lyzed separately. These results show the wide variations that may occur within the Porto Rico variety and emphasize the need for care in the selection of propagating stock. No relationship between weight, length, or circumference of the root and the total pigments, carotene, or the carotene/total-pigment ratios was evident in this variety.

TABLE 2

THE CAROTENE AND TOTAL PIGMENTS OF INDIVIDUAL ROOTS OF PORTO RICO SWEET POTATOES

	Total pigments mg./100 grams	Carotene mg./100 grams	Ratio $\frac{\text{carotene}}{\text{Total pigments}} \times 100$
Individual roots	6.95	6.39	91.94
	6.32	5.52	87.34
	5.88	5.05	85.88
	5.66	4.75	83.92
	5.48	4.60	83.94
	4.94	4.13	83.60
	4.81	3.90	81.08
	4.77	3.99	83.64
	4.64	3.90	84.05
	4.47	3.85	86.13
	4.32	3.76	87.04
	4.32	3.65	84.49
	4.21	3.43	81.47
	4.17	3.49	83.69
	4.13	3.51	84.98
	4.13	3.34	80.87
	4.03	3.41	84.61
	3.97	3.26	82.11
	3.92	3.31	84.44
	3.91	3.13	80.05
	3.72	3.11	83.60
	3.70	3.03	81.89
	3.64	2.89	79.39
	3.25	2.56	78.77
	2.82	2.25	79.79
	2.78	2.01	72.30
	2.10	1.46	69.52
Portions of same root			
stem end	6.75	6.08	90.07
center	4.46	3.87	86.77
root end	3.44	2.36	68.60
stem end	5.54	4.59	82.85
center	3.98	3.38	84.92
root end	2.76	2.08	75.36
stem end	5.43	4.72	86.92
center	3.82	3.28	85.86
root end	2.97	2.30	77.44
stem end	3.15	2.39	75.87
center	2.26	1.66	73.45
root end	1.43	.76	53.15

The position of the other pigments on the chromatogram and the tenacity with which they were held indicate that they are different from those known to have provitamin A activity. Neo-β-carotene B or any other carotene of known provitamin A value would probably be carried down in the carotene fraction as here prepared.

Discussion. Kemmerer and Fraps (*2*) have reported that carotene prepared by the usual methods, including that reported here, may contain several fractions, some of which may have no biological activity. Their method calls for adsorption on a column of calcium hydroxide. While suitable for the separation of the various carotene isomers, it is not adapted to rapid routine analysis because of the time-consuming elutions involved.

Kemmerer, Fraps, and Meinke (*3*) have recently reported that the "crude-carotene" of raw sweet potatoes (variety not named) contained, besides β-carotene, neo-β-carotene B and "impurity A." They also found 2 per cent of neo-β-carotene U in one of the five baked samples tested and up to 27 per cent (average, 5 per cent) of this isomer in dehydrated sweet potatoes. No α-carotene was found in any of the samples. "Impurity A" has little if any biological value. Neo-β-carotene B has one-half that of β-carotene, and neo-β-carotene U was reported by Kemmerer and Fraps (*2*) as of no biological value, but Deuel, *et al.* (*1*) reported it as having 38 per cent of the value of β-carotene. According to Kemmerer, Fraps, and Meinke (*3*) the total biological activity of the "crude carotene" in the three samples of raw sweet potatoes tested was equivalent to 88 per cent β-carotene, and in the baked and in the dehydrated samples it was 76 per cent. Whether these figures are representative of all varieties is not known. Kemmerer and Fraps (*2*) reported earlier that both carotenoid X (neo-β-carotene U) and α-carotene were present in fresh sweet potatoes (variety not named) to the extent of 5.4 and 1.4 per cent, respectively, but in a later paper (*3*) reported neither to be present.

Summary. The fleshy roots of the sweet potato are shown to contain appreciable amounts of yellow pigments other than β-carotene. The carotene/total-pigment ratio varies among different varieties and within varieties. In the varieties tested, the carotene/total-pigment ratio increased with increase in intensity of yellow color. Triumph, a very light-colored variety, contained a small amount of carotene shortly after harvest, but this soon disappeared in storage.

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Amino Acid and Protein Deficiencies as Causes of Corneal Vascularization: A Preliminary Report[1]

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The appearance of vessels in the cornea of rats deficient in tryptophane and lysine was first described

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by Totter and Day (*9*). Albanese and Buschke (*2*) have since observed corneal vessels in the eyes of tryptophane-deficient rats, and recently Albanese (*1*) showed that these vessels were not affected by the administration of riboflavin. We (*5*) have confirmed and extended the observations of Totter and Day with regard to the corneal vascularization which appears in lysine deficiency. This also was neither prevented nor affected by large amounts of riboflavin.

A considerable variety of conditions result in corneal vascularization. In addition to those causes mentioned above, corneal vascularization may result from riboflavin deficiency, vitamin A deficiency, zinc deficiency, thallium poisoning (*3*), or tyrosine poisoning (6). Our observations as well as the observations of others indicate that the appearance of corneal vessels is not a universal finding in all deficiencies, though there seem to be causes of corneal vascularization other than those listed above. Machella and McDonald (*8*) report a series of patients who showed the accepted clinical picture of riboflavin deficiency including corneal vascularization, but none of whom showed any marked improvement on treatment with riboflavin. Lyle, Macrae, and Gardiner (*7*) determined the degree of corneal vascularization in about 4,000 RAF personnel at 10 stations in England and 12 stations overseas. Of the vascularization observed, some was apparently due to riboflavin deficiency and responded to treatment with this factor. However, their experiments suggested that other factors present in fruits and vegetables influenced the vascularization more than riboflavin. No definite evidence of improvement was found following treatment with the other pure vitamins tried, but the most definite benefit was obtained where the diet was supplemented by a good variety of nutritious foodstuffs. They thought that the degree of corneal vascularization in their groups of subjects was a reliable index of the general state of nutrition of the group, although it seemed that the corneal vascularization of some of the subjects was due to causes other than nutritional deficiency since there was no response to a superior diet.

Recently we have investigated the effect of methionine deficiency in the production of corneal vascularization. Of 44 rats from 8 litters of Wistar rats placed on methionine-deficient diets[2] at weaning or shortly after, 8 developed corneal vessels seen with the biomicroscope. This observation was confirmed by examination of histological sections of the eyes of some of the rats. The rats were fed daily from amber glass stock bottles of the diet which were kept in the cold. The average daily riboflavin intake of the four rats on the methionine-deficient diet calculated from food consumption was 70 μg. per rat. Since 30 μg. of riboflavin per week protects rats against riboflavin-deficiency cataracts (*4*), this would appear to be an ample riboflavin intake.

Since corneal vessels seem to result in rats on lysine-, tryptophane-, and methionine-deficient diets, it occurred to us that the development of corneal vessels might be a general effect resulting from a deficiency of protein or amino acids. Accordingly, we placed a litter of four Wistar rats on a protein-free diet[3] when they were 30 days of age. Although some eye changes such as a moderate degree of corneal opacity resulted, no vessels were observed with the biomicroscope. The animals died in an average of 31 days after they were placed on the diet. We have found that with severe nutritional deficiencies the animals may die before the corneal vessels appear, even though the vessels do appear when the deficiency is less severe, so a litter of three Wistar rats 41 days of age was next placed on the same diet. Definite invasion of the cornea by vessels was observed with the biomicroscope between the fourteenth and sixteenth days on the diet in two out of three rats. When the two rats died after 36 days on the diet, vascularization and other corneal changes were well advanced. The third rat at no time showed any corneal vessels.

Since that time 15 Wistar rats from seven litters have been placed on the same protein-free diet and 11 control rats from the same seven litters were fed with a diet containing protein.[4] These rats varied in age from 50 to 62 days at the time they were placed on the diets. In from 9 to 20 days, all the rats showed definite invasion of the cornea by vessels on biomicroscopic examination, and none of the control rats over a period of six weeks have shown more than normal variation on biomicroscopic examination of their eyes. These findings have been confirmed by histological section of the eyes of some of the rats. The riboflavin intake of the rats on the protein-deficient diet as calculated from the feed records averaged 98 μg. per rat per day.

As Buschke (*3*) has pointed out, the rat seems particularly susceptible to vascularization of the cornea, and as a consequence, the question of the applicability of studies of this sort to human nutrition is a matter of interest. In this connection one of us[5] has made the following observations: In May 1945, a biomicroscopic examination was made of some 200-odd in-

[2] The composition of the methionine-deficient diets was: cottonseed oil, 30 grams; cod-liver oil, 20 grams; salt mixture, 40 grams; choline, 2 grams; calcium pantothenate, 20 mg.; thiamin chloride, 4 mg.; pyridoxin, 4 mg.; riboflavin, 16 mg. The diets contained 90, 100, or 110 grams of vitamin-free casein and sufficient sucrose to make a kilo. The salt mixture is as used by J. M. McKibben, *et al. Amer. J. Physiol.*, 1939, **128**, 102.

[3] Same as methionine-deficient diet except that sucrose was added in place of casein.

[4] Same as methionine-deficient diet except that it contained 240 grams of the casein.

[5] V. P. Sydenstricker.

habitants of Leyden, The Netherlands, who were selected at random from the population. Ten per cent were suffering from famine edema. None of the individuals examined showed corneal vessels.

From this, it would appear that while deficiencies of any of three different indispensable amino acids or of protein may result in corneal vascularization in the rat, further investigation is necessary before the significance of these findings with reference to human nutrition becomes clear.

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Dehydroascorbic Acid in Cabbage

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In analyzing school lunches for ascorbic acid content using the method of Roe and Oesterling (*3*), dehydroascorbic acid was found to be present in appreciable amounts. The average content for 55 plate lunches was 14.8 mg. of reduced ascorbic acid and 5.9 mg. of dehydroascorbic, giving a total of 20.7 mg. (*2*). Thus, there was 40 per cent more ascorbic acid present than would have been accounted for by the usual indophenol procedure.

These findings prompted us to question whether the reported losses of ascorbic acid in cabbage, when prepared as salad and allowed to stand, could be accounted for, at least partially, as dehydroascorbic acid. The destructive effect of metallic catalysts and time of chopping have been noted by several investigators, but in few studies has the reversibly oxidized form of the vitamin been reported. The method of Roe and Oesterling is sensitive to small amounts of ascorbic acid and is not open to some of the criticisms of the hydrogen sulphide method.

Values for fresh cabbage were obtained from a wedge cut from the intact head and immediately immersed in acid and then ground in a Waring blendor. The remainder of the head was cut within five minutes, either with a knife or a hand shredder, and samples taken for analyses at regular intervals. Reduced ascorbic acid was determined by the method of Loeffler and Ponting (*1*) and dehydroascorbic acid and total ascorbic acid by the diphenylhydrazine procedure of Roe and Oesterling (*3*).

Typical results are given in Table 1. When the

TABLE 1

Ascorbic Acid Content of Cabbage After Cutting and Shredding (mg./100 grams)

Flat Dutch Cabbage	Knife			Shredder		
	Reduced	Dehydro	Total	Reduced	Dehydro	Total
Before cutting	47.3	4.3	50.1	47.6	6.1	53.1
Immediately after cutting	41.5	10.4	49.4	35.5	15.1	52.2
15 minutes after cutting	40.6	10.8	46.3	35.7	17.1	52.5
30 minutes after cutting	40.4	9.1	46.1	34.9	15.7	51.5
60 minutes after cutting	41.1	8.8	49.2	35.3	15.6	52.5
120 minutes after cutting	40.9	8.5	47.2	36.3	14.3	50.6

cabbage was chopped with a knife there was a 13.5-per cent loss of reduced ascorbic acid after standing 120 minutes. However, the values for dehydroascorbic acid indicated that this was not a true loss but that a large portion was changed to the reversibly oxidized form. The total ascorbic acid at the end of 120 minutes was 5.8 per cent less than at the beginning; thus, there was a small destruction of the vitamin during this holding period. Shredding with a hand grater caused a total destruction of 4.7 per cent of the vitamin; with this method of preparation there was an increase in the amount converted to dehydroascorbic acid.

The average values for all heads of cabbage are shown in Table 2. There was a 5-per cent loss of total

TABLE 2

Per Cent of the Total Original Ascorbic Acid Present as Reduced, Dehydro- and Total Ascorbic Acid

Treatment	Knife cut (4 heads)			Shredded (5 heads)		
	Reduced	Dehydro	Total	Reduced	Dehydro	Total
Before cutting	99.9	8.4	100.0	92.9	10.3	100.0
After cutting	88.0	10.9	96.3	67.7	30.7	97.0
15 minutes after cutting	86.8	20.0	97.2	66.1	32.5	95.2
30 minutes after cutting	87.7	19.4	93.8	65.5	29.9	96.1
60 minutes after cutting	87.5	17.9	97.2	66.2	29.2	95.2
120 minutes after cutting	89.3	16.6	98.4	67.6	26.8	95.1

ascorbic acid when shredded and held for 120 minutes. The maximum loss occurred in the first 15 minutes and did not increase on standing.

When cabbage was cooked by boiling for 12 minutes and then held on the steam table for two hours, there was marked destruction of the vitamin. Only 25 per cent of the total ascorbic acid present in the freshly cooked cabbage was retained, and 50 per cent of the