THE PHYSIOLOGY OF MEMBRANE DIGESTION.

REPORT 4. INTRAVASCULAR HYDROLYSIS OF STARCH IN THE FROG

A. M. Ugolev and V. V. Chernousova

Laboratory of General Physiology (Head, Academician V. N. Chernigovskii),
I. P. Pavlov Institute of Physiology of the AN SSSR, Leningrad
(Presented by Academician V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 55, No. 5,
pp. 25-28, May, 1963
Original article submitted April 9, 1962

It has recently been shown that the hydrolysis of food substances in the gastro-intestinal tract of vertebrates may take place in two ways: 1) in the lumen of the gastro-intestinal tract by the action of enzymes secreted in the digestive juices, and 2) on the outer surface of the intestinal cells, where hydrolysis is more intensive still [1-5].

This membrane or contact digestion is clearly seen in the small intestine but absent, at least in omnivorous animals, in the esophagus, stomach, and large intestine. There is evidence, however, that a mechanism of hydrolysis similar in principle to membrane digestion is more widespread than might be supposed. In fact, it has been found that in yeasts and certain microorganisms hydrolysis of starch and disaccharides may take place by the action of enzymes adsorbed on to the outer surface of the cell membrane [6].

These findings suggest that membrane digestion appeared much earlier during evolution that the specialized digestive organs. The question accordingly arises, whether this mechanism may have been retained to some extent by the somatic cells of vertebrates, notably by the cells of the small blood vessels in which an intensive interchange of materials occurs between the circulating fluids and tissues. In the present research an attempt was made to analyze this suggestion experimentally.

We chose to perfuse the blood vessels of the frog with starch. As a substrate, starch has the advantage that it does not diffuse into the cells, so that measurement of its concentration, other things being equal, gives a reliable indication of the intensity of enzymic hydrolysis.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (Rana temporaria). Before the experiments the central nervous system of the animals was destroyed. The frogs were fixed to a special stand. The large veins entering the heart, both arches of the aorta, and the lungs were ligated. This was done to prevent artifacts which would otherwise develop, as pilot experiments showed.

A starch-saline solution was perfused at room temperature through a cannula fixed into the anterior abdominal vein. In this way the perfusion fluid passed through the arterial, capillary, and venous portions of the vascular system of the abdominal viscera and hindlimbs. The time taken for the solution to pass through the vessels was measured in each experiment by adding dye to the perfusion fluid.

The composition of the perfusion fluid and reagents was as follows: a 0.075% solution of starch prepared in Ringer's solution for cold-blooded animals, 2% HCl solution, and standard iodine reagent (0.3% I in a 3% solution of KI).

Sucrose was added to the starch-saline solution (final concentration 5%) to prevent tissue edema, the absence of which was verified in each experiment. The amylolytic activity was determined by the Smith-Roy method, as modified by ourselves [2]. Hydrolysis in vivo was determined as follows. The outflowing fluid was collected in a vessel containing 3 ml HCl, which immediately halted the enzymic reaction. The hydrolysis in vitro was determined by comparing the concentration of starch in the outflowing perfusion fluid (without addition of HCl) and after incubation for 15 and 30 min at the same temperature as in vivo. The amylolytic activity was calculated as a percentage of the hydrolyzed substrate per minute.

TABLE 1. Hydrolysis of Starch in Vivo (in the Blood Vessels) and in Vitro (by the Action of Enzymes Passing into the Perfusion Fluid)

Experiment No.	In vivo					In vitro					
	time taken for perfusion fluid to pass through blood vessels (in sec)	starch concentration		digestion during perfusion (in %)	rate of digestion (per min)	duration of incubation of perfusion (in min)	starch concentration		digestion during perfusion (in %)	rate of digestion (per min)	ratio between rates of digestion of starch in vivo/in vitro
		before perfusion	after perfusion				before incubation	after incubation			
16	30	0.65	0.59	9.2	18.5	30	0.59	0.4	32.2	1.07	17
17	16	0.65	0.56	14	52.2	30	0.56	0.48	14.3	0.48	36
21	25	0.67	0.60	10.5	25.2	30	0.60	0.49	18.34	0.63	40
24	30	0.66	0.57	13.6	27.2	30	0.57	0.48	17	0.5	50
30	30	0.52	0.45	13.5	27	15	0.45	0.28	31.8	2.1	13
34	20	0.73	0.68	6.9	16.5	15	0.68	0.47	30.9	2.06	8
37	16	0.89	0.82	7.9	20	15	0.82	0.78	5	0.33	85
38	20	0.89	0.83	6.7	20	15	0.83	0.77	6	0.4	50
39	20	0.90	0.79	12.3	36.6	15	0.79	0.70	12	0.8	46

TABLE 2. Digestion of Starch in Vivo and in Vitro (Control Experiments)

Experiment No.	In vivo					In vitro					
	time taken for perfusion fluid to pass through blood vessels (in sec)	starch concentration		digestion during perfusion (in %)	rate of digestion (per min)	duration of incubation of perfusion (in min)	starch concentration		digestion during perfusion (in %)	rate of digestion (per min)	ratio between rates of digestion of starch in vivo/in vitro
		before perfusion	after perfusion				before incubation	after incubation			
22	25	0.65	0.58	12	28.8	30	0.58	0.47	19.0	0.89	32
40	25	0.556	0.50	10	24	20	0.50	0.40	20	1.0	24
41	20	0.556	0.51	8	24	20	0.51	0.34	33.7	1.7	14
44	15	0.50	0.45	10	40	15	0.45	0.32	29	1.9	21
46	10	0.50	0.48	4	24	15	0.48	0.42	13.0	1.06	22.6

EXPERIMENTAL RESULTS

Preliminary experiments showed that the starch concentration in the outflowing perfusion fluid was significantly smaller than in the fluid when administered (Table 1). Since neither starch nor dextrins penetrate to any significant degree outside the blood vessels, and the weight of the animals remained practically constant throughout the experiment (excluding dilution of the body fluid with perfusate), there was only one possible conclusion: when the starch passed through the blood vessels of the frog it underwent enzymic hydrolysis.

It is well known that digestive enzymes (including amylase) are secreted not only into the lumen of the alimentary canal, but also into the blood stream, and it is possible that amylase entered the blood vessels during the period of perfusion. This being so, the outflowing perfusion fluid must contain a certain amount of amylase. In all the experiments we determined the activity of this enzyme in the outflowing fluid and found that it was always present (Table1).

Comparing the left side of Table 1, in which data characterizing the hydrolysis of starch within the vascular system (in vivo) are given, with the right side showing the amylolytic activity of the perfusion fluid (in vitro) reveals an important fact: hydrolysis takes place much more rapidly in vivo than in vitro. Consequently, the presence of amylase in the perfusion fluid accounts for only a small fraction of the hydrolysis taking place in the vascular system. Control experiments (in agreement with the findings of other workers) showed that hydrolysis of starch in vitro up to a limit of 30% was proportional to the incubation time.

The suggestion could be made that the secreted amylase is reabsorbed by the tissues. It is, however, much more likely that the starch may be hydrolyzed by amylase fixed in the region of the capillaries. If we remember that starch does not penetrate through membranes, the most justifiable assumption is that the enzymes are adsorbed on to the outer surface of the membranes of the endothelial cells.

Mechanisms similar to membrane digestion apparently operate on the surface of the capillary endothelium. The question arises whether the phenomenon we have described is associated with the vascular zone of the digestive apparatus, where most amylase is produced, or whether membrane digestion may also be observed in other vascular fields. We conducted a special series of experiments in which the vessels supplying the abdominal viscera were ligated and the flow of fluid through the hindlimbs only was preserved It is clear from Table 2 that in this case too there was a considerable hydrolysis of starch in unit time. It follows, therefore, that polysaccharides may undergo hydrolysis in vascular zones not connected with digestion.

Membrane hydrolysis is characteristic of primitive organisms such as bacteria and yeasts. It seems, therefore, that in the course of evolution not only did membrane digestion develop and acquire specific features in certain divisions of the alimentary tract, but it also retained some of its importance in the somatic cells and, in particular, in cells of blood vessels.

It is pertinent to remember that intracellular digestion (arising, like membrane digestion, in the early stages of evolution) remains important outside the limits of the digestive system. Admittedly, in the internal environment intracellular digestion performs a protective function, but nevertheless it retains the principal features characteristic of phagocytosis.

So far as our hypothesis is concerned, the role of membrane digestion outside the gastro-intestinal tract is still unexplained. It is possible that the passage of proteins and polysaccharides from the blood through the cell membrane is due to digestive hydrolysis. In this case, membrane digestion must be regarded as an element of intermediate metabolism.

SUMMARY

Hydrolysis of soluble starch was studied in the frog: a) during perfusion of the vascular system with starch-sucrose-salt solution and b) under the effect of amylase transported from tissues into the perfusate. A comparative analysis of the rate of hydrolysis in vivo and in vitro showed that amylase in the perfusate was responsible but little for hydrolysis in the vessels. Similar results were obtained in the experiments with the digestive system excluded from perfusion. It may be assumed that an intensive hydrolysis in the vessels may be due to the presence of enzymes fixed on the surface of endothelial cells.

LITERATURE CITED

1. A. M. Ugolev. Membrane (Contact) Digestion [in Russian]. Moscow-Leningrad, 1963.
2. A. M. Ugolev. Byull. éksper. biol., 1, 12 (1960).
3. J. H. Holt and D. Miller, J. Lab. clin., Med., 1961, Vol. 58, p. 827.
4. D. Miller and R. K. Crane, Biochim. biophys. Acta, 1961, Vol. 52, p. 281.
5. D. Miller and R. K. Crane, Biochim. biophys. Acta, 1961, Vol. 52, p. 293.
6. A. Rothstein. In the book: Fifth International Biochemical Congress. Symposium 2, Book 7 (Functional Biochemistry of Cell Structures) [in Russian], p. 16. Moscow, 1961.
