sulfite.

The *in vitro* "peroxidase-like effect" of alloxan and aldehyde[8] was inhibited by thiourea, sodium thiosulfate, and sodium sulfite, but large doses of these substances failed to inhibit the diabetogenic action of alloxan injected simultaneously or immediately afterwards; sodium metabisulfite administration also did not affect noticeably the usual alloxan effects. The maximum dosages, in mg/kg, given intravenously to rabbits were: thiourea 300, sodium thiosulfate 680, sodium sulfite 120, and sodium metabisulfite 100.

Discussion. In flavin synthesis alloxan unites with orthodiaminodimethylbenzene, and it is thought that 3,4-diaminotoluene reacts similarly with alloxan. A yellowish coloration observed along the ear veins of the rabbits after injection of the diaminotoluene and alloxan indicates that this reaction may occur in the blood of these animals. A similar reaction may explain the protective effect of orthophenylenediamine. Apparently most of the alloxan is removed by such reactions before the alloxan can damage the islet cells irreversibly. The work of other investigators[11, 12] suggests that this damage occurs within the first 5 minutes after intravenous injection of alloxan. Failure of the diaminotoluene to protect against the alloxan when the interval between injections of the two substances was greater than 5 min is confirmatory evidence for this suggestion.

Bisulfite also inhibits the diabetogenic action of alloxan, apparently by combining with it.[6] This protective action was not demonstrable consistently unless almost lethal doses of 125 mg/kg or more of sodium bisulfite were injected. An explanation of the failure of other compounds that combine chemically with alloxan to inhibit its diabetogenic action is not available from these experiments. Lack of proper concentrations or pH conditions in the blood stream for some of these reactions to occur may be responsible in some cases.

Summary. It was attempted to inhibit the selective destructive effect of alloxan on islet cells and the resultant blood sugar changes by administration of substances that combine chemically with alloxan or that inhibit some of its chemical actions. Of these substances 3,4-diaminotoluene, orthophenylenediamine, and sodium bisulfite exhibited definite inhibitory effects.

11 Hughes, H., Ware, L. L., and Young, F. G., *Lancet*, 1944, **246**, 148.

12 Leech, R. S., and Bailey, C. C., *J. Biol. Chem.*, in press.

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Serological Reactions of Levans Synthesized from Sucrose and Raffinose by Bacterial Enzymes.*

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Two classes of polysaccharides, dextrans[1-5] and levans,[6-9] have been synthesized from sucrose through the action of cell-free enzymes of bacterial origin. In the case of the leuconostoc dextrans, the enzymatically synthesized polysaccharides were shown to have serological properties similar to those of the polysaccharides formed in living cultures of the bacteria from which the enzymes came. A demonstration of the same feature for the enzymatically synthesized levans at first appeared impracticable because there were no data in the literature to indicate any serological reactivity on the part of the "natural" levans

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1 Hehre, E. J., *Science*, 1941, **93**, 237.

2 Hehre, E. J., and Sugg, J. Y., *J. Exp. Med.*, 1942, **75**, 339.

3 Stacey, M., *Nature*, 1942, **149**, 639.

TABLE I.
Serological Reactions of Products Synthesized from Sucrose and Raffinose by the Cell-Free Enzymes.

Source of enzyme	Substrate	Highest dilution of enzyme-substrate mixture that gave precipitation in tests with different antiserums: *Streptococcus salivarius* antiserum	*Bacillus N9* antiserum	Type 2 pneumococcus antiserum	Control serums
Streptococcus salivarius	sucrose	1,000	1,000	5	0
	raffinose	400	400	0	0
	other sugars	10*	10*	0	0
	buffer solution	10*	10*	0	0
Bacillus N9	sucrose	1,000	1,000	0	0
	raffinose	1,000	1,000	0	0
	other sugars	5*	5*	0	0
	buffer solution	5*	5*	0	0
Leuconostoc mesenteroides	sucrose	0	0	10,000	0
	buffer solution	0	0	10*	0

0 = no precipitation with 1:5 dilution of the antigen.
* Represents reactive material contained in the original enzyme solution, which did not increase in amount during the incubation of the test mixtures.

formed in bacterial cultures. However, upon investigation we[10] found that the levans from sucrose cultures of *Streptococcus salivarius* and of a spore-forming bacillus gave precipitation and complement fixation when tested against antiserums produced by immunization with bacteria obtained from sucrose cultures of either species. Experiments were then made to determine if the products synthesized from sucrose or raffinose by cell-free enzymes would have serological properties similar to those possessed by the levans produced in the cultures.

Sterile cell-free enzyme solutions were prepared[2] from sucrose broth cultures of the strains of streptococcus and bacillus which had been utilized in the previous[10] study. Mixtures were made of enzyme plus sterile 0.2 molar solutions of the following sugars buffered with acetate at pH 5.8; sucrose, raffinose, glucose, fructose, glucose and fructose, maltose, lactose, trehalose, xylose, arabinose. In order to compare levan-formation with dextran-formation a mixture of sucrose plus leuconostoc enzyme was included. After incubation for 10 days at 23°C under aseptic conditions, all the mixtures were examined for opalescence, for material precipitable in 65% alcohol, for free reducing sugars, and for serological reactivity. Three sorts of serum were included: (1) antiserums of *Streptococcus salivarius* and of the bacillus, produced by immunization with sucrose-grown bacteria and known to be highly reactive with the levans from cultures of the streptococcus and the bacillus; (2) type 2 antipneumococcus serum known to be highly reactive with bacterial dextrans but entirely non-reactive with levans; (3) types 1 and 8 antipneumococcus serums known to be non-reactive with either dextrans or levans, which served as routine controls. The serums were diluted 1:12 and the antigen-serum mixtures were observed for precipitation after 1 hr at 37°C.

Results. In the mixtures containing sucrose and raffinose the enzymes of the streptococcus and bacillus brought about the development of opalescent material that was precipitable in 65% alcohol and also an accumulation of free reducing sugars; neither of these changes occurred in the mixtures of the enzymes with the other substrates. The physical and chemical

[4] Hehre, E. J., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 18.

[5] Hehre, E. J., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 240.

[6] Aehsner, M., Avineri-Shapiro, S., and Hestrin, S., *Nature*, 1942, **149**, 527.

[7] Hestrin, S., and Avineri-Shapiro, S., *Nature*, 1943, **152**, 49.

[8] Hestrin, S., Avineri-Shapiro, S., and Aehsner, M., *Biochem. J.*, 1943, **37**, 450.

[9] Hestrin, S., and Avineri-Shapiro, S., *Biochem. J.*, 1944, **38**, 2.

[10] Hehre, E. J., Genghof, D. S., and Neill, J. M., *J. Immunol.*, in press.

changes in the sucrose and raffinose mixtures, and also the substrate specificity, are characteristic of levan-formation when it occurs in bacterial cultures, and are the same as those already reported[8] for the synthesis of levan by enzymes from species of *Aerobacter* and *Bacillus*.

The new fact (Table I.) is that the products formed from sucrose and raffinose by the streptococcus and bacillus enzymes had serological properties similar to those previously[10] found for the levans obtained from cultures of those bacteria. That is, the products of enzyme origin, like the levans of culture origin, had the capacity to cross-react with the antiserum of the other species as well as the capacity to react with the antiserum of its own species. This agreement in respect to the reactivities against the heterologous as well as the homologous antiserums, furnished better evidence of serological likeness between the products of enzyme and culture origins than would be furnished if the comparison were based only upon the reactivities against the antiserums of the homologous species.

The products synthesized by the streptococcus and bacillus enzymes were serologically different from those of the dextran products synthesized by the leuconostoc enzyme. The latter gave strong reactions with the type 2 pneumococcus but did not react with the two anti-levan serums, whereas, the opposite relationship was shown by the streptococcus and bacillus products. (The streptococcus enzyme did produce from sucrose a small amount of material reactive with the type 2 antiserum, but this minor exception was to be expected: a small but definite amount of dextran is formed in sucrose broth cultures of this as well as of some other strains of *S. salivarius*,[11] so that enzyme solutions prepared from them would likely contain some dextran-synthesizing enzyme along with a larger amount of levan-synthesizing enzyme.)

In addition to the tests made with the crude enzyme-substrate mixtures, experiments were made with material obtained by precipitation of a sucrose-streptococcus-enzyme mixture with 2.5 volumes of alcohol. The material was levo-rotatory and non-reducing but yielded abundant amounts of reducing sugars after mild acid hydrolysis (15 min at 60°C with 0.8N HCl); the value for fructose obtained in analyses by the Roe[12] procedure was 95% of that theoretically expected for levans. These data indicate that the enzyme derived material, although not pure, consisted chiefly of levan. When tested serologically, it gave precipitation in a dilution of 400,000 and complement fixation in a dilution of 1 million with the bacillus as well as the streptococcus antiserum, which represents essentially the same degree of serological reactivity as that shown by the purified levan of streptococcus culture origin.[10]

Summary. Cell-free, levan-synthesizing enzymes have been prepared from *Streptococcus salivarius* and from a spore-forming bacillus. The polysaccharide products formed by the action of these enzymes upon sucrose and raffinose possess serological properties similar to those recently described for the purified levans isolated from sucrose broth cultures of the living bacteria.

[11] Niven, C. F., Smiley, K. L., and Sherman, J. M., *J. Biol. Chem.*, 1941, **140**, 103.

[12] Roe, J. H., *J. Biol. Chem.*, 1934, **107**, 15.