precursor cells of the hematopoietic stroma. Under the influence of the polysaccharide, in mice with MPS deficiency there was a decrease in size of the heterotopic focus, to the level observed in intact animals (Table 3), which could be connected, under conditions of MPS deficiency, with the direct inhibitory effect of the polysaccharide on osteogenic and hematopoietic precursor cells.

Injection of the polysaccharide into recipient mice with MPS deficiency led to a decrease in the number of cells and in the weight of the bony capsule of the heterotopic focus down to the level observed in intact animals (Table 3), to a decrease in the number of hematopoietic cells in the femoral marrow, and to an increase in the number of cells in the peritoneal cavity up to its initial level (Table 1). Consequently, under these conditions the polysaccharide had an equalizing or regulatory action.

It can thus be concluded that in different states of an animal, the heteropolysaccharide studied in these experiments was characterized by an inhibitory or stimulating effect on the formation of a heterotopic focus of hematopoiesis. Moreover, the inhibitory effect of the polysaccharide under conditions of MPS deficiency restored the normal size of the enlarged heterotopic focus and the normal number of hematopoietic femoral marrow cells.

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EFFECT OF DALARGIN, A STABLE LEU-ENKEPHALIN ANALOG, ON CELL DIVISION IN THE ALBINO RAT CORNEAL EPITHELIUM

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Dalargin, a stable synthetic analog of Leu-enkephalin, is considered to be one of the most effective preparations against peptic ulcer [2, 5]. Besides its action on the endocrine system [3] and its immunomodulating properties [1], an important role in the therapeutic effect of dalargin is ascribed to its ability to act on proliferative processes. In previous investigations the writers showed that administration of dalargin stimulates DNA synthesis in epithelial tissues. In some cases, however, an appropriate increase in the mitotic index was not observed. It has been suggested that under these circumstances mitosis itself is accelerated or a circadian disturbance of coordination between DNA synthesis and entry of the cells into mitosis takes place. It was also difficult to rule out long delay of the cells in the G_2 period or polyploidization of the cells.

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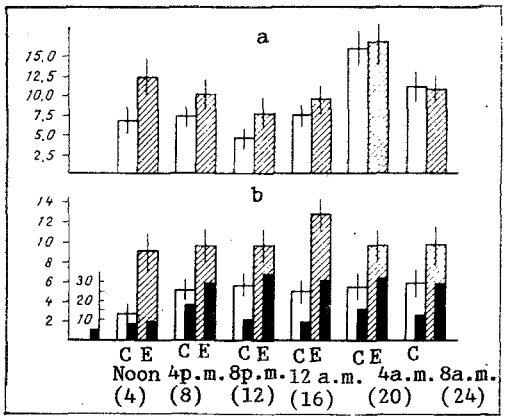

Fig. 1. Effect of dalargin on cell division in corneal epithelium of albino rats. Abscissa, clock time and (in parentheses) time after injection of dalargin (in h). C) Control, E) experiment. a) MI of corneal epithelium (in %); b) ILN of corneal epithelium (in %); black columns — LI (number of tracks per nucleus).

The aim of this investigation was to study the causes of disparity between the level of DNA synthesis and mitotic activity.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180-220 g. Dalargin was injected intraperitoneally at 8 a.m. in a dose of 10 μg/kg body weight (Fig. 1). Animals receiving an intraperitoneal injection of the same volume of isotonic NaCl solution served as the control. The experimental and control animals were killed every 4 h after injection of the preparation for 24 h. At each time of the investigation groups of 6-7 control and experimental animals were taken. Altogether 120 rats were used. To obtain autoradiographs, one cornea was incubated at 37°C in an ultrathermostat in medium 199 with ^{3}H-thymidine (2 μCi/ml), and a total preparation was obtained from the second cornea, in which the mitotic index (MI) was determined, in promille. Autoradiographs were prepared, the index of labeled nuclei (ILN, in percent) and the labeling intensity (LI) were determined, total preparations made, and MI determined by the methods described previously [4]. To rule out any change in the rate of mitosis itself, experiments were carried out on animals receiving an intraperitoneal injection of colchicine (0.2 μg/100 g) 2 h before sacrifice. The experiments with colchicine were performed 4, 12, and 24 h after injection of dalargin. Under these circumstances MI of metaphases blocked by colchicine (MI_{col}) was determined. Considering that the experiments with colchicine were not synchronized with those without colchicine, the cumulation effect was not estimated. The data were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The results are evidence that injection of dalargin induced marked stimulation of DNA synthesis in the corneal epithelium at all times of the investigation: ILN was increased by 1.7-3.1 times compared with the control, and LI by 1.2-3.5 times. An increase in MI was observed only 4, 8, and 12 h after injection of dalargin. Subsequently the changes in MI were not significant. The absence of stimulation of mitotic activity 16, 20, and 24 h after injection of dalargin in fact ruled out disturbance of circadian coordination between DNA synthesis and entry of the cells into mitosis. The parameter of disparity between the raised level of DNA synthesis and the absence of any adequate increase in MI was comparison of the mean daily values of ILN of animals of the control and experimental groups with values of the mean daily MI. Administration of dalargin led to an increase in ILN by 2.1 times ($p < 0.05$), whereas MI rose by only 1.3 times ($p > 0.3$). The ratio between the phases of mitosis and, in particular, the number of prophases, in the experimental group showed no significant changes compared with the control. This is indirect evidence against acceleration of mitosis itself, which could cancel out the increase in the number of dividing cells. However, the final answer to this question could be given by the results of experiments with colchicine, blocking any change in the velocity of mitosis in the control and experiment. Injection of colchicine caused marked stimulation of mitotic activity: at all times of the investigation there was a

considerable (by 2-2.7 times) increase in the number of dividing cells. MI_{col} 4 h after injection of dalargin was increased by 2.1 times, i.e., by the same degree in fact as MI (by 1.8 times). Evidently no significant changes took place in the velocity of mitosis at this time of the investigation. MI_{col} was doubled 12 h after injection of the opiate receptor ligand, whereas MI was increased by only 1.5 times. This difference can evidently be explained by acceleration of mitosis itself under the influence of dalargin. This was clearly confirmed by experiments 24 h after injection of dalargin.

Whereas comparison of MI in the control and experimental groups of animals revealed no differences, MI_{col} in the corneas of rats receiving dalargin was 2.7 times greater than its value in the control. This is evidence of significant acceleration of mitosis 24 h after injection of dalargin. Acceleration of mitosis evidently masks the increase in MI at the other times of investigation, which was preceded by an increase in ILN and LI. When the mean daily value of MI_{col} was calculated, it was found to be increased by 2.1 times in the experimental animals. It will be recalled that the mean daily ILN likewise was increased by 2.1 times under the influence of dalargin. These data explain the absence of an adequate increase in MI 16-24 h after injection of dalargin — it was due to acceleration of mitosis itself, masking the increase in the number of dividing cells.

Thus one of the mechanisms of stimulation of cell division by dalargin is acceleration of mitosis itself. Moreover dalargin accelerates the passage of cells through the premitotic period. According to data in the literature [6-8], the minimal duration of the G_2 period in the rat cornea in the morning is 4-5 h. The increase in MI 4 h after injection of dalargin was therefore evidently due to shortening of the G_2 period or departure of the cells from the G_2 (resting) fraction. The increase in LI taking place at all times of the investigation starting with 8 h after injection of dalargin is evidence of an increase in the rate of DNA synthesis, whereas the twofold increase in the mean daily value of ILN reflects a general increase of the proliferative fraction.

According to data in the literature [10, 11], the mitogenic effect of some neuropeptides (bombesin, substance P, vasopressin), observed in cell culture, is brought about indirectly through stimulation of inositol phospholipid turnover: through the formation of inositol triphosphate and diacylglycerol. Stimulation of this pathway is regarded [9, 12] as a universal property of various mitogens. It seems probable that the same mechanism also applies to dalargin.

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