XIX. THE ASSOCIATION OF VITAMIN A WITH THE LIPOCHROMES OF PLANT TISSUES.

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During the investigation of the conditions of formation of vitamin A in seedlings reported in the preceding paper, the association of vitamin A with lipochromes in plant tissues already emphasised by Steenbock and his co-workers (subsequent references) was being kept in view. Much of the earlier work on vitamin A consisted of the examination of such plant tissues as were commonly in use as food for human beings and farm stock. Steenbock and Gross [1919] found that, of all the roots and underground stems examined by them, the carrot and sweet potato were the only two that contained this vitamin; the other seven they described as a "complete failure." These latter included rutabaga, dasheen, red beet, parsnip, potato, mangold and sugar beet. In each experiment the root formed 15 % of the diet of the test animals, and in the case of the carrot and sweet potato, gave good growth. The minimum dose for growth of each of these was not determined, so that no exact comparison between the two was ever drawn. A rather more quantitative examination was made of the yellow and white maize seeds. When the former constituted 85 % of the ration, the test animals grew and reproduced themselves. The same ration of white maize showed a retardation of the rate of growth in varied lengths of time, which were dependent on the variety of maize used. It was also found that yellow maize in 48 % of the diet gave distinctly poorer growth than the higher ration.

Meanwhile, the close association of vitamin A with the lipochromes in animal fats led many workers to speculate on the possible identity of vitamin A with one of the lipochromes. That there was no foundation for this was, however, shown by Drummond [1919] who prepared pure crystalline carotene from carrots and demonstrated its complete lack of growth-promoting power. Later, also, Drummond and Coward [1920] and Palmer and Kennedy [1921] described many instances of animal fats which were rich in carotene and relatively poor in vitamin A, and others in which the reverse relation held. Steenbock, Sell and Bluell [1921] pointed out that the concentrations of pigment and vitamin A in butter were not closely parallel, though they were

in general agreement, and suggested that this might be due to their having the same source in the green food of the cow. Steenbock, Sell and Boutwell [1921] found that in six different varieties of dried peas, the greater the lipochrome content, the greater was the vitamin A content, though no definite comparison could be made as there is no method of measuring absolute quantities of the vitamin. Steenbock and Sell also found [1922] that the "white sweet potato and the white carrot contained little fat-soluble vitamin, which was in marked contrast to the yellow pigmented varieties. The tops of white carrot roots slightly pigmented with chlorophyll and containing a small amount of yellow pigment were found richer in fat-soluble vitamin than the bottoms containing one-half as much pigment. Green cabbage leaves taken from the heart of cabbage plants which failed to 'head' were found much richer in fat-soluble vitamin than white cabbage leaves in the head. The latter contained only one-tenth as much yellow pigment."

These observations made it seem desirable to examine the lipochrome content of the tissues used in the experiments reported in the previous paper [1923]. Accordingly, the wheat seeds and seedlings of experiment 1 were first tested. 20 g. of the seeds (460 in number) were saponified by heating on a water-bath with 50 % caustic soda for five days and with two volumes of alcohol for two days under a reflux condenser. The alcohol was then distilled off, the mass diluted with two volumes of water and extracted with light petroleum (B.P. 40–70°) three times. The extracts were combined, washed with water six times, always in an atmosphere of carbon dioxide, dried over anhydrous sodium sulphate, evaporated down in a current of carbon dioxide under reduced pressure and made up to 10 cc. exactly. The strength of the solution was estimated by means of a Hellige colorimeter, comparison being made against a 0·2 % solution of potassium dichromate. The concentration of the pigment was determined by reference to curves kindly lent by Dr O. Rosenheim and drawn according to observations made by himself on solutions of the lipochromes of known strength, in confirmation of Willstätter's work on the same point. The curves of carotene and xanthophyll are far from being identical: the curve for xanthophyll is more nearly a straight line than that for carotene. The estimation of the lipochromes in a solution cannot therefore be made exactly without first effecting a separation and then making an estimation of each independently. But where carotene and xanthophyll occur in about the same relative proportions in different tissues, one being largely in excess over the other, a fairly true comparison may be made by using the curve of the lipochrome which occurs in the greater proportion. To determine this proportion, Willstätter's phase test is applied. To a given volume of the light petroleum solution of the mixture, an equal volume of 90 % methyl alcohol is added. The xanthophyll goes into the lower alcohol layer, the carotene remains in the petroleum, and the relative strengths of the solutions may be roughly estimated. The amount of pigment in the seeds was extremely small, 0·02 mg. in the 460 seeds used, and, moreover, the pigment did not give the usual colour

reactions for lipochromes. Wheat seeds were also grown in the dark and light respectively for 12 days and the lipochrome content of the same number of shoots (460) estimated in the same way. In the 460 etiolated shoots were 1·062 mg. of lipochrome (carotene: xanthophyll = 1 : 3) estimated by Willstätter's curves as xanthophyll; while in the 460 green shoots were 1·875 mg. of lipochrome (carotene: xanthophyll = 1 : 3). Some yellow pigment was left in the remaining seeds and roots in both cases (0·064 mg. in the etiolated, and 0·1 mg. in the green), but it did not give the lipochrome reactions with strong sulphuric and nitric acids. The interesting point in the comparison is that the lipochrome content of both lots of shoots was very much greater than that of the seeds from which they had grown, and that the content of the green shoots was, roughly, nearly twice that of the etiolated shoots (Table I).

Etiolated and green shoots of yellow maize contained approximately equal amounts of lipochrome, which was greater than the amount contained in an equal number of seeds (Table I).

Table I.

	Weight in g.	No. of seeds or shoots	Lipochrome tests H_2SO_4	HNO_3	I in KI	Phase test $C : X$	Total lipochrome content mg.
Wheat:							
Seeds	20	460	−	−	−		0·02?
Etiolated shoots		460	+	+	+	1 : 3	1·062
Green shoots		460	+	+	+	1 : 3	1·875
Yellow maize:							
Seeds	20	73	+	+	−	1 : 2	0·15
Etiolated shoots		73	+	+	−	1 : 3	0·21
Green shoots		73	+	+	−	1 : 3	0·21
Peas:							
Seeds, dry	26	126	+	+	−	1 : 3	0·11
Etiolated shoots		126	+	+	−	1 : 3	0·02
Shoots without CO_2		126	+	+	−	1 : 3	0·11
Shoots without O_2		126	+	+	−	1 : 2	0·09
Remainder seeds and roots		126	+	+	−	2 : 3	0·065

A similar comparison was made between seeds and shoots of peas treated as in experiments 2 and 3 of the preceding paper; the day on which the shoots were exposed to the light was however not nearly as sunny as any one during the feeding test. The results are summarised and it is interesting to note that there was less lipochrome in the etiolated shoots than in the seeds and the amount of lipochrome remaining in the roots and seeds (not nearly exhausted of the food store by this time) was larger than that of the etiolated shoots. The amounts in the shoots exposed to light (without carbon dioxide and without oxygen respectively) showed a content quite or nearly as great as the dry seeds (Table I). It is at least suggestive to note by the way that the ratio, xanthophyll/carotene, was less in the shoots deprived of oxygen than that in the shoots deprived of carbon dioxide. But the important point to note at present is that lipochromes are present in many of these tissues before the

appearance of the vitamin. A comparison between the absolute amounts of lipochrome present in shoots which will not promote growth and those which will do so once again proves that the activity of the tissue is not measured by its lipochrome content.

Lipochrome content of:			
100 etiolated pea shoots	0·016 mg.	Inactive in dosage of one shoot	
„ pea shoots exposed to light in absence of CO_2	0·09	Active	„ „ „
„ pea shoots exposed to light in absence of oxygen	0·07	„	„ „ „
„ etiolated wheat shoots	0·23	Inactive	„ „ „
„ green „ „	0·4	Active	„ „ „
„ etiolated shoots of yellow maize	0·28	Inactive	„ „ „
„ green „ „ „	0·28	Active	„ „ „

These estimations were made on much larger numbers of shoots than those indicated but have been reduced to 100 to draw the comparison of the lipochromes.

In the meantime, an examination was being made on all available lipochrome-containing tissues from plant growths as varied as possible from a botanical point of view; and a comparison was made according to lipochrome content, growth-promoting power, and calcium content (to verify if possible the finding reported in the preceding paper on the dispensability of calcium salts in the formation of vitamin A).

A polyanthus narcissus was first investigated. The perianth of this variety is a deep rich orange and the corona even darker, but an estimation of the lipochrome content showed that the whole perianth of six lobes contained only slightly more than the corona, a much smaller structure, of the same flower. No calcium was found in 3 g. of perianth, 2 g. corona or in 0·2 g. of the flower sheath; but 0·25 % of the dry weight of the stem was calcium, estimated on 2 g. fresh material. Twenty coronas given to each of two rats daily (put in a small dish in the cage and generally eaten greedily) produced good growth as did also the perianth lobes from 20 flowers to each of two other rats (Fig. 1 and Table III).

A pure "paper-white" narcissus gave slight growth in one of three rats, none in the other two; but an equal dose of the perianth and corona of a "poetaz" narcissus (white perianth and pale yellow corona) gave very definite growth in these same rats. No calcium was found in 3 g. of perianth of the paper-white narcissus though it formed 0·53 % of the dry weight of the stems (estimated on 6 g.) and 1·8 % of the dry weight of the brown sheaths (estimated on 0·4 g.) (Fig. 1 and Table III).

On giving coronas or perianths of a large daffodil (*Narcissus Buonaparte*) to rats, they dropped in weight at once, but on discontinuing this addition, they invariably recovered their previous weight and even grew for a few days. This suggested the presence of vitamin A in this tissue and also some toxic substance. To avoid the effect of the latter, the unsaponifiable matter was extracted from the various tissues and used for the following experiment. By a comparison of the vitamin content of daffodil buds with that of flowers opened in the laboratory (the stems being cut as short as possible) it was

hoped to obtain a confirmation of the previous finding on the formation of the vitamin in a plant tissue in the presence of lipochromes and in sunlight—that is, that there should be no question of its having been transported to the flowers from the leaves. Some growth was obtained on the unsaponifiable matter from 170 buds, but no growth was obtained on the extract from 140 flowers opened in the laboratory, nor on the extract from 140 flowers opened naturally. Possibly the conditions of its storage while waiting for suitable rats for the test accounted for the inactivity but it was too late in the season to repeat the test. Again no calcium was found in the buds (5 g.), in the flowers (5 g.) opened naturally, or in the flowers (6 g.) opened in the laboratory which had been supplied with North London tap-water. But the sheaths (1 g.), leaves (4 g.), and stems (6 g.) contained 0·57, 0·39, 0·39 % calcium, respectively, of their dry weights. This is interesting botanically as no stomata were found in these perianths or in those of any of the other narcissi, yet stomata occurred on the outer (botanically "under") surface of the sheaths and in the stems and leaves. The epidermal cells of the perianths and coronas of all the yellow varieties had very definite conical-shaped outer walls, but they were not so highly developed in the white varieties. Haberlandt [1914] ascribes to this structure the function of concentrating the light on the contents of the cell (Tables II and III).

A chance of testing equally illuminated parts of the same flowers, the one containing much carotene and the other none, occurred in *Narcissus poeticus*—Barr's "White Standard," popularly known as "Pheasant's Eye." Seven hundred of these flowers were obtained, and the white perianths removed without the very small yellow patch that occurs at the base of each lobe.

The coronas were also removed and the two lots saponified separately. The unsaponifiable matter from the white perianth lobes (2·8 g. from 370 g. material) gave no growth in three rats, while the extract from the yellow coronas (0·2 g. from 11 g.) gave very good growth in three rats for eleven days (Fig. 1).

Yellow tulips gave growth on the unsaponifiable matter of their leaves and perianths, the former being stronger than the latter. Again no calcium was found in the perianths though it formed 2·5 % of the dry weight of the leaves. The stems contained no detectable calcium and in this flower there is no sheath. The lipochrome content of the perianths was low, being only 0·005 mg. in 10 g. fresh material (Tables II and III).

Other instances of the association of vitamin A with lipochromes[1] in plant tissue studied in the work are afforded by (*a*) tomato pulp, (*b*) cucumber skin, (*c*) yellow iris flower, the sepals, petals, petaloid stigmas being used in turn, but *not* the stamens or pollen, (*d*) orange juice, (*e*) red capsicum fruit, (*f*) yellow capsicum fruit, (*g*) the calyx of the ripened fruit of the winter cherry (Tables II and IV). In this connection, it should be noted that tomato

[1] A full account of the lipochromes (carotinoids) and their occurrence in animal and plant tissues has just been written by Palmer (1922).

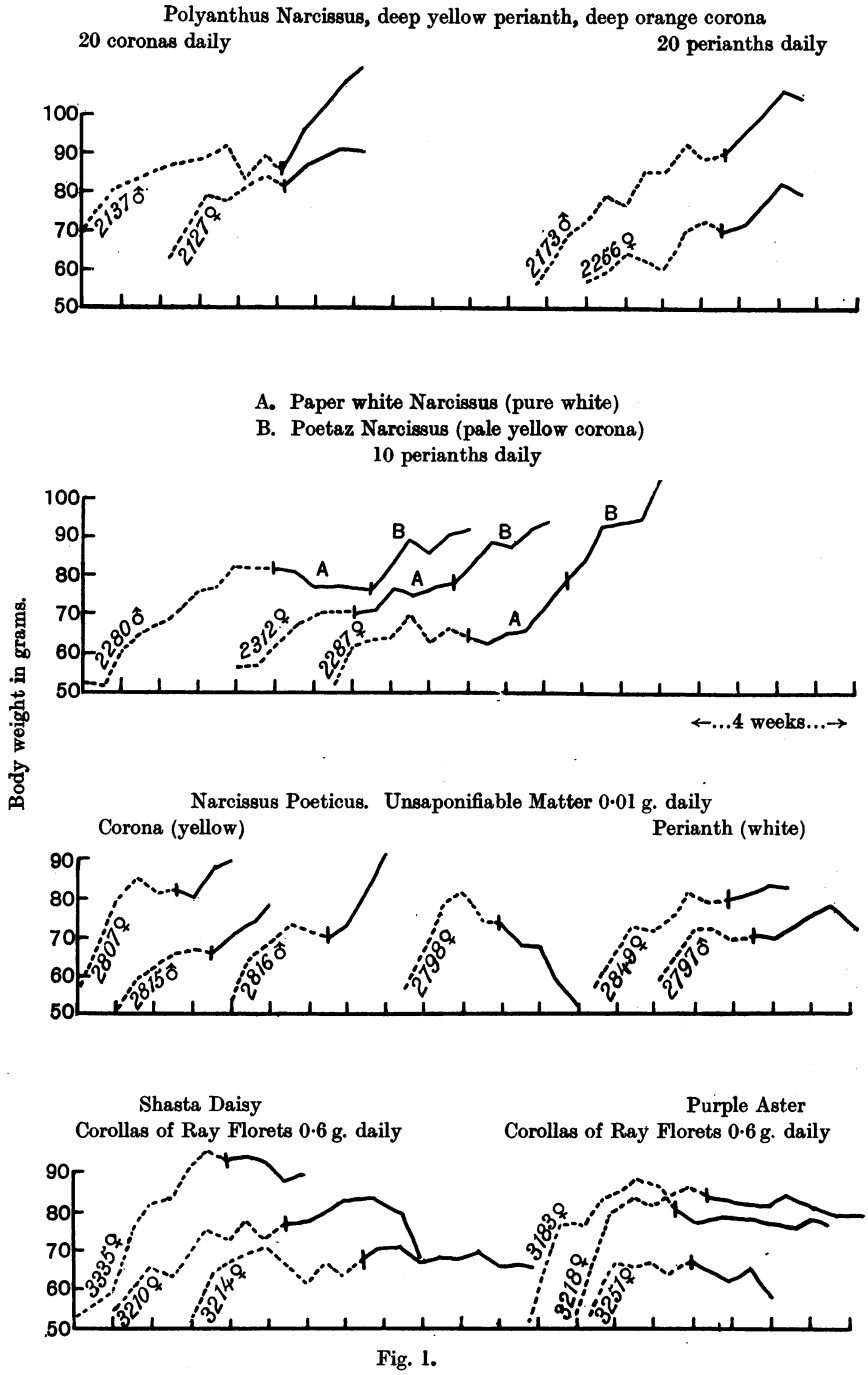

Fig. 1.

Preparatory period on A-deficient diet shown in dotted lines.

fruit has already been shown to contain vitamin A by Osborne and Mendel [1920, 1], that indications of the presence of the vitamin in orange juice have been noted by Osborne and Mendel [1920, 2] and also by Hess, McCann and Pappenheimer [1921].

The absence of vitamin A is associated with absence of lipochrome in (*a*) four varieties of mangolds (the flesh of the root only tested), (*b*) one variety of swede, (*c*) the fleshy part of the cucumber fruit (slight), (*d*) the inflorescence of the cauliflower, (*e*) the corollas (only) of the ray florets of the Shasta daisy, (*f*) the corollas (only) of the ray florets of a purple aster (Tables II and IV, and Fig. 4).

Table II.

	Daily dose. Unsap. matter	Body weight of rat in g. 0	7	14	21	28 days	
Narcissus Buonaparte:							
Perianths of buds	0·01 g.	72	72	82	82	—	Very fair
		82	89	90	94	—	
		98	100	120	124	—	
Perianths of flowers opened naturally	0·01 g.	90	89	—	—	—	Probably none (period too short)
		70	62	—	—	—	
		84	95	—	—	—	
Perianths of flowers opened in lab.	0·01 g.	99	97	97	—	—	None
		76	73	76	—	—	
		72	76	70	—	—	
		81	81	65*	—	—	
Leaves	0·01 g.	74	85	94	—	—	Very good
		102	126	142	—	—	
		89	92	112	—	—	
Yellow Tulip:							
Perianth	0·01 g.	76	74	82	90	—	Slight
		81	89	100	87 (cold)		
		75	74	76	77	—	
Leaves	0·01 g.	81	86	102	105	—	Fairly good
		69	70	79	86	—	
	Fresh matter						
Tomato pulp	0·5 g.	88	97	116	125	130	Fairly good
		79	80	92	99	102	
		82	88	86	95	99	
Cucumber skin	1·0 g.	96	109	116	122	130	Good
		79	91	110	112	112	
		62	60	70	73	70	
Yellow iris, sepals, petals and stigmas	0·2 g.	74	83	—	—	—	Probably good (period too short)
		69	74	—	—	—	
		83	101	—	—	—	
Orange juice	10 cc.	96	103	111	—	—	Good
		85	100	115	—	—	
		83	97	112	—	—	
Red capsicum, fruit	1·0 g.	94	102	116	—	—	Good
		74	82	99	—	—	
Yellow capsicum, fruit	1·0 g.	91	98	116	—	—	Good
		96	111	121	—	—	
Winter Cherry, calyx only	0·1 g.	92	103	115	—	—	Good
		78	84	90	—	—	
		83	96	107	—	—	

Table II *continued*

	Daily dose Fresh matter	Body weight of rat in g. 0	7	14	21	28 days	
Mangolds: Golden Tankard	1·0 g	98	95	78	—	—	None
		96	109	94	—	—	
		90	88	79	—	—	
Swedes: Red Queen	1·0 g.	75	78	84	—	—	None
		72	75	73	—	—	
		83	84	78	—	—	
Cucumber, fleshy part of fruit	1·0 g.	89	91	90	98	98	Slight
		75	78	84	91	86	
Cauliflower, inflorescence	1·0 g.	96	98	100	—	—	None
		78	81	80	—	—	
	Unsap. matter						
Laminaria	0·01 g.	104	118	113	—	—	Practically none
		105	105	108	—	—	
		105	105	105	—	—	
Fucus vesiculosus	0·01 g.	93	89	102	—	—	Probably none
		76	65	66	—	—	
		77	72	66	—	—	
Lilium candidum, pollen	0·01 g.	80	82	79	—	—	None
		82	92	88	—	—	
		83	88	83	—	—	
Carrot shoots, etiolated	0·8 g.	97	103	106	—	—	Fair
		90	95	101	—	—	
		82	91	91	—	—	
Carrot shoots, etiolated	0·2 g.	89	93	96	—	—	Fair
		85	91	97	102	—	
		97	111	119	119	—	
Turnip shoots, etiolated	0·2 g.	94	94	92	—	—	None
		68	70	66	—	—	
		72	70	63	—	—	
Carrot, roots	0·01 g.	63	63	78	—	—	Fairly good
		56	62	66	—	—	
		61	63	71	—	—	
Turnip, roots	0·01 g.	96	78	65	—	—	None
		86	78	81	—	—	
		71	66	61	—	—	
Polyanthus narcissus, stems	0·01 g.	102	105	—	—	—	Fair
		66	71	72	—	—	
		75	80	86	—	—	

An effort was made in the late summer to find a yellow flower whose pigments were wholly water-soluble, but in five varieties of *Nasturtium,* in *Helianthus annuus,* in the African marigold, in *Ranunculus acris,* in *Lotus corniculatus,* lipochromes were always present. Hence no example of the association of the vitamin with a yellow water-soluble pigment has been traced, and its non-occurrence in the flesh of the mangolds and swedes which contain large quantities of these pigments has been clearly demonstrated, though in this case, of course, light has had no immediate influence.

Another apparent exception to what appears to be a general rule, the association of vitamin A with lipochromes wherever they are exposed to light, was found in *Fucus vesiculosus,* a brown seaweed which grows just below high water mark; patches of this are mingled with patches of *Enteromorpha,* a green

Table III.

	Water	Ash % of dry wt.	Calcium: % of dry wt.	Calcium: estimated on approx. g.	Dose g.	Growth
Polyanthus narcissus:					Fresh matter	
Perianth lobes	90·96	9·5	0	3	1	Good
Coronas	86·61	5·82	0	2	0·5	,,
					Unsap. matter	
Stems	93·90	6·23	0·25	2	0·01	Slight
"Paper-white" narcissus:					Fresh matter	
Perianth including corona	89·49	8·17	0	3	1	Very slight
Stems	90·42	8·3	0·53	6	—	—
Flower sheaths (brown)	12·76	7·7	1·8	0·4	—	—
Narcissus Buonaparte:					Unsap. matter	
Perianths of buds	83·57	3·65	0	5	0·01	Fair
Perianths of flowers opened naturally	89·93	4·55	0	5	0·01	None
Perianths of flowers opened in lab.	92·76	6·45	0	6	0·01	,,
Flower sheaths (brown)	49·78	7·35	0·57	1·0	—	—
Leaves	85·87	7·0	0·39	4	0·01	Very good
Stems	92·74	6·05	0·39	6	—	—
Yellow Tulip:						
Perianth	89·45	5·32	0	3	0·01	Fair
Leaves	87·56	9·64	2·52	3	0·01	Good

(It is interesting to note incidentally the occurrence of calcium in those tissues in which photosynthesis (carbon assimilation) has at some time taken place and its apparent absence from those tissues where presumably this process has never been carried on.)

seaweed, on the boulders and rocks of the seashore, but on the groynes built out as breakwaters on the South Coast, the *Fucus* grows distinctly below the green weeds and therefore would be covered by the tide for a somewhat longer period than the green ones. A test on the washed fresh material previously reported [Coward and Drummond, 1921] gave no evidence of vitamin A in *Fucus*, though a green weed *Enteromorpha* had given positive results, and later tests on the extract of unsaponifiable matter of *Fucus vesiculosus* and *Laminaria*, respectively, have also detected no vitamin (Table II). The lipochromes of this species and of *Laminaria* were then examined, the method adopted being as follows.

The fresh material was ground up with solid caustic soda (about 1 g. to 5 g. fresh material) to break down the chlorophyll into components (chlorophyllins etc.) which are insoluble in light petroleum, and also to prevent possible enzyme action. A few drops of ether were also added, and, when necessary, a little silver sand to help to break down the tissue. The mixture was then ground up with anhydrous sodium sulphate to retain water, and light petroleum (B.P. 40–70°) added to dissolve out the lipochromes. In the case of *Laminaria*, the extract was scarcely coloured and a further extract with the same solvent was colourless. Ether was then tried and formed a deep yellow solution which gave a well-defined spectrum 475–450 and 420 to the end. There was only a slight indication of the most characteristic carotene band, *i.e.*

490–480. The solution gave the light blue colour reaction with 30 % hydrochloric acid characteristic of fucoxanthin according to Willstätter—and also the general lipochrome reactions with conc. H_2SO_4 and HNO_3. A second ether extract of the same preparation gave a spectrum with no trace of the first carotene band, but gave the fucoxanthin bands very well defined again. It was concluded that *Laminaria* contained only the merest traces of carotene or xanthophyll, but large quantities of fucoxanthin.

Table IV

	Daily dosage g.	Lipochrome present	Spectrum	Colour reactions with H_2SO_4	Colour reactions with HNO_3	Activity
Tomato pulp	0·5	Lycopin carotene?	Typical Masked by leycopin if present	+	+	Good
Cucumber skin	0·5	Carotene and xanthophyll	Typical	+	+	,,
Yellow Iris, flower	0·2	,,	,,	+	+	,,
Orange juice	10 cc.	Carotene and xanthophyll 4 : 1	,,	+	+	,,
Red capsicum, fruit	0·1	Lycopin carotene? no xanthophyll	,,	+	+	Very good
Yellow capsicum, fruit	1·0	Carotene, very little xanthophyll	,,	+	+	,, ,,
Winter Cherry, calyx	0·1	All carotene	,,	+	+	Good
No attempt was made in this test to find the minimum dosage for growth.						
Mangolds (flesh of root):						
Sutton's Golden Globe	1	–	–	–	–	Poor
Carter's Red Chief	1	–	–	–	–	None
Carter's Imp. Long red	1	–	–	–	–	,,
Toogood's Golden Tankard	1	–	–	–	–	,,
Swede (flesh of root):						
Sutton's Red Queen	1	–	–	–	–	,,
Cucumber, fleshy part of fruit	1	Little carotene and xanthophyll	+	+	+	Slight
Cauliflower, inflorescence	1	–	–	–	–	None
Shasta daisy, corollas of ray florets	0·6	–	–	–	–	,,
Purple aster, corollas of ray florets	0·8	–	–	–	–	,,

(The colour reactions were obtained by drying a little of the light petroleum solution on a glazed tile and adding one drop of the acid. The colour was observed at the edge of the drop of acid before charring took place.)

Fucus, on the other hand, gave a rather stronger solution of lipochromes with light petroleum; carotene or xanthophyll and fucoxanthin (traces only) were identified by means of the spectrum and 30 % HCl reaction, respectively. A further extraction with ether took out far more pigment and gave the carotene spectrum and the fucoxanthin reaction very strongly. This was repeated several times, and always the two lipochromes were obtained. A phase test of the light petroleum solution with MeOH gave a colourless lower layer, so that xanthophyll is not always present, though Willstätter and Stoll [1913] record xanthophyll and no carotene.

Pelvetia behaved like *Laminaria*, containing much fucoxanthin and very little indeed of carotene or xanthophyll.

Thus, although lipochromes are present in two brown seaweeds, fucoxanthin in *Laminaria* which is exposed only at low tide, and fucoxanthin and carotene or xanthophyll in *Fucus*, and both are exposed to a certain amount of sunlight daily, yet neither has apparently produced any vitamin A detectable by feeding tests. The specimens were examined in late summer, when the intensity of the sunlight would have been great enough in other plants, and the exposure to the sunlight would probably have been long enough, at least for the *Fucus*. The penetration of the light rays into the tissues of seaweed must be much hindered by the thickness of the cuticle and it is conceivable that the light may be adequate for the process of carbon assimilation but not adequate or not of the right quality for the formation of vitamin A.

An exception, unexplainable at present, was found in the pollen of *Lilium candidum*. This contained large quantities of carotene (no xanthophyll by the phase test) which gave a typical spectrum and the usual lipochrome reactions. The ripe pollen from four stamens was rubbed on to a small piece of diet each day and given to each test rat. A week of this test gave very little growth and the pollen began to decompose; the remainder was pounded up with anhydrous sodium sulphate extracted with light petroleum and the extract evaporated down, mixed with a little inactivated olive oil and a drop of this solution (about 50 %) used as the daily ration. This resulted in no further growth and it was decided that the pollen was probably inactive, but the test will be repeated next summer (Table II).

The association of vitamin A with lipochromes in tissues not under the influence of light.

The classic example of this association is the root of the carrot, which has long been known to contain appreciable quantities of vitamin A. Its lipochromes are almost entirely carotene. The sweet potato is also reported by Steenbock as containing both carotene and vitamin A, and yet these two structures are almost completely in the dark, during their growing period. It does not seem probable that their subsequent exposure to variable and often very dull light after removal from the soil would bring about the formation of the relatively large quantities of vitamin A reported in these tissues.

A confirmation of this association in the carrot was made on the unsaponifiable fraction obtained in the usual way, and at the same time the corresponding fraction from turnips was prepared. The carrot extract used in a dosage of 0·01 g. produced very definite growth, the turnip extract produced none at all (Table II).

The question arose whether it was possible that the vitamin had been formed *in situ* in the root of the carrot or whether it had been transported

there from the leaves, where it is known to occur (earlier references). It is, of course, impossible to cut off the current of organic matter passing down to the root of the plant without at the same time preventing the development of the root; and, although not a strictly comparable process, it was decided to see whether the vitamin in the root could be transported upwards to leaves grown from carrots in the dark. Carrots were therefore cut off about an inch from the base, the cut ends placed in water and allowed to sprout. The leaves grew with very long stems, often 5 ins. long, and very small blades, never more than $\frac{3}{8}$ in. in diameter and mostly less. The age of these shoots could not be controlled as the age of the etiolated seedlings had been, so that the material used for the feeding test was from 3 to 5 weeks old. A dose of 0·1 g. of this fresh material made no impression on the growth of the test animals, but 0·2 g. produced a definite resumption of growth. A contrast to this result was obtained by testing 0·2 g. of the leaves of turnips sprouted in the dark: this dosage gave slight growth in one rat, none at all in three (Table II). The shoots of the carrot contained carotene, as shown by the spectrum, phase test, and lipochrome reactions, and no xanthophyll; the turnip shoots contained no lipochrome. Hence, it appears that the vitamin can be transported upwards at least, and when this occurs carotene seems to be transported also. Vitamin has been found in the green stems of polyanthus narcissus flowers (Tables II and III), but the stems contained lipochromes, the epidermis had stomata and assimilatory tissue and hence was comparable to the leaf of the plant. Hence, until more accurate investigation of the different regions of these stems has been carried out, it is not possible to draw any conclusion from the last observation.

The chief point in the formation of the vitamin which is apparent from these experiments is that some lipochrome (generally carotene) is always associated with the vitamin in plant tissues; and that where carotene is found, particularly carotene exposed to sunlight, there the vitamin may be expected to be present also.

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