ENZYMIC DIFFERENTIATION AND ABSORPTIVE CAPACITY OF THE EPITHELIUM OF MACROCOLONIES FROM THE SMALL INTESTINE

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Absorption of various substances in the small intestine depends on the function of the digestive-transport conveyor on the apical membranes of the epitheliocytes [4]. Disturbances of absorption of some substances while transport of others is unaffected can take place not only within the membrane of the same cell, but also in different epitheliocytes with definite functional differentiation, and it is this which leads to the varied manifestation of changes in absorption [1, 2]. It is not yet known whether each epitheliocyte has digestive-transport pathways for all substances or whether there is definite specialization and that each cell is able to transport only a certain group of substances.

To determine whether functional differentiation of cells is possible, methods of assessing enzyme activity and absorptive capacity of the epithelium of macrocolonies of small intestine, each developing from a separate stem cell of intestinal epithelium, were used [6].

EXPERIMENTAL METHOD

Male and female Wistar rats weighing 180-200 g, kept on an ordinary animal house diet, were used. The animals were anesthetized with pentobarbital (40 mg/kg) and after laparotomy a loop of intestine 3-5 cm long was exteriorized and irradiated in a dose of 1800 rads (dose rate 332 rads/min) on the RUM-17 x-ray apparatus. In the rats of group 1 the irradiated segment of intestine was removed on the 20th day after the operation and irradiation, single isolated macrocolonies were obtained, and each separate colony was homogenized in 2 ml of Ringer's solution, pH 7.4, at 4-6°C, and activity of the following enzymes was determined in the homogenate from each colony: invertase, γ-amylase [5], and alkaline phosphatase [3]. Rats of group 2 were anesthetized on the 20th day after irradiation, and 0.1 ml of a solution of L-lysine-^{3}H (2.5×10^{-6} M, 10 μCi) or the same volume of a solution of DL-leucine-^{14}C (1.87×10^{-4} M, 1 μCi) was injected for 10 min into the lumen of the irradiated segment of intestine and to a neighboring segment of the intestine. The experimental segments of intestine were removed 10 min later, weighed samples of mucous membrane and macrocolonies were taken, washed in Ringer's solution, pH 7.4, at 4-6°C, homogenized, placed on a cardboard filter, after which their activity was determined in toluene scintillation fluid on an SL-30 (Intertechnique) liquid scintillation spectrometer. From the activity thus measured the quantity of amino acid absorbed by 1 mg tissue was calculated. The animals of both groups were starved for 20-24 h before the experiment.

EXPERIMENTAL RESULTS

Analysis of the data in Table 1 shows that each colony in different parts of the small intestine had an individual spectrum of enzyme activity, characteristic of it alone and differing even from that of a neighboring colony of the same rat (for example, colonies 1-5 growing in the proximal part of the jejunum of rat No. 1, and also the other colonies developing in different parts of the small intestine of rats No. 2-6). It will be noted that in female rats (Nos. 1 and 7) γ-amylase activity was lower than in males, both in the mucous membrane

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TABLE 1. Enzyme Activity of Epithelial Macrocolonies of Rat Small Intestine

Rat	Site of growth of macrocolonies	Enzyme	Mucous membrane 10 cm from region of colonies	Macrocolony 1	2	3	4	5	6
1 (female)	Proximal part of jejunum	Invertase	9	130	40	7,5	23	72	—
		γ-Amylase	0,04	0,9	0,15	0,04	0,1	0,2	
		Alkaline phosphatase	0,08	0,36	0,12	0,08	0,38	0,52	
2 (male)	The same	Invertase	10	36	80	90	136	—	—
		γ-Amylase	1,2	8	5	8	7		
		Alkaline phosphatase	0,06	0	1,02	0,52	0,42		
3 (male)	The same	Invertase	9	41	79	82	71	—	—
		γ-Amylase	1	8	7	6	9		
		Alkaline phosphatase	0,06	0,07	0,08	0,05	0,04		
4 (male)	Middle part of jejunum	Invertase	7	74	18	11	10	—	—
		γ-Amylase	2	20	4	4	2,2		
		Alkaline phosphatase	0,06	0,16	0,06	0,05	0,1		
5 (male)	The same	Invertase	10	20	7,5	8	5,5	50	—
		γ-Amylase	1	9	1,8	1,8	1,7	4,2	
		Alkaline phosphatase	0,06	0,05	0,06	0,07	0,02	0,06	
6 (male)	The same	Invertase	6,6	8	66	14	7	33	55
		γ-Amylase	2	2	24	4	2	14	8
		Alkaline phosphatase	0,06	0,12	0,06	0,1	0,09	0,08	0,05
7 (female)	Distal part of ileum	Invertase	10	20	24	20	21	—	—
		γ-Amylase	0,06	0,08	0,06	0,06	0,04		
		Alkaline phosphatase	0,06	0,18	0,30	0,20	0,16		

Legend. Alkaline phosphatase measured in micromoles substrate/min/mg protein; invertase and γ-amylase in mg glucose/min/100 mg protein.

and in the colonies, but this was not reflected in the individual properties of the macrocolonies, each of which preserved its own particular enzyme spectrum. Comparison of the changes in activity of these three enzymes in colonies of epithelial cells with their activity in the neighboring mucous membrane revealed an increase predominantly in the activity of one of the enzymes compared with the others. For instance, in colony 2, taken from the proximal part of the jejunum of rat No. 2, invertase activity was eight times higher than in the usual mucous membrane, γ-amylase activity was 4.1 times higher, and alkaline phosphatase activity 17 times higher. Meanwhile, in the neighboring colony 4 of the same rat, invertase activity was increased by 13.6 times, γ-amylase activity by 5.8 times, and alkaline phosphatase activity by 6.6 times. In other colonies taken from different parts of the small intestine, activity of one of the enzymes tested also was higher: invertase in colonies 5, 5, and 6 from rats Nos. 1, 5, and 6; γ-amylase in colonies 1, 1, and 1 from rats Nos. 1, 3, and 5, and alkaline phosphatase in colonies 4, 2, and 2 from rats Nos. 1, 2, and 7. No general rule for the change in enzyme activity of the macrocolonies connected with the sex of the animals could be detected.

The results of investigation of absorption of amino acids by the epithelium of the macrocolonies are given in Table 2. Analysis of these results shows that in any part of the small intestine the quantity of amino acids absorbed differs in any two neighboring macrocolonies. For instance, in colony 2 from rat No. 1 the quantity of lysine absorbed was 3.5 times greater than in colony 3 of the same rat. Similar differences could be seen in macrocolonies growing in other parts of the animal's intestine. In the mucous membrane of control neighboring segments of the intestine the lysine content deviated from the mean value by 8.3%, and fluctuated from 3.1×10^{-17} to 3.9×10^{-17} mole/mg tissue. No proximal–distal absorption gradient of the amino acid in the concentration tested could be found.

The study of absorption of leucine in macrocolonies of intestinal epithelium growing in different parts of the small intestine revealed a similar pattern: The quantity of the amino acid absorbed in one colony differed from that absorbed in a neighboring colony, often considerably (Table 2). Sometimes virtually no difference was found in the content of amino acids in individual colonies (colonies 1, 2, and 3 from rat No. 9), but in neighboring colonies this difference turned up again (colonies 3, 4, and 5 from rat No. 9). Absorption of leucine in control neighboring regions of the small intestine was considerably greater than the rate of absorption of the amino acid in the colonies and no clear proximal–distal differences were present.

TABLE 2. Absorption of Lysine and Leucine by Macrocolonies of Intestinal Epithelium in Different Parts of Small Intestine

Rat	Site of growth of macrocolonies	Macrocolony	Quantity of lysine absorbed, $\times 10^{-17}$ mole/mg	Rat	Site of growth of macrolonies	Macrocolony	Distal part of ileum
1 (female)	Proximal part of jejunum		3,6	6 (male)	Proximal part of jejunum		14,1
		1	0,4			1	2,3
		2	0,7			2	2,8
		3	0,2			3	0,3
						4	0,7
						5	1,9
2 (male)	The same		3,9	7 (male)	Proximal part of jejunum		14,3
		1	0,7			1	1,0
		2	0,5			2	0,3
		3	0,4			3	0,7
		4	0,6			4	0,6
						5	1,1
						6	1,5
3 (male)	The same		3,1	8 (male)	Proximal part of jejunum		12,4
		1	1,2			1	1,7
		2	0,9			2	1,3
		3	0,8			3	0,9
		4	0,5				
4 (male)	Middle part of jejunum		3,5	9 (male)	Middle part of jejunum		9,9
		1	1,2			1	0,5
		2	0,7			2	0,6
		3	0,6			3	0,5
						4	0,2
						5	0
5 (female)	Ileum		3,9	10 (male)	Distal part of ileum		5,7
		1	1,2			1	2,4
		2	0,3			2	1,2
		3	1,3			3	3,7

The results of these investigations indicate that in each group of cells developing from a single stem cell the activity of the three enzymes studied is strictly individual and differs from their activity even in a neighboring macrocolony. Predominance of one of the enzymes was found in many colonies. The unequal rates of absorption of lysine and leucine by the epithelium of individual macrocolonies also confirm the existence of definite functional differentiation of each macrocolony derived from the same stem cell. The lower rate of absorption of amino acids by the macrocolonies can evidently be attributed to the inadequate degree of maturity of the epithelium of the intestinal macrocolonies. The strictly individual functional specialization of each group of cells developing from one stem cell may not only depend on the regulating influences of the endocrine or nervous system but may evidently be attributable more to local regulatory changes, under genetic control. Very probably the difference in functional differentiation of the epithelial cells of the small intestine lies at the basis of the variegated pattern of changes in absorption of different substances.

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