THE EFFECT OF EXTENSIVE RESECTION OF THE SMALL INTESTINE IN RATS ON THE PHYSIOLOGICAL REGENERATION OF THE INTESTINAL EPITHELIUM

N. P. Bochkov

From the Laboratory of Growth and Development (Director – Doctor of Biological Sciences, L. D. Liozner) Institute of Experimental Biology (Director – Professor I. N. Maisky) Academy of Medical Sciences, USSR, Moscow.

(Received May 10, 1957. Presented by Active Member of USSR Academy of Medical Sciences, N. N. Zhukov-Verezhnikov)

We [1] established that within 10 to 30 days after resecting $^1/_6$th to $^1/_7$th of the small intestine of rats there was a decrease in the number of mitoses seen in epithelium of the crypts in areas adjacent to the place of anastomosis (both proximal and distal to it) when a comparison was made with corresponding areas of the intestine in unoperated rats. At the same time, the bowel of the operated animals maintained the definite reaction of increased mitotic response to unconditional reflex food stimulators. Sixty days after the operation, the mitotic epithelial activity within the crypts of the operated rats was greater than the activity shown by the control animals or by the operated animals sacrificed 10 or 30 days after the operation. By this time, hypertrophy is apparent from the increased diameter of the bowel. Ninety days after the operation, the number of mitoses in the bowel diminishes but still remains above the level observed in the control rats.

However, it was not clear what produced the alterations in the mitotic activity within the mucosa of the bowel of the operated rats: i.e., the trauma caused by the resection or the increased functional load placed upon the remaining small intestine. Moreover, it was of interest to make a more detailed study of the destructive phase of the physiological regeneration of the small intestine after a portion of it had been resected.

In the present study we examined the physiological regeneration of the small intestine following resection of almost 50% of its length. We examined areas far removed from the anastomosis, included portions of the bowel partially excluded from the digestive process.

EXPERIMENTAL METHODS

We used 16 white rats weighing 120-180 grams. Eight were used as controls, while the other eight were operated in the manner shown in the diagram.

Mentally, we separated the intestine into four equal areas. Portion No. 2, along with its mesentery, was resected. Then the distal part of portion No. 1 was approximated to the distal part of portion No. 3, marked by us as "blind," and a side-to-side anastomosis was done. In this fashion, the blind area (No. 3) along with its mesentery, remained parallel to the proximal area (No. 1). The blind area was attached to the proximal portion by three separate sero-muscular silk sutures. On subsequent examination, we were able to prove that no food would enter the blind portion of the intestine except at the orifice directly adjacent to the anastomosis. Immediately after the operation, the animals were taken to the vivarium and maintained there under the same conditions as the control rats. Both the control and experimental animals were sacrificed 30 days after surgery. This time interval was chosen because it was the time when the operated rats would reach the weight of the control rats of the same age. For two days before being sacrificed, the animals were given no food – only water. After the animals had been killed, their intestines were cut off from the mesenteries and totally fixed in Bouin's fluid.

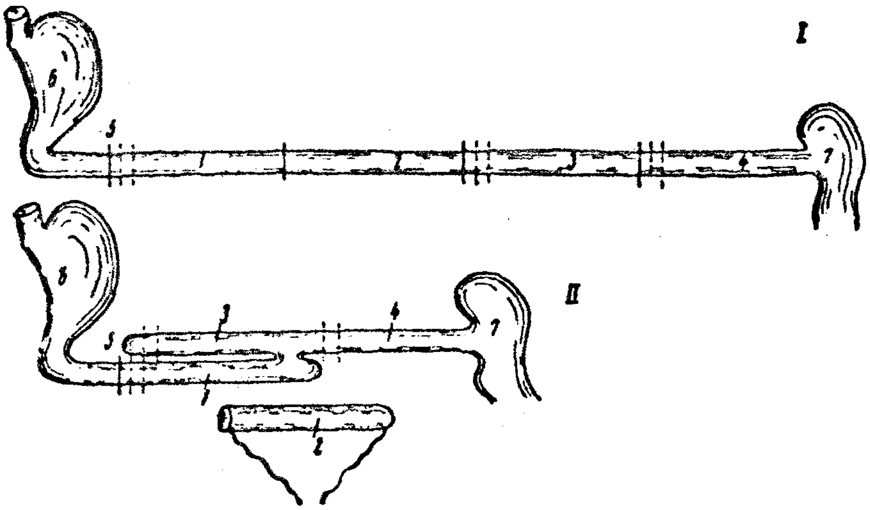

Diagram of experimental procedure. I) Before surgery; II) after surgery. 1) Proximal portion; 2) resected portion; 3) intermediate portion; 4) distal portion; 5) place where bowel passes through the mesocolon; 6) stomach; 7) cecum. Dots indicate areas taken for histologic studies.

We subjected three different areas of the intestine to morphological studies; the regions chosen were those indicated by the dotted lines of the diagram. Corresponding regions were chosen in the control animals. The material was imbedded in paraffin, longitudinal sections 7 μ thick were taken, and eosin–hematoxylin stain was used. In all the chosen areas, we counted the number of mitoses seen in 40 crypts of the sections taken longitudinally through the centers, and we then took the average number per crypt.

In addition, we qualitatively evaluated the process of epithelial sloughing and disintegration. Arbitrarily we considered it to be the first stage of sloughing either when individual cells were separating at the villous tip or when a space filled with albuminous fluid formed between the epithelium and stroma. Stage 2 was taken as being characterized by epithelial separation of a whole sheet of cells for no less than $^1/_4$ th or more than $^1/_2$ of the length of the villus. Stage 3 of sloughing was considered as having been reached when epithelial separation involved the lower half of the villus. When the epithelial sheet had already disappeared and the villus was left with only the stroma for most of its length the condition was classified as stage 4. In each rat, 100 villi were examined, each villus being categorized as pertaining to one of the 4 stages just described; then the average of the four stages of sloughing was calculated.

This qualitative evaluation of the destructive phase of physiologic regeneration is far from being a completely satisfactory method. Indeed, it is not easy to take our arbitrary division into stages as really representing the intensity of the sloughing or reflecting the various phases of the process. In addition, the end result of the secretory process depends not only on the intensity of its individual phases but also on their velocities, all these aspects of the situation being beyond the scope of our experiments at present. In spite of all this, we deemed it essential to estimate (even if only approximately) the intensity of the destructive phase of physiological regeneration in the control and experimental rats, as we did find that in the operated animals the intestinal lumen contained a larger number of sloughed cells.

EXPERIMENTAL RESULTS

The results of the mitosis count are shown in Table 1.

TABLE 1

The Average Number of Mitoses Per Crypt

Area	Rat No. 1	2	3	4	5	6	7	8	Average
	Operated Rats (experimental)								
Proximal	6.5	3.9	6.2	5.6	6.2	4.5	5.8	5.4	5.5
Middle	2.4	2.7	3.5	2.3	3.2	2.6	2.6	2.6	2.7
Distal	3.7	3.0	3.5	3.9	3.7	3.9	4.2	4.1	3.8
	Unoperated Rats (control)								
Proximal	4.5	3.6	3.1	3.0	2.9	3.6	3.3	4.2	3.5
Middle	3.0	2.5	3.1	3.1	3.6	2.5	2.0	2.1	2.7
Distal	2.8	2.0	2.8	2.5	2.9	2.1	2.2	2.8	2.5

As can be seen by examining this table, the average number of mitoses per crypt in the proximal portion of the bowel was 5.5 in the operated rats and 3.5 in the unoperated rats. In the middle area the number was 2.7 for both the operated and unoperated rats. The distal portion showed a greater mitotic activity in the operated animal as compared with the unoperated, the ratio being 3.8 mitoses to 2.5. The relation of the phases of the mitosis was the same for the operated and unoperated animals.

TABLE 2

Sloughing Stages in Arbitrary Units Per Each Villus

Area	Rat No. 1	2	3	4	5	6	7	8	Average
	Operated Rats (experimental)								
Proximal	3.33	2.59	3.75	1.91	1.68	2.09	3.2	2.72	2.68
Middle	1.9	1.76	1.41	2.0	0.54	0.73	1.45	0.55	1.29
Distal	3.45	1.73	2.76	2.35	1.76	0.55	0.92	1.82	1.91
	Unoperated Rats (control)								
Proximal	1.21	1.6	0.69	1.03	1.28	1.58	1.53	2.61	1.41
Middle	0.59	0.54	1.99	0.73	0.56	0.84	0.37	0.49	0.76
Distal	0.77	0.3	0.21	1.22	2.02	0.97	1.0	3.07	1.19

Thus, after partial intestinal resection, there is in the remaining bowel, an intensification of the processes characterizing physiological regeneration: in the operated rats, the proximal portion of the bowel shows a 36% increase in mitotic activity, while in the distal portion, the figure is 34% – all these values being taken as comparisons with similar areas studied in the unoperated animals. This difference in the mitotic counts is statistically valid as P equals 0.01. All this is true, however, of those portions of the intestine over which food passes. In the middle ("blind") portion of the intestine in the operated rats, increased mitotic activity was not observed. From this it may be concluded that the alterations within the time after the operations were not due to trauma but resulted from the intestinal resection. All the rats were sacrificed under conditions such that their digestive cycles were similar, so that the increase in the number of mitoses can be regarded as a manifestation of restorative processes attempting to compensate for the resection of almost 50% of the small intestine. The fact that the number of mitoses in that middle portion of the intestine in the operated rats over which food did not pass was similar to the number seen in a comparable area in the unoperated rats compels us to suppose that the regenerative reaction of the intestine is affected directly by the passage of food through it or, in other words, that it is a function of the imposed load. Our work does not permit a decision as to whether the increase in the number of mitoses is due to increased intestinal function or due to its structural reconstruction. The possibility that both processes are proceeding simultaneously is not excluded.

The processes of epithelial sloughing and disintegration are expressed in Table 2.

Table 2 demonstrates that in the operated animals the disintegration of the epithelium proceeds more intensively, the differences being seen mainly in the proximal and distal portions of the bowel and to a much lesser degree in the middle section. The differences in the intensity of sloughing as observed between the operated and unoperated animals are statistically valid for the proximal portion. The intensification of the destructive phases in those areas where there was food passage leads us to the supposition that in the time interval after operation the processes of functional intensification predominate over those of morphological restoration. Our results on epithelial sloughing and disintegration after partial intestinal resection are in accord with the experiments of V. P. Mandzhagaladze [2] who demonstrated an increase in the solid components of intestinal secretion from an isolated portion of the intestine after the lower division of the small intestine had been resected.

SUMMARY

Eight white rats were operated, while 8 other rats were used as controls. Approximately 50% of the small intestine was resected in the 8 operated animals and, also, a blind pouch was created as shown in the diagram depicting the procedure employed. All the animals were sacrificed 30 days after surgery. The increase in the mitotic activity and the intensification of the sloughing processes seen in the intestines of the operated animals as compared with the controls was taken to be an indication of an increase in physiological regeneration. As there was no intensification seen in the blind pouch that had been constructed, it was concluded that this intensification was not due to the surgical trauma but due to the hypertrophic and compensatory processes caused by the food passing over a smaller amount of intestine remaining and therefore producing a functional overload.

LITERATURE CITED

[1] N. P. Bochkov, Byull. Ekspti. Biol. i Med. 44, 9, 97-101 (1957).*

[2] V. P. Mandzhagaladze, Theses of the 10th Scientific Congress, Institute of Nutrition, Academy of Medical Sciences, USSR, (Moscow, 1956).

* Original Russian pagination. See C.B. Translation.