extract and the autolyzed tissue were subjected to several extractions with pure ether in an atmosphere of nitrogen. The ether extracts were combined, washed with water and dried over anhydrous sodium sulfate.

An absorption spectrum of the deep yellow ethereal solution in the visible spectrum showed bands at 420, 442, 478 and 655 mμ, respectively. In petroleum ether the same extract showed bands at 420, 444, 472 and 656 mμ, respectively, while the antimony trichloride color in chloroform showed prominent bands with maxima at 420, 495 and 610 mμ, respectively.

The sample was then saponified in an atmosphere of nitrogen, the non-saponifiable fraction taken up in ether, the ethereal solution filtered at 0° and an absorption spectrum taken of the filtrate in the visible region of the spectrum. It showed bands at 446 and 474 mμ, respectively. The spectrum of the antimony trichloride color showed bands at 410, 440, 495, 620 and in one case at 690 mμ, respectively. An ultraviolet absorption spectrum of this sample in ethyl alcohol is shown plotted in Fig. 1. A maximum is readily

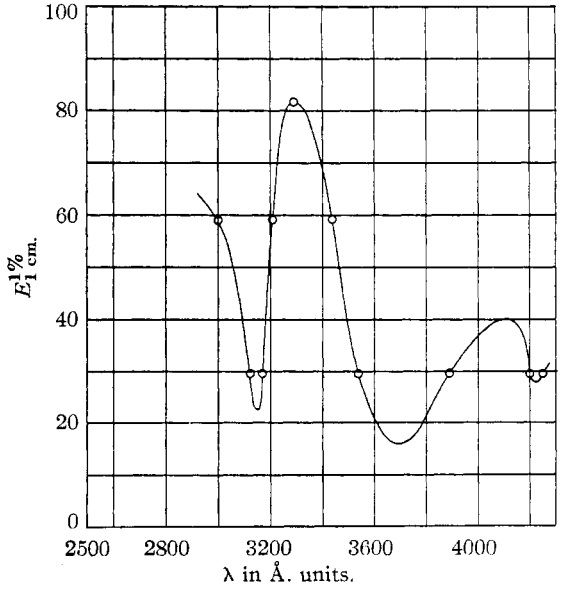

Fig. 1.

seen at 328 mμ which together with that of the antimony trichloride at 620 mμ is characteristic of vitamin A_1. The band of the antimony trichloride color at 690 mμ may be due to the presence of vitamin A_2, although the appearance of this band was not consistent. From the intensity of the maximum at 328 mμ and the concentration of the solution, we calculated $E_{1\text{ cm.}}^{1\%}$ to be 82 for our sample which, on the basis of the purest vitamin A having a potency of 3,250,000 U. S. P. vitamin A units per gram [Milas and Heggie, unpublished results] would have a potency of 116,000 U. S. P. vitamin A units per gram. In another sample taken from a single steer head we found a potency of about 76,000 U. S. P. vitamin A units per gram.

The bands of the visible spectrum at 420, 442–446, 472–478 mμ, respectively, are due to carotenoids. We have not as yet identified the other bands reported in this paper and inasmuch as we are continuing with our work, we are hoping to report a more complete account of it later.

CONTRIBUTION NO. 193 FROM THE
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RECEIVED JUNE 19, 1939

THE FORMATION OF α-ETHYLTHIOGLUCOPYRANOSIDE FROM GLUCOSE ETHYLMERCAPTAL

Sir:

It has been found two years ago [Green and Pacsu, THIS JOURNAL, **59,** 1205 (1937)] that α-ethyl- and α-benzylthioglucosides [Schneider, *et al.*, *Ber.*, **49,** 2054 (1916); **51,** 220 (1918); **61,** 1244 (1928)] are furanosides and not of "normal" (pyranoid) structure as their discoverers believed. This was shown by acid hydrolysis constants, conversion into ethylglucofuranoside, and calculations from Hudson's rules of isorotation. On account of certain irregularities observed by Green and Pacsu during the process of hydrolysis, the behavior of α-ethylthioglucofuranoside in 0.01 *N* hydrochloric acid at 100° was subsequently studied [Pacsu and Wilson, THIS JOURNAL, **61,** 1450 (1939)]. It was found that in this unprecedented hydrolysis about one-half of the α-ethylthioglucofuranoside changed into glucose and mercaptan whereas the other half escaped the hydrolyzing effect of the acid by shifting the furanoid ring into the acid resistant pyranoid ring. The ring shift resulted in the formation of α-ethylthioglucopyranoside, a new thioglucoside, which was isolated and characterized by its tetraacetate. In a recent paper [Brigl, Gronemeier and Schulz, *Ber.*, **72,** 1052 (1939)] which was submitted for publication one month later but appeared one month earlier than the article of Pacsu and Wilson, Brigl and co-workers re-

ported the preparation of the same α-ethylthioglucopyranoside and its tetraacetate. These authors proposed to use glucose ethylmercaptal for disaccharide synthesis and mixed the mercaptal in 22% hydrochloric acid with glucose. Instead of the desired disaccharide Brigl and co-workers obtained this α-ethylthioglucopyranoside in an apparently undetermined yield. As to the mechanism of the reaction the authors stated that the mercaptal lost one mercaptan residue which was partly transferred to the admixed glucose, the whole process being represented by the following two-stage reaction:

(a) $C_6H_{12}O_5(SC_2H_5)_2 = C_6H_{11}O_5(SC_2H_5) + C_2H_5SH$
(b) $C_2H_5SH + C_6H_{12}O_6 = C_6H_{11}O_5(SC_2H_5) + H_2O$

Since we had reasons to believe that this mechanism might not be the correct one, we repeated Brigl's experiment with the modification that we *omitted* glucose. From the reaction mixture we obtained α-ethylthioglucopyranoside in about 20% minimum yield. Also, we obtained the same compound but in somewhat smaller yield (15%) from the reaction of equimolecular quantities of glucose and ethylmercaptan in 22% hydrochloric acid. In our first experiment there was but a mere trace of glucose present in the acetone insoluble residue consisting mainly of barium chloride, whereas in the second experiment, when glucose was used as starting material, the acetone insoluble salt contained a fairly large quantity of unchanged glucose. In both instances the lower rotating component of the reaction mixture represented probably the acid resistant β-ethylthioglucopyranoside, since in neither case could we find glucose ethylmercaptal. These results seem to indicate that the formation in 22% hydrochloric acid of α-ethylthioglucopyranoside from the mercaptal on one hand, and from glucose and mercaptan on the other, are two distinct and independent reactions.

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RECEIVED JUNE 20, 1939

VITAMIN B_6, A GROWTH PROMOTING FACTOR FOR YEAST

Sir:

The rate of proliferation of *S. cerevisiae* in purified solutions is known to be profoundly affected by a group of substances known as bioses. The multiple nature of bios is firmly established and further evidence of the multiplicity of bios was found in the discovery of the bios action of thiamine [Schultz, Atkin and Frey, THIS JOURNAL, **60,** 490 (1938)].

We have now found that crystalline vitamin B_6 has the properties of a bios factor. This substance acts on the yeast types A and B in an advantageous manner. The work with crystalline B_6 was made possible by the gift of a few milligrams by Merck and Company.

TABLE I

TWENTY-FOUR HOUR GROWTH OF YEASTS A AND B AS INFLUENCED BY VITAMIN B_6, ETC.

Total volume in each case: 30 ml. (seeded with 1 mg. of moist yeast and rocked at 30° for 24 hours). Crop × 4.54 gives mg. of moist yeast. Supplements: inositol (1) 1 mg.; β-alanine (IIA) 0.005 mg.; bios IIB 0.13 mg.; thiamine 0.01 mg.; vitamin B_6 (VI) 0.05 mg.

Ingredients of bios tests: all C. P. sugar, salts, buffer, I and IIA, plus	24-Hour crop Type A	Type B
Nil	Trace	40
II B	15	210
II B plus thiamine	100	120
Vitamin B_6 (VI)	Trace	40
II B plus Vitamin B_6	150	200
II B plus B_6, plus thiamine	170	200

The properties of crystalline B_6 are: (1) stimulation of Type A yeast to produce a 24-hour crop of 100–120; (2) removal of the inhibition imposed on Type B yeast by thiamine; (3) stimulation of Type A yeast to give a high 24-hour crop in the absence of thiamine.

Crystalline vitamin B_6 was found to have a certain activity as a fermentation accelerator under the conditions of our fermentation test [Schultz, Atkin and Frey, THIS JOURNAL, **59,** 2457 (1937)]. The stimulation is of about the same type as the nicotinic acid effect [Schultz, Atkin and Frey, *ibid.*, **60,** 1514 (1938)] and may be overcome in the same way, *i. e.*, by adding about 0.05 mg. of B_6 to each test.

There are indications that the growth method may be useful as a method for the determination of vitamin B_6. A growth test in which all factors except B_6 are present in excess will respond to solutions or concentrates in proportion to their B_6 content as indicated by rat curative tests which were made by R. F. Light and L. J. Cracas of our Laboratory.

THE FLEISCHMANN LABORATORIES ALFRED S. SCHULTZ
STANDARD BRANDS INCORPORATED LAWRENCE ATKIN
NEW YORK, N. Y. CHARLES N. FREY

RECEIVED JUNE 10, 1939