MEMBRANE DIGESTION

PART III. A COMPARISON OF THE ENZYMATIC HYDROLYSIS OF STARCH IN THE INTESTINE AND IN VITRO

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Despite the great advances in the morphology, physiology, and biochemistry of digestion, it has not yet been possible to reproduce in vitro the high rates of digestion which occur in vivo.

Three explanations are known to us.

1. That in the lumen of the intestine, direct absorption of the breakdown products occurs at the same time as their rapid hydrolysis. Nevertheless, it is known that the accumulation of the reaction products in the mixture (which takes place during hydrolysis in vitro) leads to a slowing down of the enzymatic reaction through the formation of an inactive compound of the reaction products and the enzyme. However, this important factor concerns only the late stages of the enzymatic reaction, when a considerable amount of products has accumulated. Were this all, the initial rates of hydrolysis in vitro and in vivo would be approximately equal. As far as we know, no attention has been paid to this point.

2. In the digestive tract, enzymes act in a certain sequence, and they do not act separately, but in very complex combinations. On this account, there could be a considerable increase in the hydrolysis of food substances in vivo. It would still remain to be found whether the same combinations of enzymes, which are so effective in the digestive tract, would be equally active in a test tube.

3. We have previously proposed [1, 2], that the essential difference between hydrolysis in vitro and in vivo is that in the first case, enzymatic reactions take place only in solution, while in the intestine hydrolysis takes place both in the lumen and on the surface of the intestinal wall, which we envisage as a kind of porous reactor in which the pores are made up of microvilli.* Up till now, digestion in the lumen has been considered to be the only process. We consider that the wall of the intestine is covered with a more or less compact layer of enzymes which bring about the hydrolysis and the absorption of food materials (membrane digestion).

In our previous communications [1, 2] we showed that the presence of fragments of the intestine increases the rate of digestion in a test tube. However, these experiments had the drawback that they were performed entirely in vitro. Although the method was convenient for demonstrating the occurrence of membrane digestion, it was not possible to make quantitative measurements of the part it played during normal digestion.

The aim of the present work has been to compare the enzymatic breakdown of the food substances in vitro and in the intestine.

Despite its apparent simplicity, the comparison is actually quite difficult. The effect of absorption on the enzymatic reactions in vivo must be reduced to a minimum, and the same combinations of enzymes must be active both in vitro and in vivo.

METHOD

Acute experiments were carried out on white rats anesthetized with nembutal. The animals were placed on their backs, and a median incision was made in the abdominal wall.

*Several descriptions of the microvilli have been published [3, 4, 5].

An 8-15 cm length of the upper part of the small intestine was isolated. Polyethylene tubes were inserted into both ends, and the abdominal cavity again closed.

At one end Ringer, free from glucose, or Ringer solution containing 0.15% soluble starch was introduced. The liquid flowing out of the other tube was collected and immediately cooled.

Comparison of the Enzymatic Hydrolysis of Starch in vivo and in vitro

Series of experiment	Number of experiment	Time of perfusion of 1 ml (in seconds)	Capacity of intestine (in ml)	Starch consumed (as percentage per minute) in vivo	Incubation time (in minutes) in vitro	Starch consumed (as percentage per minute) in vitro	Ratio hydrolysis in vivo / hydrolysis in vitro
First	1	5		108	11	3.4	31.8
	2	10		30	10	1.25	23.9
	3	10	1.25	67	5	20	3.35
	4	10	2	60	5	10.8	5.55
	5	10		60	2	13	4.6
	6	10		78	10	1.95	40
	7	5	1.5	56.8	5	5.4	10.5
					10	4.7	12.8
	8	10	1.5	7.2	5	3.3	2.4
	9	5	1.5	89.6	5	13.14	6
					10	10	8.9
	10	10	1.5	93.2	5	14.72	6.3
					10	10	9.3
	11	10	1.5	32.8	5	3.9	8.2
					10	4	8.2
					15	4.1	8.2
Second	1	5	1.5	96	2	14.7	6.5
	2	10	1.5	86	5	21.4	4.1
	3	10		78	5	4.4	17.7
					15	6.4	12
					25	5.4	14.5
Third	1	10	2	47.1	10	6.28	7.4
					15	8.2	5.7
					20	7.14	6.5
	2	10	1.5	40	5	13.2	3
					10	7.4	5.4
					15	8.6	4.6
	3	10	1.5	40	10	1.8	22
					15	1.4	28
	4	10	1.5	34	10	0.6	5.6
	5	15	2	50.6	5	11.6	4.5
					10	11.6	4.5
					15	10.6	4.7

The rate of perfusion with these solutions was standard at 0.1 or 0.2 ml per second (1 ml in 10 and 5 seconds respectively).

The rate of hydrolysis in vivo was determined from the difference in the starch content between the fluids entering and leaving the intestine. In all cases, the rate of hydrolysis was calculated per minute. The amylolytic activity was measured by our modification of the method of Smith-Rowe.

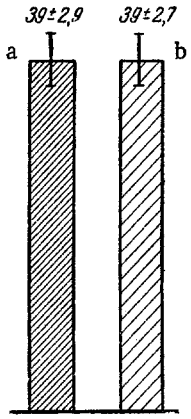
RESULTS

The classical physiological description of digestion is that starch is broken down by amylase which is liberated into the lumen of the intestine. If hydrolysis depends entirely on enzymes acting in the starch-saline solution, then it would proceed at the same rate in a test tube. In the first set of experiments, the starch solution passed through the intestine was collected in a test tube and incubated at the same temperature in a Warburg's apparatus.

It can be seen from the Table that the hydrolysis per minute in vivo was much greater than in vitro. The low rates of the reactions in vitro could not have been due principally to a slowing down of the enzymatic reaction at the late stages of hydrolysis, because the observed reduction in rate exceeded many times the amount of slowing that it would be possible for chemical reaction of the first order. Neither can it be explained as being due to the accumulation of hydrolysis products, as was shown by special experiments which will be described below.

Both objections may be met by comparing the initial rates of reaction in the intestine and in the test tube.

This study was made in the following set of experiments. Hydrolysis was studied in vivo as in the experiment described above, by perfusing the intestine with starch solution. To determine the enzymatic activity in vitro, a saline solution free from starch was used to perfuse the intestine. The same kind and the same quantity of enzymes then enter the salt solution as entered the starch solution previously. In these experiments it was found that the initial rate of hydrolysis in vitro was many times less than that in vivo. Therefore by this way it was found that starch hydrolysis takes place much more rapidly in the intestine than it does in vitro.



Absence of any reduction in the amylolytic activity after a second incubation of the intestinal perfusate. The activity is expressed as a percentage of the substrate hydrolyzed. Mean values (M ± m). a) First incubation; b) repeated incubation.

A further objection can be made to these experiments. It might be supposed that the starch present in the intestine induces an increased secretion of enzymes. However, no evidence to this effect has yet been obtained.

Therefore, in the third set of experiments, just as in the first, the enzymatic activity of the enzymes was determined in the same solution both in vivo and in vitro. As in the previous experiments in vivo, hydrolysis was determined from the loss of starch during perfusion.

The salt solution passed through the intestine was incubated at 38° until the color of the iodine-starch compound had completely disappeared. Then 1 ml of 0.3 starch solution was added to 1 ml of the perfusate, and the rate of hydrolysis was again determined (we took the maximal linear relationship between the rate of hydrolysis and the concentration of the enzyme, although special experiments had shown that this relationship is subject to the law of Borisov-Shyutts). No serious objections can be raised against these experiments, because 1) the composition of the enzymes acting in the intestinal lumen and in the intestine were identical, and 2) in both cases the initial rates of the reaction were determined.

Nevertheless, in this experiment again, hydrolysis in vivo was many times more intense than it was in vitro.

Possible objections to the third experiment must now be considered. Because the enzymatic activity in vitro was determined after preliminary breakdown of the starch introduced into the intestine, there might be some suppression of the activity of amylase by the final products of the reaction. Also, we must not ignore any possible partial inactivation of the enzymes in vitro through the action of proteases, temperature denaturation etc. However, in the control experiments it was shown that when the incubation was repeated with starch, there was no reduction in the total activity of the intestinal enzymes (under the conditions prevailing in our experiments), so that both objections are met (see Figure).

Under the conditions of our experiment it would be expected that the rates of breakdown of starch in vivo and in vitro would be approximately the same if the processes were due chiefly to enzymes which act in the lumen of the intestine. However, the results of all three experiments showed that the actual times are much greater (in some experiments several tens of times greater) than in the test tube. It cannot be supposed that this result is due to there being other combinations of enzymes in the intestine than in the test tube; (this consideration is particularly relevant to the first and third experiments).

The differences cannot depend on the accumulation of hydrolysis products, as was shown in special control experiments. This circumstance cannot be of importance in the experiments in which the initial rates of the enzymatic reactions were measured.

It must therefore be supposed that the hydrolysis of starch in the intestine is caused only partially by enzymes liberated into the lumen, and because the cells of the intestinal epithelium are impermeable to starch, intracellular breakdown of starch is also excluded.

We are therefore forced to conclude that the high rate of hydrolysis in vivo must depend on enzymes situated on the surface of the membrane of the intestinal epithelium. If this is so, it then follows that intestinal enzymes located on the membranes play a greater part than do those liberated into the lumen. It is not only that a solution with which the intestine is perfused is acted upon by only an inconsiderable proportion of the enzymes which are active in the intestine. In our opinion, it is no less important that hydrolysis at the surface established extremely favorable conditions for absorption, because owing to "membrane" digestion, hydrolysis and absorption both occur at the same place.

Finally, the results indicate that the enzymatic activity of the intestinal juice gives only a very limited indication of the part played by the intestine in breaking down food substances.

SUMMARY

The rate of enzymatic hydrolysis of starch in the intestine of albino rats was compared with the rate in vitro. It was found that although absorption produces no significant effect on the rate of hydrolysis, and although the composition of the enzymes acting in the intestine and in vitro was identical, the rate of starch breakdown in vivo was much the higher. The higher rate of digestion in vivo may be due to "membrane" digestion in the intestine.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
