# THE PHYSIOLOGY OF PARIETAL DIGESTION. A COMPARISON OF THE HYDROLYSIS OF STARCH IN THE INTESTINE AND IN VITRO BY THE SPECTROPHOTOMETRY OF IODINE-STARCH COMPLEXES

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The problem of the differences between the hydrolysis of starch in vitro and in the intestine has attracted considerable attention. Some authors are inclined to dismiss such differences altogether [6, 8], though recently it has been shown that the hydrolysis of starch in the intestine proceeds many times more rapidly than it does when the reaction is carried out in a test tube containing intenstinal enzymes [4, 5, 9]. This effect is related to the interaction of two mechanisms, digestion in the lumen of the intestine and digestion on the outer surface of the intestinal cells. Because of the existence of parietal digestion the idea has been put forward that the hydrolysis of polysaccharides in the intestine has not only quantitative but even qualitative features which distinguish it from hydrolysis in vitro [5].

It is known that α-amylases are enzymes which produce dextrin, and under their influence polysaccharides containing glucoside chains are formed from starch, and the length of these chains is reduced as the reaction proceeds. Therefore in α-amylolysis, not only is a reduction in the amount of the original substrate observed in the test tube but there is an increased dextrin content [1-3, 7, 8, 10]. If the intermediate products of hydrolysis in the intestine are really eliminated from the lumen into the brush border, then when starch breaks down in vivo there will be no very considerable accumulation of dextrin. These differences, in so far as they exist, could be detected by the spectrophotometry of iodine-polysaccharide complexes. It is known that when this method is used the reduction in the concentration of starch will be shown by a reduction of optical density at wavelengths equal to or greater than 580-600 mμ, and the accumulation of dextrins will be revealed by a shift of the maximum towards the short waves and by an increase of the relative optical density in the blue region of the spectrum [1, 3, 6, 11].

## EXPERIMENTAL METHOD

Acute experiments were carried out under nembutal anesthesia on white rats weighing 150-160 g. The isolated rostral and caudal parts of the small intestine for a length of 20-25 cm were perfused. The perfusion fluid was starch-Ringer solution (concentration of soluble starch 0.075%), the pH was 7.0, and the rate 2-5 ml/minute. The collected perfusate was rapidly cooled to arrest the enzymatic reaction, and it was then diluted (1 ml of perfusate + 5 ml $H_2O$ + 0.5 ml 1 N HCl). To obtain iodine-starch complexes, to samples prepared in the manner just described we added 0.5 ml of 0.1% iodine in 1% KI. Control tests were carried out in a similar manner, but instead of the perfusate we used 1 ml of 0.075% starch (taken as 100%). We then determined the optical density by means of a SF-4 spectrophotometer, and used wavelengths from 400 to 660 mμ at 20 mμ intervals. In some of the experiments the optical density of the iodine-polysaccharide complexes were studied by means of a recording SF-2M spectrophotometer.

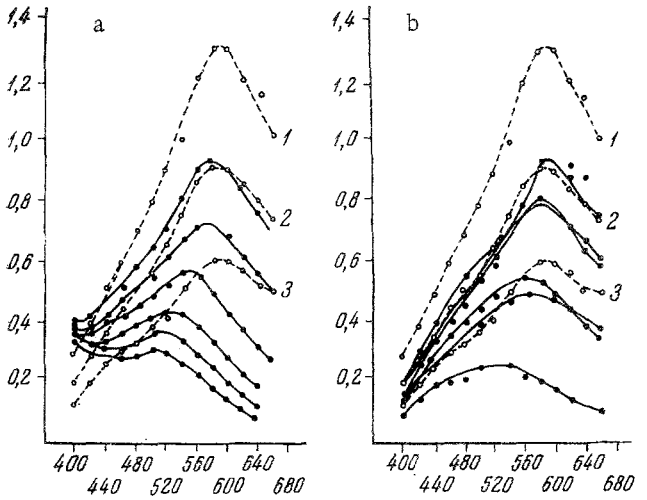


Fig. 1. Absorption spectra of iodine-starch complexes. a) At various stages during the breakdown of soluble starch in vitro; b) at various stages of breakdown of soluble starch in the intestine. Dotted curves – absorption spectra of intact starch. 1) 0.075%; 2) 0.562%; 3) 0.0375%. Ordinate – optical density in units of the SF-4 instrument; abscissa – wavelength.

In order to measure the hydrolysis in vitro the collected perfusate was incubated in water in an ultrathermostat at 38° until the reaction for iodine failed completely. Then to the perfusate we added an equal amount of 0.15% starch solution (so that the concentrations of starch in vivo and in vitro were the same), and we incubated the solutions in a ultrathermostat. After every 1-2 min we took 1 ml of solution and added it to a test tube containing 5 ml water, 0.5 ml 1 N HCl, and 0.5 ml $I_2$ in KI. We then made the spectrophotometric measurements.

## EXPERIMENTAL RESULTS

Because in the hydrolysis of starch changes in optical density occur either because of an excess of the original substrate or because of the formation of various dextrins, it is essential to distinguish between these products. This can be done by comparing the absorption curves of starch during its hydrolysis with the curves of starch which has not been broken down, taken in various concentrations.

As can be seen from Fig. 1 the absorption spectrum of iodine-starch complexes as recorded in our experiments was close to what has been described by other authors [7, 11]. The maximum optical density corresponded to a wavelength of 580-600 mμ; a comparison of solutions with various concentrations of starch showed a linear relationship between the concentration of the substrate and the optical density.

When starch breaks down in vitro under the influence of enzymes collected in the perfusate the results obtained resembled those which have been obtained earlier. According to the degree of breakdown (Fig. 1a) there is a reduced extinction at a wavelength of 580-600 mμ and a displacement of the maximum towards the shortwave end of the spectrum. Special attention should be paid to the relative increase of optical density at a wavelength less than 580 mμ in comparison with a reduction corresponding to the amount of starch in this sample. As has been pointed out previously these phenomena indicate an accumulation of dextrins during starch hydrolysis.

Entirely different results were obtained in the determination of the absorption curves for starch hydrolyzed in vivo. As can be seen from Fig. 1b, the curves here show but little difference from the controls. A marked displacement of the maximum was recorded only when more than 60-70% of the original substrate had been hydrolyzed. As was shown by a comparison of the optical densities of the experimental and control samples, in intestinal hydrolysis the dextrins accumulate in very small amounts. The relationship between the degree of hydrolysis of starch and the accumulation of dextrins may conveniently be considered by comparing the optical densities of the control samples (starch which has not been broken down) and the experimental sample (starch which has been broken down in the test tube or intestine); the comparison is made at wavelengths sensitive to the iodine-starch and iodine-dextrin complexes

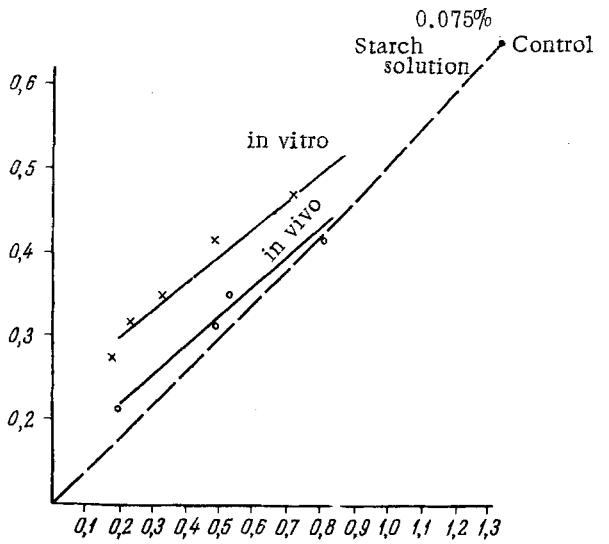


Fig. 2. Relationship of the optical densities of the iodine-polysaccharide complexes at various stages in the hydrolysis of starch in vivo and in vitro, and also for the unbrokendown starch (dotted line). Ordinate – optical density as indicated on the scale of the SF-4 instrument at a wavelength of 460 mμ (indicating chiefly the dextrin content); abscissa – at a wavelength of 580 mμ (indicating chiefly the starch content).

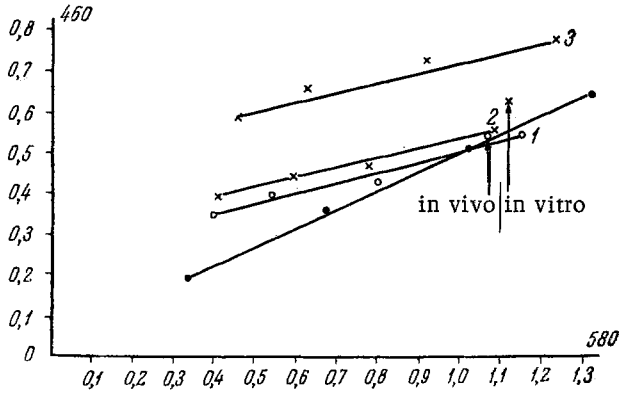


Fig. 3. Accumulation of dextrins at various amylase concentrations: 1) undiluted perfusate; 2) perfusate diluted three times; 3) diluted ten times, and for equalized rates of hydrolysis in vivo and in vitro. Remaining indications as in Fig. 2.

Fig. 2). An increase of optical density at a wavelength of 460 mμ relative to the control line indicates an accumulation of a red component (mixture of dextrins), and affords grounds for drawing the following conclusions.

In hydrolysis in a test tube there is a considerable accumulation of dextrins which increase to a certain amount during hydrolysis. In hydrolysis in vivo dextrins accumulate in small amounts. Therefore the process of digestion of starch in the intestine is associated with the accumulation of only small amounts of dextrins. This effect is brought about partly because hydrolysis in vivo takes place more rapidly than in vitro. From Fig. 3 it can be seen that when soluble starch breaks down in the test tube the accumulation of dextrins is greater the smaller the amylase content. At first sight these differences between hydrolysis in vivo and in vitro might be attributed to the difference in

hydrolysis rates. However more careful consideration shows that such a conclusion is inadmissible. It is known that during prolonged perfusion of the rat intestine amylase is released from the surface of the intestine and reduces the rate of hydrolysis almost to zero. If the characteristic features of the breakdown of starch depend only on the rate of the enzymatic reaction then the absence of dextrins should exert an effect only at the start of the perfusion. Actually however even by the end of the experiment when the rate of hydrolysis in vivo has been greatly reduced the accumulation of dextrins in the perfusate could not be arrested. Also, in special experiments where a comparison was made of the hydrolysis of starch in the intestine and in the test tube, for equal rates of the reactions and for equal degrees of hydrolysis marked differences were maintained (see Fig. 3).

Because the intestinal wall is impermeable (or almost impermeable) to dextrins, the conclusion reached is that the intermediate products of hydrolysis are eliminated by passage into the brush border, where they are broken down and absorbed. Analysis of samples of starch broken down in the intestine or in a test tube shows that the greatest difference lies in the region of the spectrum between 480 and 460 mμ, and at shorter wavelengths. We must therefore suppose that parietal digestion is closely associated with the breakdown of dextrins, starting from ten glucose residues or less, although previously we have shown that soluble starch may be broken down upon the surface. Confirmation of these results by other methods gives reason to suppose that more than 80% of the glucose bonds are broken down in the region where parietal digestion has taken place. These results also help us to understand one of the mechanisms of the facilitatory influence of parietal digestion in the early stages of hydrolysis, which occurs in the lumen of the small intestine. During hydrolysis in a test tube amylase is distributed between the starch and the intermediate hydrolytic products, but because during intestinal digestion these products do not accumulate in the chyme, but enter the brush border, an additional effect develops for the reason that amylase now acts chiefly on the original substrate.

The method here described may be useful for the diagnosis of the condition of parietal digestion and digestion in the lumen.

## SUMMARY

A comparison was made of the hydrolysis of starch and the increase in the amount of dextrins during digestion either by perfusion of the small intestine of albino rats or "in vitro," under the influence of enzymes introduced into the perfusate. It was shown that at corresponding stages of the hydrolysis in vivo and in vitro there was far less accumulation of the breakdown products of starch in the former case. It is concluded that hydrolysis of dextrins takes place in the brush border of the villi. The removal of the dextrins from the chyme into the region of parietal digestion leads to an intensification of the initial stages of starch hydrolysis. This mechanism may be of general significance.

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