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EFFECT OF PRECEDING STRESS ON MITOTIC ACTIVITY OF THE REGENERATING LIVER

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For 2 h male rats were immobilized 14 h or 24 h before partial hepatectomy. Some of these animals received subcutaneous injections of theophylline immediately after immobilization and next day. Under the influence of stress a tendency was observed for mitotic activity of the regenerating liver to be increased. This effect was particularly marked in rats receiving theophylline additionally.

KEY WORDS: regeneration of the liver; mitotic activity of hepatocytes.

Numerous investigations have shown that stress leads to inhibition of mitotic cell activity. This has been found mainly by the use of objects such as the corneal epithelium. However, stress does not inhibit but increases the number of mitoses in the epithelial cells of the crypts of the small intestine [2, 3, 5]. It has also been found that the action of a stressor before partial hepatectomy increases DNA synthesis in the regenerating liver [8, 9].

The object of this investigation was to study the effect of stressor action preceding partial hepatectomy on mitotic activity of the regenerating liver and also to investigate the action of theophylline, an inhibitor of cyclic AMP phosphodiesterase, on the result of such stress.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats with a mean weight between 180 and 231 g. The animals as a whole were divided into five groups (Table 1). The animals of group 1 acted as the control and underwent partial hepatectomy with the removal of about 70% of the mass of the liver. The animals of the other groups were immobilized 14 or 24 h before hepatectomy for 2 h by fixation to the bench. Some rats immediately after the end of immobilization and also next day (before resection of the liver) received subcutaneous injections of theophylline solution in a dose of 10 mg/100 g body weight. All the animals were killed 30 h after resection of the liver, which was always performed at 3-4 p.m. Pieces of liver were fixed in Carnoy's fluid and embedded in paraffin wax. Sections were stained by Feulgen's method. The total number of mitoses and the various phases of mitosis separately were counted in 100 fields of vision of the microscope (objective 40, ocular

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TABLE 1. Effect of Preceding Stress Combined with Injection of Theophylline on Mitotic Activity of Regenerating Liver ($M \pm m$)

Group of animals	Procedure	Mitotic index	Distribution by phases (%)				Phase coefficient
			prophases	meta-phases	anaphase	telo-phases	
1	Resection of liver (8)	$4,25 \pm 0,7$	57,82	16,45	9,55	16,18	2,88
2	Resection of liver 24 h before stress (8)	$6,9 \pm 0,95$ $P < 0,005$	60,12	18,07	7,79	14,02	3,58
3	Resection of liver 24 h before stress and injection of theophylline (8)	$8,02 \pm 0,7$ $P < 0,001$	59,77	22,84	5,31	12,08	4,75
4	Resection of liver 14 h before stress (7)	$6,92 \pm 1,42$	65,83	16,22	6,3	11,65	4,57
5	Resection of liver 14 h before stress and injection of theophylline (8)	$11,4 \pm 1,0$ $P < 0,005$	60,48	25,17	4,4	9,95	5,96

Legend. Number of animals in parentheses.

10). The mitotic index (the number of mitoses per thousand cells) was calculated. The number of mitotic phases was expressed as a percentage of the total number of mitoses. The phase coefficient (the ratio of the number of prophase and metaphase to the number of anaphase and telophase, in %) also was calculated.

I. Spirin assisted with the experiments.

EXPERIMENTAL RESULTS

The results are given in Table 1.

As Table 1 shows, in the experiments with immobilization for 2 h 14 and 24 h before resection of the liver a tendency was observed for the mitotic index and phase coefficient to increase. However, in one experiment in which stress was produced 14 h before resection of the liver, this increase was not statistically significant (compare groups 4 and 1). In both groups in which stress was combined with injections of theophylline there was a highly significant increase in the number of dividing cells. It is considered that theophylline facilitated the accumulation of cyclic AMP, which caused a well-marked increase in the number of mitoses. This hypothesis is in agreement with the results of investigations showing the role of cyclic AMP in liver regeneration [1, 6, 7, 10].

How can the results be interpreted? Presumably stress initially inhibited the passage of the hepatocytes into mitosis by blocking cells at the g_2 stage. Stress, of course, is coupled with the liberation of adrenalin, which can itself delay the cells at this stage [4]. Later, after the inhibitory effect of stress had ceased, a "rebound phenomenon" arose, marked by synchronized entry of the cells into mitosis. However, this explanation is contradicted by the long time interval between stress and the beginning of counting mitosis. In the first experiment the interval was 54 h (24 h elapsed after the beginning of stress to the operation and 30 h after partial hepatectomy). In another experiment this interval was 44 h (14 h + 30 h respectively). The "rebound phenomenon" could hardly persist for so long. In addition, as investigations have shown, preceding stress stimulates DNA synthesis, and this took place even when the stress preceded resection of the liver by 3 days [8]. In addition, in the present experiments the increase in the mitotic index was not accompanied by a decrease in the phase coefficient; this coefficient also increased. That is why the writers incline to the view that the increase observed in the number of mitoses was not a "rebound phenomenon," but the expression of stimulation of mitotic activity of the hepatocytes. Another factor responsible for this stimulation could be an increase in the secretion of adrenalin and glucagon under the influence of stressor action.

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AUTORADIOGRAPHIC INVESTIGATION OF THE RATE OF COLLAGEN SYNTHESIS DURING STIMULATION OF WOUND HEALING

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The rate of tropocollagen synthesis by fibroblasts of granulation tissue and of its liberation into the intercellular space was investigated in control animals and during stimulation of the wound process by potassium orotate, a pyrimidine derivative. Under the influence of the stimulator tropocollagen synthesis was observed to be increased. The precursor of collagen fibers appeared in the intercellular space and was incorporated into the fibrous structures of the granulation tissue sooner under these circumstances than in the control, a fact which correlated with the intensification of RNA synthesis in the fibroblast nuclei and with the more rapid liberation of the newly synthesized RNA from the nucleus into the cytoplasm of the cells under similar conditions. No sharp excess of collagen was observed in the granulation tissue of the animals receiving potassium orotate, however.

KEY WORDS: tropocollagen; proline-³H; potassium orotate; fibroblasts; rate of collagen synthesis.

Investigations during the last 10-15 years have resulted in the opinion that collagen is synthesized by fibroblasts and then transported into the intercellular space [1, 2, 4]. So far as wounds are concerned, the periods of maximal synthesis of collagen molecules and formation of collagen fibers have been established [8-11]. It has also been shown that under conditions of hypo- and avitaminosis C the quantity of newly formed collagen in wounds of guinea pigs is sharply reduced but it returns quickly to normal if the animals are transferred to a diet containing sufficient vitamin C [7, 14, 15]. However, hardly any work has been done to discover the effect of stimulation of the wound process on the rate of formation of collagen and the quantity of it formed de novo. By applying the method of autoradiography using the labeled precursor of collagen such an analysis can be undertaken.

With these facts in mind it was decided to investigate the rate of formation of tropocollagen in fibroblasts and of liberation of the newly formed precursor of collagen fibers into the intercellular space during stimulation of the wound process by potassium orotate, a pyrimidine derivative, and also to try to establish quantitative differences in the content of newly synthesized collagen in the granulation tissue of experimental and control animals.

EXPERIMENTAL METHOD

Experiments were carried out on 30 noninbred albino mice weighing 25 g. In the region of the anterolateral surface of the thigh an incision 1 cm long was made in the skin and subcutaneous cellular tissue and a piece of muscle measuring 2 × 2 × 2 mm was removed. The wounds healed beneath a scab. The animals were divided into two groups. Mice of one group immediately after the operation began to receive potassium orotate by mouth as a 2% solution in a dose of 0.2 ml daily and the animals of the other group acted as the control.

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