determinant and a tendency for color thresholds to remain constant under varied environmental conditions, indicated that the defect probably would not respond to vitamin treatment. However, until just ten years ago, we were not fully aware of the intimate and essential role of vitamin A in the normal function of the rods. The discovery of the relationship between vitamin A and rhodopsin could suggest that it might also be required in some similar but unknown way by the cones.

Following the first report by Dunlap and Loken, some preliminary observations were made on 16 college students who had defective color vision. Most of them had failed to pass tests of the Army Air Corps, but were quite anxious to do so. Three tests were used with this group: the Ishihara, the American Optical Company's pseudoisochromatic plates and the Westcott lantern slide, which is a modification of the yarn test principle for the purpose of group testing. Vitamin A (as purchased locally in the form of concentrated fish oil) was given to these subjects in doses of 25,000 units daily for eight weeks or more. One of this group took 250,000 units daily on prescription of a local physician. Fourteen of these cases, including the one just mentioned, showed no improvement, but two of them finally achieved almost perfect scores. Both of these subjects subsequently passed the Army Air Corps tests and are now training in that service.

Had all these preliminary tests been negative, it is unlikely that further observations would have been made, but it seemed difficult at the time to account for the improved performance of these two individuals except as being a result of vitamin A treatment. In the light of the results reported below, it may be necessary to accept another hypothesis. It should be said, however, that the original defect in these two cases was of slight degree.

In order to check the possibility that some benefit could be derived from vitamin A by a few individuals, some extensive observations were made under more rigid conditions. Group tests of 897 R.O.T.C. freshman cadets at Louisiana State University showed 65 who had various degrees of weakness in color sensitivity. Individual tests were then given to 58 subjects who began taking 50,000 units of vitamin A on alternate days. This schedule was continued for eight weeks. After having taken 1,400,000 units, each subject was retested under the same conditions as before.

Because there are many reasons why subjects, especially those who are not volunteers, might fail to follow instructions for taking vitamin A, it was considered important that this part of the test be carefully supervised. Accordingly, the subjects were required to swallow the capsules at regular hours at a dispensing station.

No significant improvement in color sensitivity was shown by any individual in the group of 41 who finished the eight weeks period of treatment. Most of the records of response to the 62 plates of the American Optical and Ishihara tests were practically identical before and after taking vitamin A. The maximum improvement shown by any individual was a correction of three previous errors. The lantern slide test gave essentially the same results, although there was more variability in the responses. An analysis of the reliability of these tests and their value as a convenient means of detecting color "blindness" will be presented in a later report.

The procedure in the present experiment differed from that of Dunlap and Loken in a few respects. We used a large number of subjects of approximately the same age (median, 17 years, 9 months) and living under very similar conditions throughout the period of testing. We are able to assert positively that all subjects actually took vitamin A, because this was done regularly in the presence of the experimenter or an associate. The material used was a vitamin A ester of high potency, determined spectrographically and confirmed by bio-assay.

It may be concluded from these tests that vitamin A in doses of 25,000 I.U. daily for eight weeks fails to produce any significant improvement in color sensitivity. It seems improbable that administration of the vitamin for longer periods of time would change this result, although observations are being continued on several subjects.

Murray[4] warns against the unfortunate consequences which could follow acceptance of vitamin cures for color deficiency until the permanency of results is thoroughly tested. The present study does not entirely dispose of the possibility that a few men, perhaps with minor color vision defects, may improve slightly; but the number who could use vitamin A for this purpose is so small as to be negligible. We need not, therefore, be concerned about the numbers who can pass the test temporarily.

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VITAMINS IN DEHYDRATED SEEDS AND SPROUTS

THE common use of sprouted seeds in the diets of oriental peoples appears to rest on a sound nutritional basis, if we are to judge by the vitamin content of such food materials. It has already been reported that significant increases in the concentration of riboflavin, nicotinic acid and biotin occur during germination of

many kinds of edible seeds.[1] Wheat and barley show increases of thiamine during germination, but several other species appear not to change appreciably in vitamin B_1 content. The present brief report summarizes certain earlier data obtained on cereals, and presents some new observations for pantothenic acid, pyridoxine, folic acid and inositol in seeds and sprouts of several common species of edible plants.

The general methods of investigation which were reported earlier have been continued in this work. Seeds were germinated at 25° C in a greenhouse or in peat moss and after 5 or 6 days the whole plants, including seed, shoot and root, were harvested, washed clean and dehydrated at 70° C. The dormant seeds were dried in a similar manner. All dried samples were ground in a small Wiley mill, and aliquots were taken for assays. For determinations of pantothenic acid, pyridoxine, folic acid and inositol, 0.5 gm of dry material was placed in 30 ml of buffer solution at pH 4.5. The buffer solution contained 3.75 gm glacial acetic acid and 5.0 gm anhydrous sodium acetate per liter. Twenty milligrams of papain and 20 mg of Taka-diastase were added to each half gram sample, and the mixture was incubated at 37° C for 24 hours. A few drops of benzene were used to inhibit growth of microorganisms. The digested material was heated in steam at 100° C for 30 minutes, made up to a volume of 50 ml, filtered with Super-cel in a Büchner funnel and subsequently extracted twice with ether. The filtration and ether extraction were adopted for the purpose of removing fatty substances which might have interfered with the microbiological assays.[2] Pyridoxine was assayed with a yeast growth method according to a procedure developed in the Yale laboratories.[3] The other vitamins were tested in accordance with methods published by R. J. Williams *et al.*[4] The losses in dry matter which occurred during germination were determined for the purpose of making certain calculations regarding the change in vitamin content. Vitamin values of the plant materials were corrected for errors introduced by the presence of small amounts of vitamins in the enzyme preparations.

A summary of the data obtained for four kinds of plants is presented in Table I. The ratio of dry matter in the dormant seeds to that found in 6-day-old sprouted seeds ranged in the different species from 1.06 to 1.33, indicating some loss in dry material in metabolic processes accompanying germination. The vitamins expressed as micrograms per gram of dry matter, show much greater gains in sprouting seeds than can be accounted for on the basis of increased concentration through loss of dry matter and mere maintenance of vitamins stored in the seeds. The data presented for riboflavin, nicotinic acid, biotin and thiamine are averages taken from experimental work reported in a previous paper. The observations on pantothenic acid, pyridoxine, folic acid and inositol, reported here for the first time, offer further evidence for the increase of vitamins during germination. It appears also that considerable differences in content of these vitamins exist among species. In addition to the kinds of plants listed in the table, certain others were also studied. Germinated peas and buckwheat showed gains in pyridoxine and folic acid ranging from 3 to 10 fold and smaller increases in pantothenic acid.

TABLE I

VITAMIN CONTENT OF DORMANT AND SPROUTED SEEDS. MICROGRAMS PER GRAM OF DRY MATTER*

	Oats		Wheat		Barley		Corn	
	Dormant	Germinated	Dormant	Germinated	Dormant	Germinated	Dormant	Germinated
Dry matter mg per seed	19.3	14.8	28.5	21.4	35.6	30.2	315.0	271.3
Riboflavin	0.8	11.6	1.3	5.4	0.9	7.2	1.1	4.3
Nicotinic acid	7.5	44.0	62.0	103.0	67.5	115.0	9.5	39.5
Biotin	0.9	1.4	0.17	0.36	0.31	0.91	0.21	0.54
Pantothenic acid	7.6	21.9	7.6	12.6	5.4	10.0	4.2	7.7
Pyridoxine	0.3	1.8	2.6	4.6	0.2	0.5	0.7	0.8
Folic acid	22.0	143.0	28.0	106.0	14.5	50.0	10.0	45.0
Inositol	630.0	1290.0	1460.0	2100.0	1240.0	1370.0	800.0	1640.0
Thiamine	11.3	12.2	7.0	9.0	6.8	9.0	5.5	5.1

*The values are calculated as riboflavin, nicotinic acid, biotin methyl ester, calcium pantothenate, pyridoxine HCl, inositol and thiamine HCl. Folic acid is expressed as micrograms of concentrate having a potency of 3100, according to Dr. R. J. Williams, who so kindly supplied this vitamin material.

It seems probable that not all species may be expected to exhibit such increases in vitamin content as are indicated for the sprouting cereals. It would be desirable to have these preliminary determinations, which are based entirely upon microbiological assays, checked with other methods. The data obtained thus

[1] Paul R. Burkholder and Ilda McVeigh, *Proc. Nat. Acad Sci., U.S.A.*, 28: 440, 1942.

[2] F. M. Strong and L. E. Carpenter, *Ind. Eng. Chem. Anal. Ed.*, 14: 909, 1942.

[3] Paul R. Burkholder, *Amer. Jour. Bot.*, 30: 206, 1943.

[4] R. J. Williams, University of Texas Publication No. 4237, 7, 1942.

far strongly support the view that many seeds gain in vitamin content during germination. Of considerable importance for animal and human nutrition is the fact that the vitamins which appear to be synthesized in sprouting seeds are preserved during subsequent dehydration.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE CONSTRUCTION OF TISELIUS ELECTROPHORESIS CELLS

INASMUCH as the attention of manufacturers of optical equipment is at present directed almost exclusively toward war production, the procurement of suitable cells for the electrophoresis apparatus of Tiselius has become a rather difficult problem. Because of the wide application of the Tiselius instrument to problems of biological and colloid chemistry it was thought that the experiences of the authors in constructing these cells might prove to be of general interest. The methods should also prove applicable to the construction of other types of glass cells.

The cells have been constructed of one eighth inch thick color-clear plate glass. After a little practice no difficulty was encountered in grinding the glass parts to the proper size on the face of a rotating iron disc fed with carborundum and water in the usual way. The rectangular holes in the horizontal plates were cut by grinding through the faces of the plates from both sides with the edge of a small iron disc mounted in a lathe and fed with carborundum. In this operation the glass was mounted on a plate hinged to the compound tool rest of the lathe. The holes were then squared up by hand with carborundum and a strip of metal. The horizontal sliding surfaces were ground flat on plate glass after the sections were assembled. The center sliding section was of the double length design described by Longsworth, Cannan and MacInnes.[1] It was assembled in two steps. First, the rectangular tubes were cemented and ground on the ends until square and of equal length. During the grinding the tubes were temporarily fastened together with beeswax. Second, the tubes and horizontal plates were assembled and cemented. The top and bottom sections were each cemented in a single operation.

The principal difficulty was, of course, the cementing of the glass parts. Numerous cements of various types were tried without success until a low-melting glass-like material described by von Angerer[2] was used. The cement is made by fusing together 5 parts of washed silicic acid, 16 parts of red lead (minium, Pb_3O_4) and 4 parts of calcined borax, using a blast lamp furnace. While still molten, the material was poured out into water, dried, ground fine in a Mullite mortar and put through a 500-mesh screen. The powder was mixed with water to form a thin paste, which was applied evenly with a brush to the surfaces to be cemented. After the cement dried the parts were assembled in a suitable steel jig, using weights to apply pressure to the joints, placed in an electric muffle furnace, and heated to about 500° C—approximately one hour was required for the furnace to reach this temperature. The proper temperature imparts a slight glow to the furnace, perceptible only in a darkened room. After three hours the furnace was turned off and allowed to cool, about six hours being required for it to reach room temperature. In designing the jig it was found that any metal part which touches the glass over any considerable area should be in contact with the entire glass surface, otherwise the metal conducts heat to local areas and cracks the glass. The joints frequently contain numerous small bubbles, but seem to be essentially as strong as the glass itself. If the cement has been applied evenly a tight seal is obtained.

Although no further polishing of the optical surfaces was attempted, the optical properties of the tubes have been found to be quite satisfactory. No irregularities could be observed in the base lines produced by the cells even though the Tiselius apparatus in use in this laboratory is a rather sensitive one. The joints have been found to be permanent and substantial. Preliminary experiments have indicated that pyrex glass may also be cemented by the same method.

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[3] Fellow of the National Research Council.

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