

Utilization of Limit Dextrins by the Animal Body

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Starch in nature appears to contain two types of polymers of glucose, the amyloses, with a linear type of molecule showing a blue color with iodine, and the amylopectins, with highly branched structures, which give a red color with iodine. *Beta* amylase, capable of splitting maltose from the non-aldehydic end of a chain, can complete the hydrolysis of amyloses, but can carry the splitting of the amylopectins apparently only to the points of branching, producing limit dextrins, residual substances of high molecular weight which give colors with iodine. *Alpha* amylase causes quick fragmentation of amyloses, amylopectins, or limit dextrins into reducing dextrins which yield no color with iodine.

Since *alpha* amylase is labile to acid whereas *beta* amylase is stable to acid at low temperatures, it is possible selectively to inactivate *alpha* amylase by treating a diastase preparation at 0°C. for 20 minutes in an acetate buffer solution at pH 3. Insoluble materials, including inactivated *alpha* amylase, were removed in a Sharples supercentrifuge after adjusting with NaOH to pH 4.6 and adding an equal quantity of ethyl alcohol. Increasing the alcohol content to 80% precipitated *beta* amylase which was filtered off and suspended in acetate buffer at pH 4.6-4.8. Overlaid with toluene, such a preparation was kept in the refrigerator until used. Several preparations of *beta* amylase were made in the laboratory, and one of high purity was obtained from a commercial source.

In connection with some studies of enzymatic splitting of starches, it has seemed of interest to investigate the utilization in the white rat of limit dextrins administered orally or parenterally. Limit dextrins in the alimentary tract would be expected to be readily digested, but those administered by another route might escape utilization.

Rice starch was selected as the source of limit dextrins because of its small granules and its relatively high yield of limit dextrins of higher molecular weight. The starch was defatted by stirring and refluxing it with 85% alkaline methyl alcohol for about 15 hours. Although washed with 95% methyl alcohol, the air dried defatted starch gave a distinct alkaline reaction when dissolved. From this there were prepared several ten liter batches of 2% sol with pH adjusted to 4.6 by addition of glacial acetic acid. The sols were incubated several days at 37°C. with *beta* amylase preparations. When little additional maltose was being produced, as shown by the Shaffer-Hartmann determination (3), additional portions of enzyme were added to insure that the hydrolysis by the *beta* amylase had gone to essential completion. For the Shaffer-Hartmann determination, 30 ml. of ethyl alcohol were added to 10 ml. of the digest

to precipitate starch and dextrans. Twenty ml. of the filtrate was evaporated to about 5 ml., transferred to a 10 ml. volumetric flask and made up to volume with ethyl alcohol. Aliquots of this solution were treated according to the Shaffer-Hartmann procedure. The amount of reduction increased rapidly in a linear manner and suddenly leveled off. After digestion was discontinued, retrograded amylose and any flocculent precipitate present were filtered off and part of the dextrans was precipitated by adding an equal volume of 95% ethyl alcohol to the filtrate. After several reprecipitations and after grinding the product in a mortar with absolute alcohol and then with anhydrous ether, a light tan powder was obtained which was dried. This powder gave violet-red colors with iodine, was soluble in hot water, only slightly soluble in cold water, and was thought to have relatively high molecular weight. The filtrate was concentrated under vacuum and after further addition of alcohol a fraction of medium molecular weight was obtained. It was soluble in cold water and gave a red complex with iodine. Digests containing small amounts of *alpha* amylase yielded in addition small amounts of limit dextrans very soluble in water and which gave only a light red color with iodine. These were thought to have low molecular weight.

Young male white rats weighing 280-380 g. were deprived of food for 24 hours. Limit dextrans were administered in 5 ml. of water via stomach tube or subcutaneous injection. If the preparation of the limit dextrans was not sufficiently soluble, the dose was administered at hourly intervals in several more dilute portions. After 8 hours the livers were removed from the anesthetized animals and the glycogen determined (Table I) by the method of Good, Kramer, and Somogyi (1), as modified in this laboratory (2). Control experiments with rats fed starch, glucose, or maltose (Table II) and with rats sacrificed after a 36 hour fast were obtained.

TABLE I. Glycogen in the Liver of the White Rat After Administration of Limit Dextrans

Mol. Wt.	Mode of Administration	Dosage g/100 g. Body Wt.	No. of Rats Used	% Glycogen
Low	Stomach tube	0.48	1	1.75
Medium	Stomach tube	0.57	2	1.15
Medium	Stomach tube	0.55	2	1.61
Medium	Subcutaneous	0.53	1	1.37
High	Stomach tube	0.47	1	2.05
High	Stomach tube	0.28	1	0.24

It appeared that glycogen was deposited in the liver of rats after the oral or subcutaneous administration of the limit dextrin preparations employed. Intestinal *alpha* amylase in the rat presumably continued the hydrolysis of limit dextrans. Van Genderen and Engel reported that a large quantity of *alpha* amylase was always found in the rat duodenum (5). The mode of introduction into the organism of the limit dextrans employed seemed to cause but little variation in the amount of

TABLE II. Glycogen in the Liver of the White Rat After Administration of Carbohydrates

Carbohydrate	Mode of Administration	Dosage g./100 g. Body Wt.	No. of Rats Used	Glycogen Av. %
None			7	0.26
Glucose	Stomach tube	0.62	1	2.77
Maltose	Subcutaneous	0.60	1	1.94
Starch	Subcutaneous	0.59	3	1.33
Starch	Stomach tube	0.86	1	1.37
Limit Dextrin	Both methods	0.54	8	1.21

apparent liver glycogen deposited. The possible role of tissue, particularly liver, enzymes in promoting the utilization of injected polysaccharides is not clear. Formation of glycogen without any hydrolysis of the limit dextrins, or deposition of limit dextrins as such is conceivable. The fate of injected starch is analogously obscure.

Conclusion

After the oral or subcutaneous administration of several preparations of limit dextrins, glycogen or a similar polysaccharide was deposited in the livers of white rats.

Bibliography

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