DYNAMICS OF NUCLEIC ACID SYNTHESIS IN NUCLEI OF THE CIRCULAR MUSCLE LAYER OF THE ALBINO RAT STOMACH AFTER RESECTION OF 50 % OF THE FUNDAL REGION

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After resection of 50% of the wall of the fundus of the stomach in noninbred rats nucleic acid synthesis was studied in the nuclei of the smooth-muscle cells of the outer muscle coat and muscle cells of the blood vessels at various intervals. Experiments with thymidine-H^3 showed that the number of smooth-muscle cells of the circular and longitudinal layers of the outer muscle coat of the stomach synthesizing DNA is increased by 30 times on the second to third day after the operation. Many (up to 10%) labeled muscle cells were observed in the muscle coat of the vessels. Cytospectrophotometric investigations showed that the relative DNA content in the nuclei of the smooth-muscle cells of the outer muscle coat was substantially unchanged between one week and six months after the operation whereas the RNA content was increased by one-third over the control level one month after resection of the stomach.

In recently published investigations the state of the resected stomach has been studied qualitatively and quantitatively at various stages of its regeneration [1, 3-7]. So far as the state of the muscle coat of the stomach is concerned, Verzhbitskaya [2] found an increase in the number of mitoses in the connective-tissue and smooth-muscle cells on the third day after formation of a small defect. Regeneration ended with

TABLE 1. ILN (in %) of Smooth-Muscle Cells of Outer Muscle Coat of Albino Rat Stomach after Resection of the Fundus (M ± m)

Times after resection (in days)	Circular layer	Longitudinal layer
Control	0,1±0,07	0,2±0,05
Mock operation	0,3±0,06	0,3±0,02
1	0,2±0,10	0,3
2	3,4±0,68	6,2±0,52
3	3,3±0,45	5,9±1,70
5	0,5±0,21	—
10	0,4±0,24	0,5±0,50

TABLE 2. Relationship between Content of DNA (Feulgen's method) and DNA plus RNA (gallocyanin) in Sections through Nuclei of Smooth-Muscle Cells of Resected Stomachs (in % of control)

Times after resection	Relative DNA content	Relative RNA + DNA content
Control	100,0	100,0
1 week	92,5	117,1
2 weeks	95,5	107,0
1 month	110,0	144,7
3 months	107,5	106,4
6 months	—	105,0

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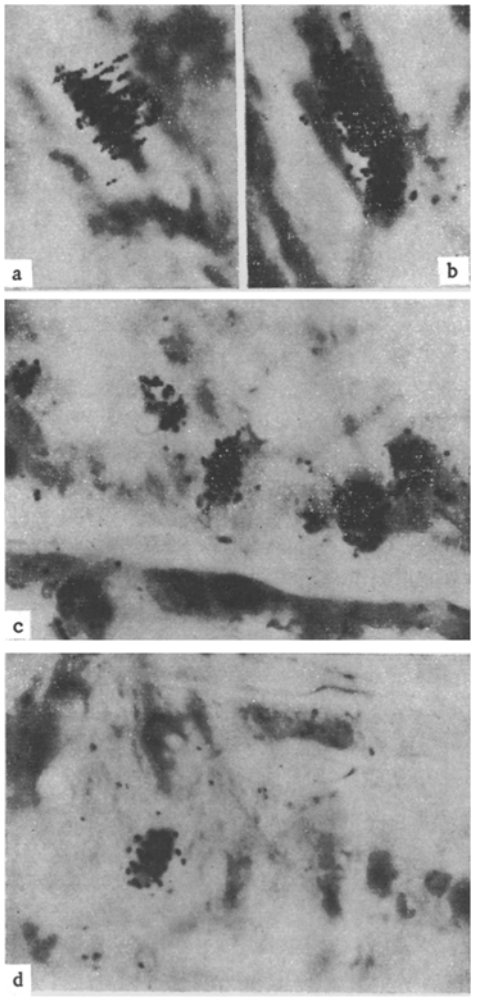

Fig. 1. Smooth-muscle cells of the circular layer of the outer muscle coat (a, b) and the muscle coat of an artery (c, d) of the resected rat stomach synthesizing DNA on the second day after operation. Dominici-Kedrovskii strain, 1350×, immersion.

the formation of a scar. Timashkevich [6] found marked hypertrophy of the outer muscle coat of the fundus of the resected stomach six months after resection of half of this portion.

However, the processes responsible for hypertrophy of the muscle coat and, in particular, changes in the intensity of synthesis and the content of DNA and RNA in the nuclei of the smooth-muscle cells of the outer muscle coat of the stomach have not been studied. An attempt was made to rectify this omission in the investigation described below.

EXPERIMENTAL METHOD

About 50% of the wall of the fundus of the stomach was resected in the region of the greater curvature in noninbred male rats weighing 200-280 g [6]. A mock operation included laparotomy, mobilization of the stomach, followed by its replacement and suture of the abdominal wall. In series I the gastrectomized rats (five animals at each time on the first, second, third, fifth, and tenth days after resection), 10 animals (two at each time) at the same times after the mock operation, and five intact control rats all received an intraperitoneal injection of thymidine-H^3 with specific activity 1.4 Ci/mmole and in a dose of 0.5 μCi/g body weight at 9 A.M., 1 h before sacrifice. Paraffin sections 5 μ in thickness were coated with type M emulsion. Exposure lasted one month. After development, the specimens were stained by the Dominici-Kedrovskii method. The number of nuclei of the labeled smooth-muscle cells and the number of grains of silver above the nucleus were counted in not less than 1,000 cells in the circular muscle layer and 500 cells in the longitudinal muscle layer. The index of labeled nuclei (ILN) was calculated in percent. In series II the rats were killed one and two weeks and one, three, and six months after the operation, three animals at each time, together with control animals of the same age. After fixation of the stomach with Carnoy's mixture [6] pieces of the stomach were embedded in paraffin wax so that an area from the stomach of a gastrectomized and a control animal were together in the same section. Sections were stained with gallocyanin by Einarson's method [8] or by the Feulgen method. To differentiate between RNA and DNA, besides comparing sections stained by these two methods, RNA was extracted with ribonuclease or mild acid hydrolysis carried out. Comparative cytospectrophotometry of the total nucleic acid (staining with gallocyanin) and DNA (staining by Feulgen's method) content was carried out on sections through the nuclei of the circular layer of the outer muscle coat, using Morozov's method [9] and a frame cytophotometer. The mean content of RNA and DNA per nucleus in the control series was taken as 100% and changes in these parameters in the gastrectomized animal were expressed as percentages of the control.

EXPERIMENTAL RESULTS

On the first day after resection a narrow zone of necrosis and injury to the cells and fibers of the stomach wall could be seen along the line of the anastomosis. On the second-third day a few labeled fibroblast-like cells appeared in this zone and also on the serosa. On the next three days granulation tissue developed in the zone of the anastomosis. During the first week edema of the submucosa and its infiltration by a few leukocytes, accompanied by loosening of the structure and edema of the interstitial tissue of the whole muscle layer, especially near the anastomosis, were observed. On the seventh day the neutrophils infiltrating along the line of the anastomosis, on the serosa, and in the granulation tissue were accompanied

by numerous lymphoid cells. Starting from the second week the granulation tissue became mature, its blood vessels were emptied, and a collagen scar formed. After one month and later a dense scar was found along the line of the anastomosis, but the tunica propria and the outer muscle layer had not regenerated in the zone of the scar.

The number of smooth-muscle cells of the outer muscle coat synthesizing DNA was not increased 24 h after resection (Table 1). On the second day their number rose sharply (by almost 30 times, $P < 0.001$; Fig. 1a, b). Similar relationships were observed in the longitudinal muscle layer. Admittedly, it was difficult to count the labeled cells in the latter, because of well-marked fibrinous deposits containing proliferating fibroblast-like cells. DNA synthesis at this time also appeared in the endothelium and muscle cells of the blood vessels of the resected stomach. For instance, in a medium-caliber artery of muscular type 28 labeled muscle cells were found among 295 counted (Fig. 1c, d), whereas in the intact rats and rats undergoing the mock operation no smooth-muscle cells synthesizing DNA could be found in the vessels. On the third day the number of cells of the circular muscle coat of the stomach in the S-phase of the mitotic cycle was increased by the same degree ($P < 0.001$). In some cells labeled mitoses were found. By the fifth day the number of nuclei of smooth-muscle cells synthesizing DNA was sharply reduced but considerable individual variation was observed, thus accounting for the large error of the mean. A similar picture was observed ten days after resection of the stomach. After the mock operation (Table 1) the mean level of DNA synthesis in the circular layer of the smooth-muscle cells was not increased. In the longitudinal muscle layer in some cases an increase in the number of muscle cells in whose nuclei DNA was being synthesized could be detected, but this was combined either with deposition of fibrin on the serosa or with microtrauma to the muscle layer. No differentiation into zones could be seen in the distribution of the smooth-muscle cells in the S-phase in the stomach after resection. By contrast, in the immediate vicinity of the zone of necrosis along the line of resection, no muscle cells with signs of DNA synthesis in the nuclei were found. On the third day very many labeled fibroblasts were found in this zone. In the rest of the muscle coat of the resected stomach cells synthesizing DNA were distributed relatively uniformly (within 800 μ of the line of resection ILN was 1.57%, within 1500 μ it was 4.84%, and further away still it was 3.12%). An increase in the number of smooth-muscle cells in the S-phase was also observed in the muscle coat of the forestomach. In one case, for instance, ILN in the muscle coat of the forestomach on the third day after resection was 3%.

Together with labeled nuclei in the outer muscle coat of the stomach on the third day there were solitary labeled mitoses which could not be definitely identified. This problem will be specially studied later. No mitoses were found at any time of observation in the smooth-muscle cells of the tunica media of the arteries or arterioles.

Cytospectrophotometric investigation showed that between one week and three months after resection of the stomach the DNA content in sections through nuclei of the smooth-muscle cells of the circular layer was substantially unchanged (Table 2). The results for the total nucleic acid (RNA + DNA) content in sections of the smooth-muscle cell nuclei (Table 2) indicate an increase in their RNA content compared with the control one month after resection of the stomach, which may evidently reflect the state of protein synthesis at this period.

Although marked activation of DNA synthesis in the population of gastric smooth-muscle cells occurred on the second to third day after resection, the thickness of the muscle layer at this period was indistinguishable from the control. For instance, the circular muscle layer, in both the control measurements and until the fourteenth day after resection, had a mean thickness of about 100 μ. Starting from one month after resection the thickness of the circular muscle layer was increased by 2.5-3 times. This is in agreement with previous findings [6]. During this period RNA accumulated in the nuclei of the smooth-muscle cells.

These observations show that some smooth-muscle cells of the stomach are in the G_0 period and pass into the S-phase of the mitotic cycle after resection of 50% of the wall of the fundus. This period in regeneration of the muscle coat of the stomach was clearly restricted to the two and three days after resection. The synchronous reaction of the smooth-muscle cells in different parts of the muscle coat of the stomach (circular and longitudinal layers, forestomach), the muscle coat of the gastric vessels, and the epithelium of the mucous membrane is noteworthy [7]. It is evidence of the absence of tissue specificity in the activity of the factors stimulating DNA and RNA synthesis in the regenerating organ. It will be recalled that these periods of greatest activation of DNA synthesis in the population of gastric smooth-muscle cells

coincide with the times of severest disturbance of the blood and lymph circulations and with marked proliferation of fibroblasts in deposits on the serosa, in the zone of injury, and in the interstitial tissue of all coats of the stomach.

Hypertrophy of the muscle coat takes place later, and the times of development of hypertrophy of the muscle coat coincide with the times of increase in RNA content in the nuclei of the smooth-muscle cells.

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