TABLE I

SUBSTRATE SPECIFICITY AND KIND OF ACTION ON SUCROSE

Test conditions	Test mixture	Properties of test mixtures after incubation: Opalescence	Serological reactivity[1]	Precipitation with 1.25 volumes of alcohol[2]	Reducing sugars mg/cc	Properties of solutions of the material precipitated by alcohol: Opalescence	Reducing sugars after acid hydrolysis[3] mg/cc
1 part extract plus 20 of substrate; incubated 9 days at 23° C.	sucrose + extract	+++	1000	++++	3.05	+++	2.76
	" + heated extract	0	1	0	.05	0	.00
	extract + water	0	1	0	.01	0	.00
	sucrose + water	0	0	0	.04	0	.00
	raffinose + extract	0	5	±	.20	0	.03
	other sugars[4] + extract	0	1	0	–	0	.00
1 part extract plus 1 of substrate; incubated 2 hours at 37° C.	sucrose + extract	+	150	++	.62	+	.52
	" + heated extract	0	10	±	.05	0	.06
	extract + water	0	10	±	.04	0	.05

[1] Highest dilution which precipitated 1:15 dilution of type 2 pneumococcus and leuconostoc antiserums after 1 hour at 37° C.
[2] Test mixture diluted 1:10 in 10 per cent. sodium acetate before precipitation with alcohol.
[3] No reducing sugars present before hydrolysis.
[4] Lactose, maltose, arabinose, xylose, galactose, dextrose, fructose and mixture of dextrose and fructose.

two representative experiments: in the first 1 part of extract plus 20 parts of various substrates were incubated for 9 days at 23° C. and in the second equal parts of extract and of sucrose substrate were incubated for only 2 hours at 37° C. The substrates were 10 per cent. solutions of the sugars in 0.1 molar acetate buffer pH 5.5 and were sterilized by Berkefeld filtration. The serological tests were made with 1:15 dilution of type 2 pneumococcus and of leuconostoc rabbit antiserums; the specificity of the reactions with those antiserums was controlled with 1:6 and 1:15 dilutions of types 1 and 3 antipneumococcus and normal rabbit serums. All the tests with the control serums were negative and are omitted from the data which are given in Table 1.

It is evident (Table 1) that when the unheated leuconostoc extract was incubated with sucrose there was a development of opalescence, of specific serological reactivity and of material precipitable with 1.25 volumes of alcohol, together with an accumulation of free reducing sugar. The alcohol precipitable material which in other analyses was proved a polysaccharide can be considered as the product responsible for the opalescence and for the serological reactivity of the sucrose-extract test mixture; the reducing sugar found in the same test mixture represents another product of the reaction. It is noteworthy that there was a close correspondence between the amount of free reducing sugars which accumulated in the test mixtures and the amount of reducing sugar obtained by acid hydrolysis of the alcohol precipitated material. This correspondence suggests that the action of the extract upon sucrose is analogous to that which has been proposed for the living leuconostoc bacteria; that is, that X molecules of sucrose are converted into X molecules of fructose plus a dextran polymer of X glucose anhydride units.

The serological specificity of the dextran polysaccharide was indicated in these experiments by the negative reactions given by all the control serums; more detailed data on the close agreement in serological properties between the polysaccharide formed by the sterile extracts and that produced in the usual living cultures will be given in the later paper. The substrate-specificity of the active principle is indicated by the fact that with exception of the slight action upon raffinose, none of the set of phenomena which occurred with sucrose was observed with any of the other sugars which were tested.

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ON VITAMINS IN WHEAT GERM

IN the course of our work on vitamin contents of wheat and bread, we found that wheat germ made from hard spring wheat contains a component, which stimulates the action of yeast to a greater extent than can be explained by its known vitamin contents. This was true even when the vitamins so used were present in much greater amounts than calculated from the known vitamin contents of wheat germ. A,[1] B_1[2] (thiamine), B_2 (riboflavin), PP (nicotinic acid) and E (tocopherol) are known to be present in wheat germ. All these were tried in comparative experiments, although the effects of A and E, both being fat-soluble, seem of no interest in this connection. In

[1] Through the courtesy of American Chlorophyll Company.
[2] Through the courtesy of Merck and Company, Inc.

addition, the following were also used similarly: calcium pantothenate (dextrorotatory),[3] a pyrimidine component of B_1 (2-methyl, 5-ethoxymethyl, 6-amino pyrimidine) and vitamin B_6 hydrochloride.[4]

The wheat germ used was highly purified (about .5 per cent. of wheat grain) and contained per gram 24γB_1 and 7.5–10γB_2.[5] The equipment and general procedure was similar to that already described for oxydase determinations.[6] Reactions were carried out in apparatus similar to that already described.[7] It differed from it in having three compartments, one for the yeast suspension, one for the nutrient solution[8] and one for the vitamin solution or the wheat germ extract.[9] The apparatus was provided with glass hooks, both on the top and bottom parts of the ground joint. Rubber bands held the two parts together and made pressure measurements possible.

1 cc of a .4 per cent. yeast suspension and 1 cc of the nutrient solution were used in all the experiments. Of the vitamin-containing solutions, .2 to 1 cc were used and additional H_2O to bring the volume to 1 cc. The total volume of the apparatus, exclusive of the monometer tube, was 15 cc, leaving 12 cc of air space. Thus at 31.0° C. (the temperature of the thermostat) an increase of 1 millimeter of mercury pressure represents very closely .028 milligrams of CO_2. Rate of shaking was two complete excursions per second. Shaking was interrupted at 15-minute intervals for reading. A typical experiment is given.

Nutrient solution	1.00 cc	1.00 cc	1.00 cc	1.00 cc
Yeast suspension .4 per cent.	1.00 cc	1.00 cc	1.00 cc	1.00 cc
Wheat germ extract .5 per cent.		.20 cc	1.00 cc	
Vitamin A				40 units
Wheat germ oil (E)				.01 cc
Vitamin B_1				1 γ
Vitamin B_2				50 γ
Vitamin PP				10 γ
Additional H_2O	1.00 cc	.80 cc		.60 cc
After 150′ of shaking mm of pressure	72	90	148	78
Mg of CO_2 produced	2.016	2.520	4.144	2.184
Increase due to addition		25 per cent.	106 per cent.	8 per cent.

It appears that wheat germ contains a water-soluble heat-stable component, which stimulates yeast cells to greater activity in a manner usually attributed to vitamins. The effect is much greater than that of other vitamins studied. It may be due to a single or a mixture of essential food factors as yet not recognized.

This effect becomes particularly significant because nutritional authorities are endeavoring to establish certain levels for vitamins in flour and bread. It is expected that flour would contain 1.6 mg of thiamine, 1.2 mg of riboflavin, 10 mg of nicotinic acid and approximately 20 mg of iron per pound.[10] The additions would be made to offset the removal of vitamins by depriving the wheat of the wheat germ. The discovery here outlined may lead to making the reconstitution of the flour more complete.

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[3] Through the courtesy of Dr. R. R. Williams.

[4] Through the courtesy of Dr. K. G. Falk.

[5] Determinations made by Dr. D. J. Hennessey, of Fordham University.

[6] Bul. No. 238, B.P.I., U. S. Dept. of Agriculture, 1912.

[7] *Jour. Biol. Chem.*, XVII, p. 409, 1914.

[8] A. J. Schultz, L. Atkin and C. N. Frey, *Jour. Am. Chem. Soc.*, 59: 2547, 1937.

[9] Catox apparatus can be secured from Palo-Myers, Inc., New York City.

[10] Personal communication from Dr. Russell M. Wilder, chairman of the Committee on Food and Nutrition, National Research Council.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR MAKING SNOWFLAKE REPLICAS

THE exquisite beauty and infinite variety of the snowflake described in the early writings of Tyndall and others was brought to popular attention by the pioneer crystallographic work of Wilson A. Bentley, whose photomicrographs of snowflakes are classic in this field of study.

The technique of photographing snowflakes, while not exceedingly difficult, is rarely attempted because of the equipment needed and the essential factor that camera, slides, microscope and other apparatus must be kept below 0° C.

The technique for preserving snowflakes is extremely simple. A strip of velvet, or other dark material, fastened to a board or table, a few glass microscope slides, a toothpick or wire and a weak solution of a suitable resin are all the equipment necessary. The only other essential detail is an occasional snow flurry. The above-mentioned material is kept outdoors in a sheltered place ready for appropriate precipitation.

As soon as a suitable flake is observed on the dark background a drop of solution is placed on the glass slide with the toothpick or wire, and the flake is lifted with the same tool and placed in contact with the drop.