HETEROGENEITY OF FUNCTIONAL PROPERTIES OF RAT SMALL INTESTINE EPITHELIOCYTES

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UDC 616.341-001.29-092.9-07.616.341-008.931-092.18

KEY WORDS: macrocolonies of rat small intestine epitheliocytes; invertase; dipeptidase; ^{3}H-thymidine; functional differentiation.

The study of enzyme activity of epitheliocytes in macrocolonies of the small intestine developing from a single stem cell has shown their strictly individual functional differentiation, with predominance of one particular enzyme in most colonies [1]. The dimensions of the colonies studied also varied considerably. It was accordingly suggested that correlation may exist between enzyme activity of the epithelium of the macrocolonies and their size. To test this hypothesis it was decided to compare the weight of test colonies of epitheliocytes and the activity of certain enzymes concerned with contact digestion of the cells of these colonies, in order to identify any possible relationship between the degree of enzyme differentiation of the macrocolonies and their size.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-200 g, kept on the ordinary animal house diet. The animals were anesthetized with pentobarbital (40 mg/kg), an operation was performed to exteriorize a loop of intestine 5-8 cm long, and the loop alone was irradiated in a dose of 18 Gy* (1 Gy = 10^{-2} rads, dose rate 0.055 Gy/sec) on the RUM-17 x-ray apparatus. On the 20th day after the operation and irradiation, the irradiated portion of intestine was removed, single isolated macrocolonies developing in the zone of irradiation from single stem cells were separated [4] and weighed, after which each separate colony was homogenized in 2 ml of Ringer's solution (pH 7.4, 4-6°C), and activity of the following enzymes was determined in the homogenate of each colony: invertase (EC 3.2.1.48) and dipeptidase (EC 3.4.13.11) by the method in [2]. Glycylglycine (from Merck, West Germany) was used as the substrate for determination of dipeptidase activity. For the 20-24 h before the experiment the animals were deprived of food. As an additional characteristic of the epitheliocytes of the macrocolonies, incorporation of ^{3}H-thymidine into the crypt cells, was investigated. On the 20th day after irradiation of the isolated segment of intestine, ^{3}H-thymidine was injected into the animals intraperitoneally in a dose of 3.7 Bq†/g body weight. The rats were decapitated 1 h after the injection, and the separate colonies and control areas next to them in the intestinal mucosa were removed. The colonies were homogenized in Ringer's solution to count the number of cells in each sample, after which the cells were disintegrated in 1 N KOH solution applied to a cardboard filter, after which the activity of each specimen on the filter was determined in toluene scintillation fluid on the SL-30 spectrometer (from Intertechnique, France). The quantity of ^{3}H-thymidine per cell was calculated from the activity thus obtained. Altogether 52 colonies in different parts of the small intestine of 10 rats were studied.

EXPERIMENTAL RESULTS

As Table 1 shows, enzyme activity of the colonies diminished with an increase in their size. Colonies weighing only a little (2-4 mg) had the highest enzyme activity, mainly of one of the two enzymes studied. In colonies weighing over 5 mg invertase and dipeptidase activity were far lower, nearer to the levels of enzyme activity in the control areas of the mucosa. In macrocolonies of this type, predominance of one of the two test enzymes was virtually absent, i.e., enzyme differentiation did not take place.

*Abbreviation for Gray, the SI unit of absorbed radiation (translator's note).
†Abbreviation for Becquerel, the SI unit of radioactivity; 1 Bq = 3.7 Ci.

Department for Research into Combined Procedures, Research Institute of Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk. (Presented by Academician of the Academy of Medical Sciences of the USSR G. A. Zedgenidze.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 6, pp. 741-743, June, 1981. Original article submitted May 19, 1980.

0007-4888/81/9106-0825$07.50

TABLE 1. Enzyme Activity and Weight of Macrocolonies of Rat Small Intestinal Epithelium

No. of rat	Parameter studied	Intact mucosa 10 cm away from region of colonies (control)	Macrocolonies						
			1	2	3	4	5	6	7
1	Invertase activity	3,5	47	31	17	7	6	—	—
	Peptidase activity	3,5	21	12	8,2	7,6	7	—	—
	Weight of macrocolonies	11	2	3	4	5	6	—	—
2	Invertase activity	3,4	26	28	12	5	2,6	—	—
	Peptidase activity	3,3	28	19	9,6	5	3	—	—
	Weight of macrocolonies	36	2	3	4	8	24	—	—
3	Invertase activity	3,1	28	12	25	4,5	6	4	3,5
	Peptidase activity	3,6	23	13	11	6	4	3	2,6
	Weight of macrocolonies	17	2	3	3	8	10	15	19
4	Invertase activity	3,0	25	19	8	3	2,6	2,5	2,6
	Peptidase activity	3,1	20	11	9	2,9	3,1	3	2,8
	Weight of macrocolonies	17	2	3	5	17	18	20	25

Legend. Invertase activity given in mmoles/-min/g, dipeptidase activity in μmoles/min/mg, weight of macrocolonies in mg.

TABLE 2. Incorporation of ^{3}H-Thymidine into Cells of Macrocolonies

No. of rat	Place of growth of macrocolonies	Parameter studied	Intact mucosa 10 cm away from region of colonies (control)	Macrocolonies							
				1	2	3	4	5	6	7	8
1	Small intestine	Weight of macrocolonies	6	2	4	6	6	7	—	—	—
		Incorp. of ^{3}H-thymidine	24	5	8	22	24	28	—	—	—
2	" "	Weight of macrocolonies	10	3	4	5	6	6	6	6	7
		Incorp. of ^{3}H-thymidine	24	14	12	25	20	27	30	26	34
3	Ileum	Weight of macrocolonies	4	1	2	7	—	—	—	—	—
		Incorp. of ^{3}H-thymidine	19	2	4	21	—	—	—	—	—
4	"	Weight of macrocolonies	7	1	3	7	—	—	—	—	—
		Incorp. of ^{3}H-thymidine	18	3	16	18	—	—	—	—	—
5	"	Weight of macrocolonies	8	3	3	4	5	7	15	—	—
		Incorp. of ^{3}H-thymidine	22	9	9	10	14	16	17	—	—
6	"	Weight of macrocolonies	10	6	14	15	—	—	—	—	—
		Incorp. of ^{3}H-thymidine	22	14	18	21	—	—	—	—	—

Legend. Weight of macrocolonies given in mg, incorporation of ^{3}H-thymidine in cpm $\times 10^{-4}$ per cell.

Investigation of incorporation of ^{3}H-thymidine into cells of the macrocolonies also revealed considerable heterogeneity, associated with colony size (Table 2). In small macrocolonies (1-4 mg) the rate of incorporation of the label was low and varied from 2×10^{-4} to 16×10^{-4} cpm per cell. With an increase in size of the colonies (over 5 mg) activity of the label in the cells increased, varying from 14×10^{-4} to 34×10^{-4} cpm per cell of the macrocolony, about the same as the rate of incorporation of ^{3}H-thymidine into cells of the control areas of mucosa (18×10^{-4}-24×10^{-4} cpm per cell).

These results are evidence of functional heterogeneity of macrocolonies of rat small intestinal epitheliocytes developing from single stem cells. Small colonies (weighing under 5 mg) have high enzyme activity with well-defined differentiation – predominance of activity of one of the test enzymes. DNA synthesis, mea-

sured from the rate of incorporation of ^{3}H-thymidine, was retarded in the cells of these colonies, further confirmation of their particular biological features. Cells of macrocolonies weighing over 5 mg possessed enzyme activity and also a rate of DNA synthesis close to those in control areas of the mucosa. Colonies of this type constituted the overwhelming majority, and it is therefore likely that when the functional characteristics of the mucosa as a whole are studied, their indices may mask the functional indices of the small number of highly differentiated cells of "small" colonies (under 5 mg). The existence of functional heterogeneity of the epitheliocytes of the mucosa evidently has definite biological significance, for it endows it with greater resistance to the action of unfavorable factors. For example, in radiation injury to the intestine, cells with lower mitotic activity will be more radioresistant and will ensure the more rapid recovery of the mucosa after irradiation. The cause of the "decrease" in proliferative activity of the epitheliocytes of the mucosa during prolonged fractional irradiation of the abdominal region may perhaps be the greater survival rate of the slowly proliferating cells of "small" macrocolonies [3].

The author is grateful to Corresponding Member of the Academy of Sciences of the USSR A. M. Ugolev for interest in and help with these investigations.

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METABOLISM OF ERYTHROCYTIC CHALONE IN VITRO

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UDC 612.111.014.3.018:612.6

KEY WORDS: chalone; myelokaryocytes; metabolism.

It was shown previously that the chalone activity of erythrocyte extracts is associated with protein fraction 1 [2].

In the investigation described below the metabolism of this fraction was studied during culture of bone marrow cells.

EXPERIMENTAL METHOD

Fraction 1 was isolated from erythrocyte extracts and concentrated to 11 mg/ml by the method described in [2]. The isolated protein, corresponding to a single peak on the densitogram, was conjugated with fluorescein isothiocyanate (FITC, from Serva, West Germany), using 0.5 M carbonate buffer, pH 9.5, for this purpose [3]. The labeled protein was separated from the unbound fluorochrome on a column (1.2 × 30 cm) packed with Sephadex G-25 and reconcentrated to the original volume. The labeled protein was added to bone marrow cells isolated from the femors of mice, pipeted in Hanks' solution, and centrifuged for 15-20 min at 1000 rpm. The sedimented cells were suspended in Hanks' solution and poured in equal numbers into centrifuge tubes. Bone marrow from 20 noninbred albino mice weighing 18-20 g was used. A mixture of cells from five animals corresponded to each experimental point.

The following series of experiments were carried out with the cell suspensions thus obtained.

Series I. Labeled protein was added to the myelokaryocytes at the rate of 0.05 ml to 2 ml of suspension. After 1 min the cells were shaken, washed 3 times with cold Hanks' solution to remove unbound protein, and

Department of Pathological Physiology, Moscow Medical Stomatologic Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 6, pp. 743-745, June, 1981. Original article submitted May 6, 1980.