RELATION BETWEEN EPITHELIAL MITOTIC ACTIVITY IN CRYPTS OF SMALL INTESTINE AND STATE OF NERVOUS SYSTEM

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Investigations conducted in recent years, mainly on corneal epithelium, have revealed the important role of the central and peripheral divisions of the central nervous system in the regulation of mitotic activity [1,4,5, 8,11]. The relation between the mitotic activity in the epithelium of Lieberkuhn's crypts and the state of the nervous system has been studied by relatively few investigators [2,3,6,11], and this question cannot be regarded as adequately clarified.

The problem of the present investigation included a study of the effect of changes in the peripheral divisions of the nervous system on the mitotic activity in the epithelium of Lieberkuhn's crypts.

Experimental Method

The experiments were conducted on 104 sexually mature white male rats weighing 180-200 g each. In animals of the first group, we studied the mitotic activity after partial compound denervation of different portions of the intestine, and in animals of the second group after alteration of the state of the autonomic divisions of the nervous system by the injection of pharmacological agents.

With animals of the first group, we conducted two series of experiments. In the first series, the innervation was destroyed by separation and transection of two or three of the intestinal nerve branches which pass in neurovascular bundles through the mesentery to the portion of the ileum located 6 cm from its junction with the cecum. On the 5th, 10th, and 15th postoperative days, we studied the mitotic activity of this portion (distal) and a portion situated 5 cm proximal of the first (first proximal).

In the second series of experiments, the destruction of innervation was effected by incision of the mesentery at its site of attachment to the intestinal loop over a distance of 2 cm along the intestine. In some of the animals of this series we destroyed the innervation of the same portion of the intestine as in the previous series of experiments (distal), and in the rest of the animals we denervated the portion situated 5 cm from the pyloric part of the stomach (second proximal). In all the animals of this series, we studied the mitotic activity in the epithelium of the second proximal and distal portions of the intestine on the 10th postoperative day.

The second group of investigations also included two series of experiments. In the first series we studied the mitotic activity in the case of enhanced tonicity of the sympathetic division of the nervous system (a single subcutaneous injection of adrenalin in a dose of 1 mg per 1 kg of body weight), and in the second series of experiments – in the case of enhanced tonicity of the parasympathetic division [single subcutaneous injection of proserine (neostigmine) in a dose of 0.1 mg per 1 kg of body weight]. In both series, the intestine was studied $1\frac{1}{2}$ hr after injection of the autonomotropic substances.

TABLE 1

Change in Mitotic Activity After Transection of Intestinal Nerve Branches

Time after operation	Subgroup	Number of mitoses ($M \pm m$)		Difference between control and experiment ($M_e - M_c$)		Criterion of significance of difference of means $t = \frac{(M_e - M_c)}{(m_e^2 + m_c^2)^{\frac{1}{2}}}$	
		proximal portion	distal portion	proximal	distal	proximal	distal
5th day	Control (5 animals)	389 ± 18.4	359 ± 19	113	81	3.1	2.9
	Expt. (8 animals)	276 ± 31.2	278 ± 20.3				
10th day	Control (7 animals)	364 ± 16.1	367 ± 14.4	42	129	1.55	5.81
	Expt. (10 animals)	322 ± 21.7	238 ± 16.9				
15th day	Control (6 animals)	348 ± 20	370 ± 26.1	49	77	1.48	2.0
	Expt. (9 animals)	299 ± 26.3	293 ± 27.4				

TABLE 2

Change in Mitotic Activity After Incision of the Mesentery at the Proximal and Distal Portions of the Intestine (10th Postoperative Day)

Operation	Number of mitoses ($M \pm m$)		Difference between 2nd proximal and distal portions in absolute units ($M_{prox} - M_{dist}$)	Criterion of significance of difference of means $t = \frac{(M_{prox} - M_{dist})}{(m^2_{prox} + m^2_{dist})^{\frac{1}{2}}}$
	in second proximal portion	in distal portion		
Destruction of innervation in second proximal portion (9 animals)	275 ± 26.2	406 ± 31	131	3.2
Destruction of innervation in distal portion (9 animals)	383 ± 19.9	282 ± 18.7	101	3.7

In addition to the experimental animals, each series of experiments included approximately the same number of control animals. All the animals before the experiment and after it were kept in the same conditions. In the series of experiments involving partial compound denervation, we opened up the abdominal cavity of the control animals and stitched it up again. In the series in which the autonomotropic substances were used, we injected an equal quantity of physiological saline into the control animals. In every case, the experimental and control animals were sacrificed by decapitation at the same time of day, three to four hours after being fed. The portion of the intestine, cut up lengthwise and spread on thin card, was fixed in Maximow's fluid or alcohol-formalin. In paraffin-embedded hematoxylin-stained 8μ sections prepared from this portion, we counted the number of mitoses in 100 fields in every case. The field of view was delimited by a 3.8 × 5.5 mm diaphragm inserted in the ocular. Under magnification by ocular 7 and objective 90, the diaphragm isolated a region of mucous membrane containing the entire width of the longitudinally sectioned crypt and a 68.75μ portion of its length. By gradually shifting the preparation in one direction along the length of the section, we used crypts in which the bottom and all of the lumen right up to the mouth were visible. Since the diaphragm delimited only

TABLE 3

Mitotic Activity $1\frac{1}{2}$ Hr After Subcutaneous Injection of Adrenalin and Proserine

Series	Subgroup	Number of mitoses ($M \pm m$)	Difference between control and experiment ($M_e - M_c$)	Criterion of significance of difference of means $t = \frac{(M_e - M_c)}{(m_e^2 + m_c^2)^{\frac{1}{2}}}$
Third (adrenalin)	Control (7 animals)	400 ± 12.9	129	7.3
	Experiment (7 animals)	271 ± 12		
Fourth (proserine)	Control (14 animals)	421 ± 29	4	0.12
	Experiment (13 animals)	417 ± 19		

part of the length of one crypt, we counted the mitoses successively in the region next to the bottom, then in the region situated above, and so on over the whole length of the crypt. In this way, we counted 100 diaphragm-limited fields. The results were subjected to statistical treatment.

Experimental Results

The results of the mitotic count in animals of the first series of the first group of investigations are given in Table 1 in the form of mean numbers for the experimental and control of animals.

An analysis of these results shows that in animals with destroyed intestinal innervation, the number of mitoses in the crypt epithelium was reduced on the 5th postoperative day both in the intestinal portion (distal) supplied directly by the transected intestinal nerve branches, and in the portion (first proximal) adjacent to it. In the distal portion the reduction was 23%, and in the first proximal portion 29%, of the number of mitoses in the corresponding portions of the intestine of the control animals (the difference was statistically significant in the first proximal portion, and almost significant in the distal portion of the intestine).

By the 10th day, the number of mitoses in the crypts of the distal portion of the intestine had fallen still further [the difference in the numbers of mitoses in this portion of the intestine in the experimental and control animals was 35% (statistically significant)]. At the same time, the number of mitoses in the first proximal portion approached normal (the difference in the number of mitoses in the first proximal portion in the control and experimental animals was now 12%). Consequently, in the experimental animals, there was now a difference in the numbers of mitoses in the distal and first proximal portions. The mitotic activity of the portion supplied by the transected nerve branches was 26% lower on the average than the mitotic activity of the adjacent portion (statistically significant difference). In the control animals, the difference in the numbers of mitoses in these same portions did not exceed 6%.

By the 15th day after partial denervation of different loops of the intestine, the mitotic activity in the crypt epithelium in the experimental animals had returned to normal. On statistical analysis of the figures the difference in the numbers of mitoses in the control and experimental animals was found to be insignificant (in the distal portion the number of mitoses in the experimental animals was 17% lower, and in the first proximal portion 14% lower). There was no difference in the numbers of mitoses in the crypt epithelium in the distal and first proximal portions of the intestine.

Thus, in the portion of the intestine with destroyed innervation, the number of mitoses was reduced. A change of similar nature, only less pronounced, occurred in the adjacent portion. In the latter case, the change was probably due to the proximity to the portion with destroyed innervation and the close nervous links between these portions.

In the second series of experiments, we counted the number of mitoses not in the adjacent portions of the ileum, as in the first series, but in the portion of the ileum (distal) and in the portion located 5 cm from the pyloric part of the stomach (second proximal). According to published results [9] and the results of our own counts, there are no differences in the numbers of mitoses in these portions of the intestine in intact animals. The numbers of mitoses in these portions after the destruction of innervation in one of them are shown in Table 2.

It follows from Table 2 that the number of mitoses in the epithelium of Lieberkuhn's crypts of intestinal portions with destroyed innervation, irrespective of whether the portion is the distal or second proximal, was lower than in the portions situated at a considerable distance from the site of damage: In the second proximal portion the reduction was 32%, and in the distal portion it was 26%.

In cutting the mesentery in the second series of experiments, we damaged the tiny blood vessels as well as transecting the nerve branches. However, the changes in the numbers of mitoses in these animals did not differ from the changes in animals in which only the nerve branches were cut.

We can infer from this that a relatively slight disturbance of the blood supply has no particular effect on the mitotic activity on the 10th postoperative day.

The first group of investigations shows that partial denervation of different portions of the intestine causes a reduction of mitotic activity in the epithelium of Lieberkuhn's crypts in these portions. The reduction is most pronounced on the 10th postoperative day. This kind of change in mitotic activity occurs also in other organs [5,8,12], and indicates the role of the state of the nervous system in the regulation of cell division.

In the second group of investigations (third and fourth series), we tried to determine the role of certain parts of the peripheral nervous system in the regulation of cell division.

The literature contains some information on the nature of the part played by the sympathetic division of the nervous system in the regulation of mitotic activity in corneal epithelium: The sympathetic division of the nervous system has an inhibiting effect on mitotic activity, and the parasympathetic division has a stimulating effect [7,10].

The results of our counts of the numbers of mitoses in the epithelium of Lieberkuhn's crypts in the case of enhanced tonicity of the sympathetic or parasympathetic divisions of the nervous system are shown in Table 3.

Table 3 shows that the injection of adrenalin leads to a mean reduction of 32% in the mitotic activity, while the injection of proserine produces no changes in mitotic activity (the autonomotropic agents were injected in doses which affect the mitotic activity of corneal epithelium) [10].

An analysis of the cited results shows that the mitotic activity in the epithelium of Lieberkuhn's crypts depends on the state of the peripheral divisions of the nervous system. In its main features, this relationship is of the same nature as the relationship between the mitotic activity and the state of innervation in the tissues of other organs.

In addition, we should note a certain special feature in the regulation of the mitotic activity in the epithelium of Lieberkuhn's crypts by the autonomic system. This is the similarity between the response of cell division in the epithelium of the intestinal crypts, and the response of cell division in other organs to change in the sympathetic nervous system, and the absence of such response to change in the parasympathetic nervous system.

SUMMARY

Experiments were performed on albino rats. Two to three intestinal nerve branches were cut in the mesentery of the small intestine, and the mesentery was cut (2 cm) at the site of attachment to the intestinal loop. Mitosis was reduced by 26-35% on the 10th postoperative day as compared with the intestine of control animals, and the intact intestinal loop in the operated animals. Enhanced sympathetic tonicity (adrenalin administration) reduces the mitotic activity in the epithelium of the small intestine, whereas enhanced parasympathetic tonicity (proserine administration) has no effect on this activity. Changes of the mitotic activity in the epithelium of the crypts following surgical intervention on the peripheral nervous system point to the relationship of this activity to the state of the nervous system.

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