DIURNAL CHANGES IN THE REACTION OF THE CORNEAL EPITHELIUM TO HYDROQUINONE

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The reaction of the corneal epithelium to hydroquinone varies during the 24-h period. Hydroquinone increases the mitotic index in the epithelial cells only during the morning. This increase is caused by a delay in cell division at the metaphase stage. In the evening this phenomenon is more marked, but there is a simultaneous decrease in the number of prophases. The number of pathological mitoses induced by hydroquinone is higher during the evening.

There has been a recent increase in the number of publications describing the study of reactions of the body as a whole and of single cells to various factors, including drugs. The results of these investigations have shown that the effect of the particular factor differs depending on the time of day or night. For example, in experiments with Escherichia coli endotoxin and with strophanthin, the highest mortality in mice was observed when the substance was administered during the afternoon, and the lowest at night [8, 9].

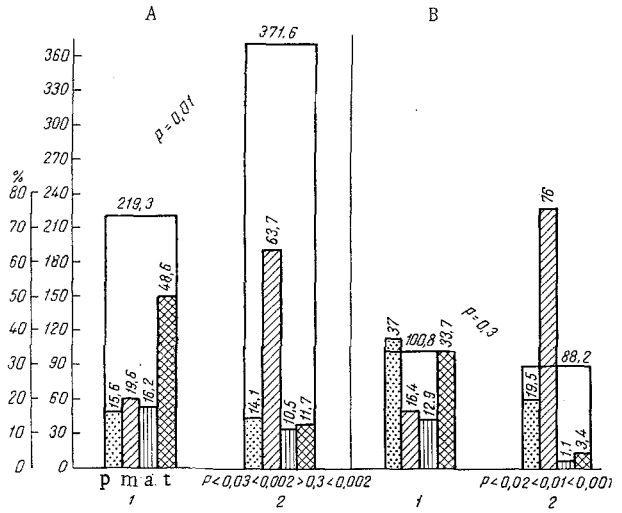

Fig. 1. Diurnal changes in mitotic activity and in relative percentages of phases of mitosis in corneal epithelium after treatment with hydroquinone during morning (A) and evening (B): 1) control; p) prophases; t) telophases; 2) hydroquinone; m) metaphases; a) anaphases. Unshaded columns denote mitotic activity.

Interest in the study of pathological mitoses and the mechanisms of their occurrence has also increased, and this is not accidental. The appearance of pathological mitoses is connected with the development of chromosomal diseases and the initial stages of carcinogenesis [4, 6]. Radiation sickness, virus infections, and the growth of tumors are always accompanied by anomalies of mitosis [3, 5, 10, 13]. Substances capable of producing pathological mitoses experimentally have been found. They include hydroquinone [12, 14]. Investigations have shown that the action of hydroquinone even for only a very short time on a culture of hamster cells or of mouse intestinal epithelium can cause a definite increase in mitotic activity, an increase in the relative number of metaphases, and an increase in the number of pathological mitoses, with predominance of three-group metaphases [7].

The object of the present investigation was to study the reaction of the corneal epithelium, a tissue with a well marked diurnal mitotic rhythm [1], to hydroquinone in the morning and evening.

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TABLE 1. Changes in Mitotic Activity of Corneal Epithelium after Application of Hydroquinone in Morning and Evening

Time of day	Group of animals	Number of animals	Mitotic activity	Percentages of different phases of mitosis				% of pathological mitoses	% of individual forms of pathological mitoses		
				prophases	metaphases	anaphases	telophases		three-group metaphases	chromosomal deletions	other forms
8 a.m.	Control	5	175.8	23.9	18.2	11.2	46.7	0			
	1st experimental	5	252	16.4	64.1	6.7	12.8	20.6	11.7	8.8	0.1
6 p.m.	Control	5	62.6	27.2	14.4	10.1	48.5	0			
	2nd experimental	5	59.1	22.5	68.3	1.8	7.4	33.3	19.2	11.8	2.2

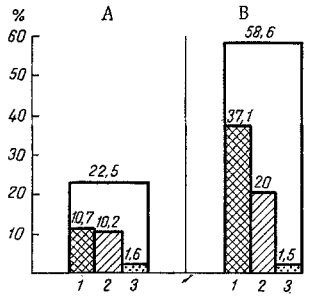

Fig. 2. Diurnal changes in number of pathological mitoses in corneal epithelium after application of hydroquinone during morning (A) and evening (B). Unshaded columns represent percentage of pathological mitoses; 1) percentage of three-group metaphases; 2) percentage of chromosomal deletions in metakinesis; 3) percentage of other forms of pathological mitoses.

EXPERIMENTAL METHOD

Experiments were carried out on albino mice aged 1.5-2 months. At 8 a.m. (experimental group 1) and at 6 p.m. (group 2) physiological saline (the solvent) was applied to the left (control) cornea and 5% hydroquinone solution to the right cornea. Mitotic activity was estimated by the number of dividing cells in a constant area (1.65 mm^2) of cornea. Pathological mitoses were defined by Alov's classification [2]. The statistical analysis of the results was carried out by the Fisher–Student method.

EXPERIMENTAL RESULTS

The reaction of the corneal epithelium to hydroquinone differed in the morning and evening. After application of 5% hydroquinone solution to the cornea at 8 a.m., when mitotic activity of the epithelium is high, an increase in the number of mitotically dividing cells ($P = 0.003$) and in the number of metaphases ($P = 0.001$) was observed, indicating delay of mitosis at this stage of division. The percentage of anaphases and telophases was reduced by 50-67% ($P = 0.003$). The action of hydroquinone at 6 p.m. when proliferative activity of the epithelium is low, differed to some extent. The mitotic activity of the corneal epithelium in the experiment showed no significant change ($P = 0.5$). The percentage of metaphases, just as in the morning, was increased by almost four times ($P = 0.06$). The number of cells at the stages of anaphase was reduced by six to seven times ($P < 0.05$).

Pathological mitoses were extremely rare in the epithelium of the control corneas. Under the influence of hydroquinone, their number increased sharply, and it was especially high in the evening (33.3%; $P = 0.02$). The increase in number of pathological mitoses was due primarily to the increase in number of three-group metaphases and to deletion of chromosomes in metakinesis. The class of three-group metaphases included cells in which, besides a metaphase plate, one, two, or more chromosomes were found at each pole. Other types of pathological mitoses were rare (0.1-0.2%). They consisted mainly of K-mitoses, scattered metaphases, and cells with swollen centrioles at the poles.* As Table 1 shows, the percentage of

*The number of pathological mitoses and of the individual types of mitosis appearing under the influence of hydroquinone showed considerable individual variation. Instability of the action of hydroquinone has been reported by other workers [7].

three-group metaphases was higher when the corneal epithelium was treated with hydroquinone during the evening.

More demonstrative results were obtained in the second experiment. After application of hydroquinone in the morning, both the mitotic activity and the number of metaphases in the corneal epithelium increased. The number of telophases was reduced by more than half. Pathological mitoses accounted for 22.5% of the total number of mitotically dividing cells. The number of three-group metaphases and the number of chromosomal deletions in metakinesis were almost equal (10.7 and 10.2%). When applied during the evening, hydroquinone reduced the mitotic activity in the corneal epithelium slightly. The increase in the number of metaphases was greater than after morning application. Under the influence of hydroquinone the number of prophases fell and cells at the anaphase and telophase stages disappeared almost completely. The number of pathological mitoses in the experimental corneas in the evening rose to 58.6% of the total mitotic activity ($P = 0.06$). Of this number, 37.1% were three-group metaphases ($P = 0.2$). Chromosomal deletions in metakinesis amounted to 20% (Figs. 1 and 2).

Comparison of these results shows that hydroquinone increases mitotic activity in the corneal epithelium during the morning only, and that this increase is mainly due to delay in division of the epithelial cells at the metaphase stage. During the evening the metaphase delay is more marked, as shown by the sharp decrease in the number of anaphases and telophases in the corneal epithelium. Furthermore, the changes in mitotic activity on the evening are associated with delay of the cells in starting on mitosis, as was confirmed by the decrease in mitotic activity and by the decrease in the number of prophases. Nevertheless, hydroquinone induces pathological mitoses more intensively in the evening than in the morning. A parallel is seen between the metaphase delay and the number of pathological mitoses. The percentage of three-group metaphases was higher during the evening.

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