EXPERIMENTAL RESULTS

Unlike bilayer agar cultures, growth of colonies was not found in a single monolayer agar system. Consequently, clonal growth of granulo-monocytic precursor cells depends on the quantity of colony-stimulating factor in the nutrient layer (feeder). In the presence of hypothalamic polypeptide factor both ClFA and CFA of the granulo-monocytopoietic precursor cells were increased; the number of medium and large colonies was significantly increased in this case (Table 1).

During culture of hematopoietic cells with pineal polypeptide factor a moderate decrease in CFA of the precursor cells of granulo-monocytopoiesis was found, mainly on account of precursor cells forming large colonies. Polypeptides isolated from the hypothalamus and pineal gland thus differ in their regulating effect on precursor cells of granulo-monocytopoiesis.

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ACTION OF HEPATIC CHALONES ON HEPATOCYTE PROLIFERATION IN THE INTACT AND DENERVATED LIVER

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A central place in the problem of constancy of cell composition of organs and tissues is currently occupied by the question of interaction between regulatory factors at different levels — from tissue to organism. The proliferative program of a tissue and the initiation and completion of reparative regeneration also are evidently the integrative result of interaction between various factors and the reacting tissue. A matter of great importance in this context is elucidation of the role of the nervous factor in the regulation of cell proliferation and, in particular, its interaction with endogenous tissue regulators of cell divison. The basis for this approach to the problem is formed by data on intensification of proliferative activity of cells in organs with disturbed innervation [1, 5, 9]. However, the mechanisms of this phenomenon are not yet clear.

Accordingly, in the investigation described below, it was decided to study the action of tissue-specific inhibitors of cell proliferation (chalones) on DNA synthesis and mitotic activity in the regenerating denervated animal liver.

EXPERIMENTAL METHOD

Male Wistar rats with an average weight of 140-160 g were used. The rats were divided into two groups: the animals of group 1 served as the control, those of group 2 were subjected to subdiaphragmatic vagotomy 1 week before the experiment. Two-thirds of the liver

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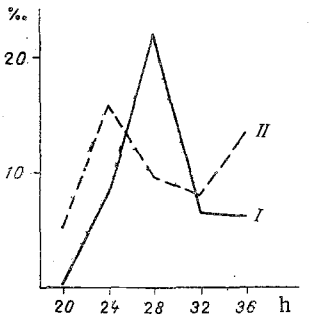

Fig. 1. Changes in number of DNA-synthesizing cells and mitoses in regenerating rat liver at different times after partial hepatectomy: I) MI; II) ILN.

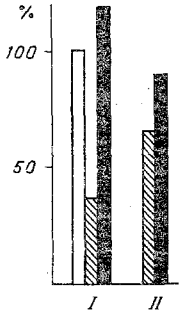

Fig. 2. MI (I) and ILN (II) in regenerating rat liver after injection of chalones (in % of control). Unshaded columns — hepatectomy; shaded) hepatectomy + chalones; black columns) hepatectomy + vagotomy + chalones.

was removed from all the rats under ether anesthesia at the same time of day (from 10:15 to 10:45 a.m.). The zero point for counting the times of sacrifice was 10:30 a.m. There were three series of experiments. In series I the number of DNA-synthesizing and dividing hepatocytes was determined at different times after partial hepatectomy. The reason for this series of experiments was the need to establish the kinetics of cell proliferation in regenerating liver so that optimal times for the experimental procedures could be chosen. The available information in the literature on this question is not absolutely consistent [12, 13]. For that reason, starting from 12 h after the operation, every 4 h for 36 h pieces of regenerating liver tissue were taken for investigation; in histoautoradiographs the number of mitoses and the number of nuclei synthesizing DNA were determined in them. The rats were given an injection of [^{3}H]thymidine 1 h before sacrifice.

In the experiments of series II mitotic activity (MA) was investigated in the liver after resection of the organ in control and vagotomized animals. Rats of both groups in this series were given an intraperitoneal injection of colcemid 18 and 24 h after partial hepatectomy, and they were killed 3 h after the injection. In the experiments of series III DNA synthesis and cell division were studied in the regenerating liver of control and vagotomized rats after injection of chalone-containing preparations into the animals. Rat liver extract containing G_1- and G_2-chalones was obtained by precipitation from an aqueous homogenate in a concentration gradient of ethyl alcohol [14] with modifications [7].

The control and vagotomized rats received three injections of hepatic chalones — 12, 20, and 28 h after partial hepatectomy, in a single dose of 50 mg/100 g body weight. To monitor any possible action of protein, serum albumin was injected into animals of another group at the same time, in a dose equivalent to the protein content in liver extract (35 mg). The animals were killed 48 h after partial hepatectomy, and [^{3}H]thymidine was injected into them 1 h before sacrifice. In all series of experiments [^{3}H]thymidine was injected in a dose of 1 μCi/g and colcemid in a dose of 1.5 mg/kg. The mitotic index (MI), index of colcemid-blocked mitoses (MI_{col}), and the index of labeled nuclei (ILN) were determined after examination of 8000-10,000 hepatocyte nuclei. The indices of proliferation were expressed in promille. The numerical results were subjected to statistical analysis by the Fisher–Student method.

TABLE 1. MA of Hepatocytes of Intact and Vagotomized Rats after Partial Hepatectomy

Time after hepatectomy, h	MA ($MI_{col} \pm m$, ‰)		P
	control	vagotomy	
18—21	0,0 $n=5$	0,2±0,1 $n=5$	—
24—27	25,05±9,98 $n=5$	92,83±20,15 $n=6$	0,015

EXPERIMENTAL RESULTS

The results of the experiments of series I are given in Fig. 1. No labeled nuclei or mitoses were found 12 h after partial hepatectomy. A low level of [^{3}H]thymidine incorporation (0.15%) was observed after 14 h, and it lay within the limits of circadian fluctuations in the number of DNA-synthesizing cells in the liver of intact rats. An appreciable level of DNA synthesis and the first mitoses were observed after 20 h. ILN rose rapidly and reached peak values 24 h after the operation (between 20 and 24 h, P = 0.002), fell until 28 h (P = 0.019), and rose again until 36 h (P = 0.048). The number of mitoses reached a maximum 28 h after partial hepatectomy.

The time of appearance of the wave of DNA-synthesizing and dividing hepatocytes after partial hepatectomy thus agrees with data obtained on Wistar [12] and noninbred [2, 10] rats and differs from results obtained on August rats [13]. Later after the operation the wave-like character of entry of the hepatocytes into the phase of DNA synthesis, described previously in rats only for changes in MI during regeneration of the organ [12], was observed. Data on proliferation of hepatocytes after partial resection of the liver in vagotomized animals (experiments of series II) are shown in Table 1. In the vagotomized animals MA increased earlier after partial hepatectomy, and was reflected in higher values. The ploidy of the hepatocyte nuclei changed compared with the control. During regeneration of the denervated liver the number of diploid nuclei fell from 34.5% in the control to 30% in the experiment, and the relative proportion of nuclei in higher classes of ploidy (4n and 8n) increased. Whereas in the control the total number of polyploid nuclei was 56.5%, in the control it was 66.0%. Intensified proliferation in the liver of vagotomized animals was thus manifested as more rapid polyploidization of the hepatocytes.

The results of the study of hepatocyte proliferation in the regenerating liver of the control and vagotomized rats after three injections of hepatic chalones (experiments of series III) are given in Fig. 2. Chalones effectively reduced the number of DNA-synthesizing cells and mitoses in the control animals: Even 20 h after the last injection of the preparation ILN was 1.5 times less, and MI 2.7 times less, than in the rats receiving serum albumin. The difference in the degree of inhibition of DNA synthesis and of MA may be evidence that the blocking action of chalones on DNA synthesis at this stage of the investigation was beginning to weaken, but restoration of DNA-synthesizing activity of the hepatocytes was not yet reflected in MI.

Meanwhile the lower values of the indices of proliferation compared with the control 20 h after the last injection of chalones probably indicate that the chalones remained bound with the cells and altered their proliferative properties for a longer time than is usually considered [15].

These data agree with the results of a study of proliferation, after repeated injection of chalones, in other objects: hepatoma 22a and Ehrlich's ascites carcinoma [11]. Restoration of the level of proliferation in the investigations cited to the control values was observed after an interval of 1 to 2 days.

Analysis of the data on hepatocyte proliferation in the regenerating liver after vagotomy (Fig. 2), in our opinion, deserves special attention because it can be concluded from them that hepatic chalones do not affect the intensity of proliferation in the denervated liver. Chalones are regarded as integration factors [12]. At the same time, we know that if the innervation of tissues is disturbed, the degree of their integration is reduced [13] In the light of these observations it can be postulated that structural and functional disorganization of the liver arising as a result of disturbance of its innervation abolishes or drastically reduces the sensitivity of hepatocytes to tissue proliferation regulators (chalones)

There are thus grounds for considering that for the action of even such a phylogenetically old system of tissue regulation as the chalone system to be realized, an essential condition is that the adequate trophic influence of the nervous system should be preserved. At the same time, the effect of a sharp decrease in sensitivity of the denervated tissue to the action of an adequate biological agent, revealed by these experiments, is not an exclusive effect. For example, a denervated muscle loses its ability to respond by intensification of glycogen synthesis to injection of hydrocortisone [6]. It can be concluded from facts such as these that the well-known Cannon–Rosenblueth law of increased sensitivity of denervated structures is not a universal phenomenon.

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