

Type localities and stratigraphic data have been checked in the field by specialists in nearly all cases and will be given explicitly and in an up-to-date manner. Therefore, the cards will contain much more information than is available in the literature to-day.

There will be running numbers for the whole catalogue, one for each card. These numbers will begin with 1 and continue as long as new catalogue cards are being published. Also, there will be running numbers restricted to each class. Thus the class Gastropoda will have its own numbering apart from the numbers for the entire catalogue. The numbers will make it easy to arrange the cards and to refer to them in the literature.

The following cards are ready for publication:

- Tetrabranchiate Cephalopoda (Nautiloidea)  
28 species on 43 cards
- Gastropoda—Genera Cryptochorda and Lapparia  
12 species on 12 cards
- Gastropoda—Family Turritellidae  
81 species on 81 cards
- Brachiopoda  
27 species on 28 cards

The cards may be obtained from the Bureau of Economic Geology, Austin, Texas. Any further information will be furnished gladly.

H. B. STENZEL,  
*Editor of the Catalogue*

## SPECIAL ARTICLES

### PRODUCTION FROM SUCROSE OF A SEROLOGICALLY REACTIVE POLYSACCHARIDE BY A STERILE BACTERIAL EXTRACT

THIS report deals with the production from sucrose of a serologically reactive polysaccharide by an enzyme or some similar heat labile principle contained in sterile filtered extracts prepared from cultures of *Leuconostoc mesenteroides*. The possibility that polysaccharides possessing serological properties might be synthesized from the proper substrates by enzymes obtainable from appropriate bacteria would seem indicated by the recent proof of the enzymatic syntheses of glycogen and of starch. The production of gum-like material from sucrose by sterile preparations derived from various species of spore-forming bacilli has been reported by others.<sup>1</sup> But in these earlier studies the products were not identified chemically nor tested serologically, whereas in our studies the product formed by the enzyme or active principle has been proved to be an immunologically reactive polysaccharide similar in both chemical and serological properties to the product formed in cultures of the living bacteria.

*Leuconostoc mesenteroides*, which is a Gram-positive coccus widely distributed on plants, seemed especially suitable for the investigation because the production of reactive polysaccharide by these bacteria can be referred to a known constituent of the medium: not only are abundant amounts formed apparently only in the presence of sucrose, but also the polysaccharide product (dextran) has been proved<sup>2</sup> to be composed entirely

of units (glucose anhydride) which sucrose could supply. An additional advantage was that the leuconostoc polysaccharide was not only recognizable by its chemical properties but could also be identified by its capacity to react with the antisera of types 2 and 20 pneumococci<sup>3</sup> as well as with the antiserum of the homologous bacteria.

We have not yet obtained the active principle entirely free of preformed polysaccharide. However, although more highly purified extracts would be desirable, the present ones are adequate to establish the general mechanism of the reaction and to permit the isolation of the purified polysaccharide product for chemical as well as serological study.

During the past 12 months 14 different lots of leuconostoc extract have been tested and all were found to have the capacity to form the reactive material from sucrose. The extracts had been filtered through Berkefeld W candles and had been subjected to rigorous tests for sterility. Aseptic technique was used in the preparation and subsequent handling of all the enzyme-substrate test mixtures, and their sterility was controlled at appropriate intervals during the incubation periods by microscopic examination and by culture in a series of liquid and solid mediums which were known to be adequate for detection of small numbers of leuconostoc bacteria. As a result of these controls we feel certain that the observed reactions occurred in the absence of microorganisms. The results of the tests of the isolated polysaccharide and a description of the preparation of the extracts, together with data on the influences of temperature and of pH and on the serological differences in the products yielded by extracts prepared from different strains of leuconostoc will be given in a later paper now in preparation.

The substrate specificity and the general mode of action upon sucrose can be illustrated by the data on

<sup>3</sup> J. Y. Sugg and E. J. Hehre, unpublished manuscript.

<sup>1</sup> M. W. Beijerinck, *K. Akad. v. Wetensch.*, Amsterdam, Proc. sect. sc, 12: 635, 1910; F. C. Harrison, H. L. A. Tarr and H. Hibbert, *Canadian Jour. Research*, 3: 449, 1930; L. Dienes, *Jour. Inf. Dis.*, 57: 12, 22, 1935.

<sup>2</sup> F. L. Fowler, I. K. Buckland, F. Brauns and H. Hibbert, *Canadian Jour. Research*, 15 B: 487, 1937; S. Peat, E. Schluchterer and M. Stacey, *Jour. Chem. Soc.*, 581, 1939; W. Z. Hassid and H. A. Barker, *Jour. Biol. Chem.*, 134: 163, 1940.

TABLE I  
SUBSTRATE SPECIFICITY AND KIND OF ACTION ON SUCROSE

Test conditions	Test mixture	Properties of test mixtures after incubation				Properties of solutions of the material precipitated by alcohol	
		Opalescence	Serological reactivity <sup>1</sup>	Precipitation with 1.25 volumes of alcohol <sup>2</sup>	Reducing sugars mg/cc	Opalescence	Reducing sugars after acid hydrolysis <sup>3</sup> 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
1 part extract plus 1 of substrate; incubated 2 hours at 37° C.	other sugars <sup>4</sup> + extract	0	1	0	—	0	.00
	sucrose + extract	+	150	++	.62	+	.52
	" + heated extract	0	10	±	.05	0	.06
	extract + water	0	10	±	.04	0	.05

37° C. <sup>1</sup>Highest dilution which precipitated 1:15 dilution of type 2 pneumococcus and leuconostoc antiserums after 1 hour at 37° C.

<sup>2</sup>Test mixture diluted 1:10 in 10 per cent. sodium acetate before precipitation with alcohol.

<sup>3</sup>No reducing sugars present before hydrolysis.

<sup>4</sup>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,<sup>1</sup> B,<sup>1</sup> B<sub>2</sub> (thiamine), B<sub>2</sub> (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

<sup>1</sup>Through the courtesy of American Chlorophyll Company.

<sup>2</sup>Through the courtesy of Merek and Company, Inc.