood
	.15	11.5	76.	3.78	1.55	2.23	460.	89.2
	.07	5.5	87.	1.85	.62		220.	
	.26	20.5	83.	2.24	2.95		820.	
	.25	19.5	82.	2.38	2.70		780.	
	.25	19.	87.	1.85	2.66		760.	
	.11	8.5	79.	3.22	.98	2.24	340.	89.6
	.22	16.6	78.	3.44	2.30	1.14	664.	45.6
	.20	15.4	79.	3.22	2.16	1.06	616.	42.4
	.15	11.5	77.	3.58	1.53	2.05	460.	82.
	.14	10.5	79.	3.22	1.37	1.85	420.	74.
	.18	13.8	78.	3.44	1.95	1.47	552.	59.6
	.10	7.5					300.	
ERAGE							532.6	68.9
rage International Units							887.	295.2

Lot V

Plymouth Rock laying hens on basal ration

le	O.D.	γCarotene per al.	% trans- mission	Vitamin A and carotene	Blue color produced by carotene	γVitamin A per al.	γCarotene per 100 ml plasma	γVitamin A per 100 ml blood
	.09	7.	77.	3.58	.78	2.80	280.	112.
	.09	7.	73.	4.26	.78	3.48	280.	139.2
	.18	13.4	59.	7.	1.89	5.11	536.	204.4
	.28	22.0	64.	5.91	3.10	2.81	880.	112.4
	.06	4.9	75.	3.92	.54	3.38	196.	135.2
	.23	17.8	78.	3.44	2.50	.94	712.	37.6
	.23	17.8	74.	4.06	2.50	1.56	712.	62.4
	.13	9.5	80.	3.08	1.23	1.65	380.	66.
	.14	10.5	58.	7.28	1.40	5.88	420.	235.2
	.02	1.6	57.	7.45	1.00	6.45	64.	258.
ERAGE							446.	136.4
erage International Units							743.	586.5

Lot VI

This study consisted of two 12-weeks old cockerels. One was kept as a control bird, on the basal ration for growing chickens.

The other bird received the basal ration to which had been added 50 mg. of diansythexene stilbesterol per pound of feed. The chickens were bled on 9 consecutive days and analyses made.

ple	O.D.	✓Carotene per al.	% trans- mission	Vitamin A and carotene	Blue color produced by carotene	Vitamin A per al.	✓Carotene per 100 ml plasma	✓Vitamin A per 100 ml blood
trol bird								
day	.11	8.5	79.	3.22	1.03	2.19	340.	87.6
day	.16	12.2	79.	3.22	1.63	1.59	488.	63.6
day	.08	6.2	96.	.42	.71		248.	
day	.13	10.	87.	1.85	1.33	.52	400.	20.8
day	.16	12.2	90.	.87	1.63		488.	
day	.14	10.8	95.	.56	2.52		432.	
day	.15	11.	93.	.84	1.47		440.	
day	.14	10.5	93.	.84	1.38		420.	
day	.11	8.5	83.	2.52	1.03	1.49	340.	59.6
RAGE							400.	57.9
rage International Units							66.6	
l receiving stilbesterol								
day	.28	22.	79.	3.22	3.10	.12	480.	4.8
day	.16	11.8	79.	3.22	1.59	1.63	472.	65.2
day	.14	10.2	89.	1.54	1.61		408.	
day	.21	16.2	85.	2.24	2.30		648.	
day	.09	9.	87.	1.85	.78	1.07	280.	42.8
day	.33	26.	86.	2.06	.24	1.82	640.	72.8
day	.28	22.	81.	2.90	3.10		880.	
day	.12	8.8	81.	2.90	1.00	1.90	352.	76.
day	.28	22.	61.	6.58	3.10	3.48	880.	139.2
AGE							604.4	66.8
age International Units							1007.	287.24

Lot VII

This group consisted of 12-week old cockerels. Group I received 50 mg. of diansythexene stilbesterol per pound of basal ration for growing birds. Group II received estrogen in pellet form.

ple	O.D.	γCarotene per al.	% trans- mission	Vitamin A and carotene	Blue color produced by carotene	Vitamin A per al.	γCarotene per 100 ml plasma	Vitamin A per 100 ml. blood
up I								
	.59	51.5	57.	7.45	7.45		2060.	
	.80	85.	39.	12.10	11.46	.64	3400.	25.6
	.50	42.5	63.	6.16	7.3		1700.	
	.37	29.7	65.	5.74	4.31	1.44	1588.	51.6
	.81	87.	41.	11.6	6.	5.6	3480.	224.
RAGE							2517.	100.4
rage International Units							419.5	
up II								
	.53	45.5	41.	11.6	6.7	4.9	1820.	196.
	.84	94.	36.	12.8	11.9	.9	3760.	36.
	.68	63.7	53.	8.5	5.3	3.2	2580.	128.
AGE							2720.	120.
rage International Units							4533.	516.

SUMMARY

Carotene and vitamin A were determined in chicken blood. The blood was taken from seven lots of birds and determinations were made on ninety-five samples.

The carotene and vitamin A content in the blood of young cockerels is high, compared to laying hens.

The addition of fresh green clover as a green supplement to the basal ration for laying hens, apparently increased the carotene content of the chicken blood, but did not increase the vitamin A content.

As compared to the light weight breed of laying hens, the blood from the heavier laying hens contains more carotene and vitamin A.

Stilbesterol given in the feed and in pellet form increases the carotene content of the blood in cockerels, but apparently does not increase the vitamin A content. The carotene content of the blood of the cockerels receiving stilbesterol in pellet form was slightly higher than that in the blood of the cockerels receiving stilbesterol in the ration.

This study gave evidence that blood analysis is a satisfactory method of comparing carotene and vitamin A in chickens of different breeds, ages and sex, and birds on different rations.

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