# EXPERIMENTAL BIOLOGY

## INVESTIGATION OF PHYSIOLOGIC REGENERATION IN THE RAT SMALL INTESTINE FOLLOWING ITS PARTIAL RESECTION

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Following resection of part of the intestine definite morphologic changes take place in the remaining part, viz. there is enlargement of the lumen, thickening of the mucosa and the annular muscular layer, hypertrophy of the lymphatic elements, increase in the number and size of villi (Monari [14], Stasov [9], Bornstein [13], Novikov [7]). The stomach, duodenum and the colon also participate in compensatory processes which occur after intestinal resection. Both the motor and secretory functions of these organs undergo changes: the passage of food along the gastro-intestinal tract is slowed (Stasov [9], Lampert [4]). Mandzhgaladze [5] established on dogs that following partial resection of the intestine the solid part of the digestive juice secreted by the isolated part showed marked increase of activity of the main intestinal juice enzymes (enterokinase, phosphatase, saccharase). After resection of the lower part of the small intestine the isolated part of the intestine secretes juice in which the percentage of solid constitutents is higher than prior to resection.

Depsite the large number of papers devoted to "functional and hypertrophic compensation" (B. D. Stasov) which occurs after resection of part of the intestine, none of the investigators has carried out detailed analysis of the course of this process, restorative in character, or undertaken to clarify the mechanisms underlying it; nor has the functioning of the intestine during this period been studied.

In approaching the study of restorative processes occurring in the intestine after resection we started with the concept that the enzyme-secreting function of the intestine was reflected morphologically in manifestations of its physiologic regeneration [3]. The destructive phase of physiologic regeneration is reflected in exfoliation and sloughing of epithelial cells and sheets into the lumen of the intestine [8, 14], the sloughed epithelium, which constitutes the solid part of intestinal juice, forming the main source of the latter's enzymes [6, 10, 12]. S. L. Blyakher [2] noticed that fasting rats showed predominance of sloughing and disintegration of epithelium whereas in the case of animals which had been fed the epithelial sheet was continuous. As regards restorative processes in intestinal epithelium, it has been established that the number of mitoses in the crypts of animals which had been fed and "conditionally fed" (teasing with food) increases as compared to fasting animals (Khrushchev [11], L. Ya. Blyakher [1]).

The problem with which the present communication is concerned was to discover how the physiologic regeneration of the intestine was altered following its partial resection and what were the restorative processes occurring in it during this period.

Attention was mainly concentrated on the study of the restorative phase of physiologic regeneration. Comparison was made of the number of mitoses in the intestinal crypts of fasting animals and those which had been fed, both unoperated and subjected to resection of 1/7th of the small intestine. Such a comparison made it possible to find out whether an increase of the number of mitoses in the crypts following feeding, such as was consistently seen in non-operated animals, also occurred in the operated ones. Moreover, an attempt was made to discover whether the operated rats preserved their ability to react to natural conditioned food stimuli by means of increasing number of mitoses in the crypts.

## EXPERIMENTAL METHODS

Experiments were performed on white rats weighing 200-250 g. Nine rats served as controls, the remainder (25 animals) were subjected to operation. The abdomen was opened under ether anesthesia and 8-10 cm of the intestine was resected; this constituted 1/6th-1/7th of its length. The anterior limit of the resected portion always passed 3 cm below the point of passage through the mesocolon. "End to end" anastomosis was made with the help of silk sutures. The period of observation varied in different series. When the operated animals were sacrificed a length of intestine measuring 3 cm and including the anastomosis was dissected out; a length of intestine corresponding to the site of resection in the experimental animals was removed from the control animals when they were sacrificed. The tissues were fixed in Bouin's mixture and paraffin blocks were made. Sections 7μ in thickness were stained with hematoxylin-eosin. Mitoses were counted in central longitudinal sections of 30-20 crypts; the average number of mitoses per crypt was calculated.

## EXPERIMENTAL RESULTS

Six rats were sacrificed 10 days after operation; of these 3 had been fasting for 50 hours and 3 had fasted 48 hours and were then fed during 2 hours. The number of mitoses above and below the anastomosis was counted (Table 1).

TABLE 1

Number of Mitoses per One Crypt 10 Days After Operation

| Number of rat | 1 | | 2 | | 3 | | Average | |
|---|---|---|---|---|---|---|---|---|
| Nature of experiment | above the anastomosis | below the anastomosis | above the anastomosis | below the anastomosis | above the anastomosis | below the anastomosis | above the anastomosis | below the anastomosis |
| Fasting for 50 hours | 2.8 | 3.0 | 2.9 | 2.9 | 2.7 | 2.7 | 2.8 | 2.9 |
| Fasting for 48 hours + feeding for 2 hours | 6.1 | 6.4 | 6.6 | 6.6 | 6.6 | 6.7 | 6.5 | 6.6 |

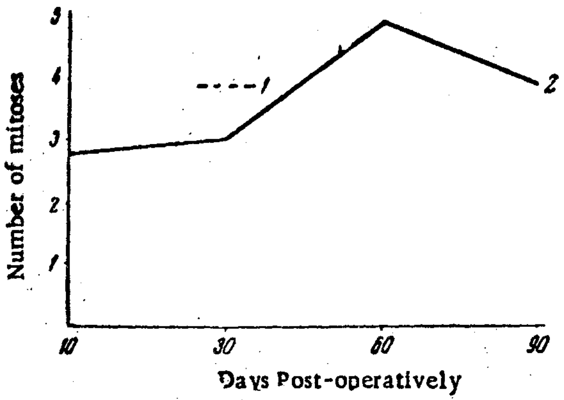
As can be seen from Table 1 the average number of mitoses per one crypt was equal to 2.8 in fasting rats and 6.5 in animals which had been fed. The number of mitoses in the regions above and below the anastomosis was approximately the same. In the case of rat No. 2 (fed) the number of mitoses in both segments was the same and equalled 6.6; in rat No. 3 (fed) there were 6.7 mitoses per crypt in the anterior portion of the intestine and 6.6 in the posterior portion; in rat No. 3 (fasting) the number of mitoses was 2.7 in both portions of the intestine.

Microscopic examination of the villi showed that marked sloughing of epithelium was taking place in the operated fasting rats, whereas in the case of the animals which had been fed almost all the villi were covered by an uninterrupted sheet of epithelium.

Analysis of data provided by the 10-day postoperative period reveals that the epithelium of the small intestine mucosa reacts to fasting and intake of food similarly to normal as early as 10 days postoperatively. Apparently holocrine secretion of enzymes occurs in the anterior and posterior portions of the intestine, which is comparable to events taking place in the unoperated animals. This can be deduced from the presence of approximately equal numbers of mitoses in the crypts of these segments as well as from the morphological features of the villi characteristic for the normal.

Consequently, 10 days postoperatively apparently no substantial changes in the processes of physiologic regeneration take place.

Thirty days following operation 18 rats were sacrificed (9 operated and 9 control). Six rats (3 operated and 3 control) had fasted for 50 hours, 6 had fasted for 48 hours and were then exposed for 2 hours to the sight and smell of food (kept under netting) which they could not reach. These formed the "conditionally fed" group of animals. In the experimental rats the mitoses were counted in the segment above the anastomosis. Results of the mitotic counts are presented in Table 2.




Changes in mitotic acitivity in the process of regeneration in the rat.
1) control; 2) experiment.

As Table 2 indicates, the average number of mitoses per one crypt in operated fasting animals is 3.0, in animals which had been fed 5.8 and conditionally fed ones 4.5; in unoperated rats the number of mitoses is 3.9, 5.5, 5.0 respectively. The epithelium of the intestinal mucosa in the operated rats thus reacts to intake of food or being "teased" by food in exactly the same way as that which is characteristic for unoperated rats. Statistical treatment of the data showed that the differences between the values for fasting rats on the one hand and fed ones on the other were beyond the limits of chance deviation. Investigation carried out on conditionally fed animals demonstrated that the part of the small intestine remaining after resection retained the ability to react in the usual manner to the regulating influences of the control nervous system.

Two fasting and 2 fed rats were sacrificed on the 60th postoperative day (Table 3).

As in the earlier stages of the experiment, the intestinal epithelium was found to have reacted to intake of food by increasing numbers of mitoses. It is interesting that in the fasting operated rats the number of mitoses was considerably increased by comparison with their number in fasting operated animals examined 10 and 30 days after resection. This increase was statistically significant. The difference in the number of mitoses in the intestinal crypts of the operated and unoperated rats can be interpreted as an indication of the presence of hypertrophy in the portion of the intestine remaining after resection. This interpretation is confirmed by manifestations of hypertrophy, observed both macro- and microscopically. The external diameter of fixed intestine was measured. Measurements were made every 3 cm, 20 measurements on the average for every rat. The figures obtained are presented in Table 4.

TABLE 2

Number of Mitoses per one Crypt 30 Days after Operation

| Nature of experiment | Operated | | | | Unoperated | | | |
|---|---|---|---|---|---|---|---|---|
| | № 1 | № 2 | № 3 | average | № 1 | № 2 | № 3 | average |
| Fasting for 50 hours | 2.7 | 3.3 | 3.1 | 3.0 | 3.7 | 4.2 | 3.7 | 3.9 |
| Fasting for 48 hours + feeding for 2 hours | 6.75 | 5.45 | 5.2 | 5.8 | 5.8 | 5.7 | 5.0 | 5.5 |
| Fasting for 48 hours + conditional feeding for 2 hours | 3.5 | 5.5 | 4.5 | 4.5 | 5.0 | 5.1 | 4.9 | 5.0 |

It was found that the diameter of the intestine of fasting unoperated rats was equal, on the average, to 2.7 mm and that of fasting operated rats 3.5 mm. In the case of animals which had been fed the figures were 3.5 mm for unoperated rats and 4.3 mm for operated ones. The diameter of the intestine was thus increased in the operated animals. Statistical examination of the differences in intestinal diameter showed that for fasting rats R = 5.8 and for fed rats R = 7.7, i.e., the differences were not chance. In order to discover how long

TABLE 3

Number Mitoses per one Crypt 60 Days after Operation

| Nature of experiment / Number of rat | 1 | 2 | Average |
|---|---|---|---|
| Fasting for 50 hours | 4.8 | 5.0 | 4.9 |
| Fasting for 48 hours + feeding for 2 hours | 5.7 | 6.1 | 5.9 |

intensified proliferation of epithelial cells in the small intestine crypts persisted in the operated rats 6 of the latter were examined 90 days after operation. These rats were kept fasting for 50 hours prior to being sacrificed. The number of mitoses in the crypts of these rats is given in Table 5.

Data shown in Table 5 indicate that 90 days after operation the number of mitoses in the intestinal epithelium continues to be high, although some tendency to decrease begins to appear.

Data on the number of mitoses in fasting rats sacrificed at various periods are summarized in the figure.

TABLE 4

Rat Intestine Diameter (in millimeters)

| Rats | Unoperated | | | Operated | | |
|---|---|---|---|---|---|---|
| | № 1 | № 2 | average | № 1 | № 2 | average |
| Fasting | 3.0 | 2.5 | 2.7 | 3.5 | 3.6 | 3.6 |
| Fed | 3.4 | 3.3 | 3.4 | 4.5 | 4.2 | 4.4 |

Postoperative days are marked along the abscissa, the average number of mitoses per one crypt – along the ordinate. The curve rises until the 60th day after operation, beginning to decline from then on.

TABLE 5

Number of Mitoses per one Crypt 90 Days after Operation

| Nature of experiment / No. of rat | 1 | 2 | 3 | 4 | 5 | 6 | Average |
|---|---|---|---|---|---|---|---|
| Fasting for 50 hours | 4.6 | 4.0 | 3.5 | 4.9 | 2.8 | 3.8 | 4.0 |

On the basis of the data obtained it may be taken as established that the rat intestine, after resection of 1/7th of its length, shows definite reaction to unconditioned and conditioned food stimuli quite soon after operation, the response manifesting itself by increased number of mitoses in the crypts. The increased number of such mitoses in fasting operated rats as compared with unoperated rats (fasting) can be taken as an indication of enhanced functioning of the remaining intestine and hypertrophy of its mucosa. Following resection of part of the small intestine its remaining part thus shows the occurrence of restorative processes.

## SUMMARY

1/7 part of small intestine was resected in 25 white rats in its superior portion. Intestines of rats reacted in 10-30 days after the operation by increased number of mitoses in response to unconditioned and conditioned food stimuli (as in normal condition). On the 60th day after the operation, the number of mitoses in the intestine of fasted operated rats was increased in comparison with fasted animals which were not operated,

as well as in comparison with fasted operated rats, which were sacrificed earlier. Mitosis was still pronounced in the intestine of operated rats in 90 days after intestinal resection.

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* In Russian.