EXPERIMENTAL BIOLOGY

ROLE OF THE ENVIRONMENT IN REGULATING THE MITOTIC REGIME OF THE BODY

PART III. EFFECT OF VARYING THE NUMBERS OF GROUPS OF MICE ON CELL DIVISION IN THE CORNEAL EPITHELIUM

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It has been shown by one of us [3] that the mitotic regime of the corneal epithelium of mice is to a considerable extent determined by the condition of the animal, and by environmental factors (maintenance factors, visual and auditory stimuli, etc.). In our subsequent work we found still more distinct effects on cell multiplication of such environmental factors as at first sight seemed to us to be negligible. In particular, we observed that the number of animals in a group, its uniformity, and other such factors could have a well-defined effect on mitotic activity. Since these effects were of interest from the methodological aspect and for the study of the effect of natural environmental stimulants on the process of cell division in the cornea, we undertook a special experimental study of them.

EXPERIMENTAL METHODS

Male white mice were taken for the experiments. The corneas, after fixation in Carnoy's fluid followed by staining, were cut into serial horizontal sections. The number of mitotic phases were counted for each cornea, down to the late stages of nuclear reconstruction of daughter cells, which were counted separately, and a chart showing distribution of mitoses over the epithelial surface was prepared by our previously described method [3]. The mitotic activity of the epithelium was derived from the number of mitoses per 100 fields of vision, which with the ocular diaphragm used by us corresponded to 1 sq. mm. of corneal surface. In order to obtain a fuller picture of the changes taking place in the corneal epithelium, we calculated the mitotic index in each case for the cornea as a whole as well as for its individual layers: outer, central, and inner. The experiments were performed at different seasons of the year, comprising 8 series of experiments on 92 mice.

In the first three series we investigated the effect of varying the number of mice in a cage on the mitotic activity of their corneal epithelium, taking into account the length of time that the various groups remained in the cages.

The experiments of the 1st series were done on two groups of mice kept for a long time in two adjacent cages, 5 mice to each. One day before killing the mice and removing the corneas, the mice of one group were isolated, each in its separate cage. No changes were made for the second group, which served as a control.

The mice of the second two series were also divided into two groups, one of 5, and the other of 15.

In one case (2nd series) the mice remained in their cage 4 days, and in the other (3rd series) 15 days, before killing and taking of material; both groups of each series were killed on the same day. Only 5 mice of the groups of 15 were taken for counts of mitoses.

The results of these experiments are presented in Table 1.

TABLE 1

Effect of the Number of Mice in a Group on the Mitotic Activity of the Corneal Epithelium

Stage of mitosis	Number of days the groups were kept together in one cage					
	1		4		15	
	number of mice in group					
	1	5	5	15	5	15
	number of mitoses					
Prophase	81	70	72	66	84	83
Metaphase	174	141	187	160	181	196
Anaphase	42	35	41	39	40	40
Telophase, early	50	43	56	49	54	53
" late	39	35	36	36	38	38
Total	386±26	324±21	392±10	350±28	397±20	410±18

The results show that the longer the mice are kept together in their individual groups, the smaller becomes the difference between the mitotic activities of the corneal epithelium in small and large groups. The most marked increase in the total number of mitoses (all stages) is found in the group of mice in which the individuals were isolated from each other a day before the experiment (119% of the control figure). The differences between the mitotic indices are smaller (12%) for the second series, while in the third series there is good agreement between the values of all the indices for both groups of mice (difference 3%).

Whereas prolonged association of mice in numerically different groups has no significant effect on the mitotic activity of their corneal epithelium, changes in the strength of the groups (removal of part of the group) have an immediate and marked effect on the mitotic index of the remaining animals. A more detailed examination of this phenomenon was made in a number of series of experiments, as follows.

In the 4th series of experiments we placed 15 male mice of about the same weight into a fairly small cage, and killed them 4 days later, at the following times: 5 mice at 10:05, 5 at 10:40, and 5 at 12:05 hours. Thus the second lot was killed about 35 minutes after removal of the first, and the third lot of 5 mice 1.5 hours after removal of the second.

The next series differed from the 4th in that the mice were killed in an adjoining room, so as to exclude the possibility of any effect produced by noises incidental to removal and fixation of the histological material.

The 6th series was treated identically, except that the animals were killed 30 minutes earlier, so as to check whether the effects found were due to differences in the time of day, rather than in the experimental procedure.

In the 7th series, the second group of mice was killed 15 minutes after the first, instead of 30-35 minutes, as in the preceding groups.

As appears from Table 2, the same result is found in all the series. Irrespective of the time of day, the removal of part of a more of less uniform group of mice invariably leads to a regular and relatively abrupt inhibition of mitotic activity in the corneal epithelium. This inhibitory effect is most marked in the outer zone, less so in the middle zones, and least of all in the inner zones.

The strict sequence of changes in the phases of mitosis is of special interest. The greatest effect 15 minutes after removal of the first group of mice is seen in the remaining ones of series 7 in the prophase of mitosis, the fall in the number of cells in metaphase being somewhat smaller. We could find no change in the number of cells in later stages of mitosis, in this series; series 4, 5, and 6, however, give an idea of changes taking place

TABLE 2

Effect of Sudden Change in the Number of Mice in a Group on the Mitotic Activity of the Corneal Epithelium

Corneal zone	Stage of mitosis	Series of experiments										
		4th series			5th series			6th series			7th series	
		time at which mice were killed										
		10.05 hr	10.40 hr	12.05 hr	10.14 hr	10.47 hr	12.17 hr	9.32 hr	10.09 hr	11.39 hr	10.05 hr	10.20 hr
outer	Prophase	58	72	52	54	64	37	57	62	50	83	27
	Metaphase	155	50	121	122	60	80	140	50	100	204	101
	Anaphase	30	20	39	35	18	28	39	19	39	43	45
	Telophase, early	39	28	39	36	29	44	42	30	45	54	53
	Telophase, late	22	30	37	26	21	30	28	27	33	38	47
	Total	304 ±35	200 ±18	288 ±30	273 ±13	192 ±18	219 ±17	306 ±20	188 ± 9	267 ±28	422 ±21	273 ±19
middle	Prophase	66	81	64	62	66	56	71	63	68	82	35
	Metaphase	144	97	139	133	74	131	165	88	112	190	145
	Anaphase	29	36	47	34	37	37	45	37	42	36	41
	Telophase, early	47	46	51	40	55	72	47	44	54	47	55
	Telophase, late	24	41	44	33	37	42	35	29	33	36	45
	Total	310 ±21	301 ±15	345 ±20	302 ±30	269 ±17	338 ±23	363 ±26	261 ±23	319 ±35	391 ±34	321 ±12
inner	Prophase	66	58	56	50	60	65	84	55	84	106	36
	Metaphase	146	119	137	103	72	140	121	113	114	157	121
	Anaphase	22	33	55	23	36	35	38	65	37	28	43
	Telophase, early	36	47	46	32	39	72	30	43	48	44	47
	Telophase, late	20	29	45	29	34	55	32	20	34	40	43
	Total	290 ±28	286 ±27	339 ±25	237 ±50	241 ±22	367 ±23	305 ±38	296 ±17	317 ±26	375 ±35	290 ±32
Mean mitotic index for all corneas		304 ±23	238 ±17	308 ±23	276 ±17	214 ±15	267 ±16	321 ±19	217 ±13	283 ±27	410 ±18	288 ± 8

at longer intervals after the removal of part of the animals of group, viz., 33-37 minutes. By this time the later stages of mitosis are the more inhibited. This effect is most marked in the metaphase, but is also evident in the anaphase and in early telophase; the latter applies only to the outer zone of the cornea. After 30-40 minutes the number of cells in prophase exceeds the initial value.

These changes in the mitotic phases show that inhibition of mitosis takes place immediately after removal of the first lot of mice from the groups. It first affects the prophase, and goes on progressively to affect the later stages of mitosis. A result of inhibition of entry of cells into division is not only to reduce the number of mitotic phases visible, but also to reduce the total number of cells undergoing mitosis. A statistical treatment of the experimental material confirmed the validity of the changes observed in the mitotic activity of the corneal epithelium. The changes in the number of cell divisions in the middle and inner zones of the cornea are too small to be regarded as statistically significant. Inhibition of mitosis due to reduction in the number of mice in a group is apparently only a transient phenomenon, since a new wave of mitoses appears 30-40 minutes later, as is shown by the increase in number of cells in prophase at that time.

As is evident from the data of Table 2, at about 1 1/2-2 hours after the beginning of the experiments the level of mitotic activity rises, approximating to the initial value, and the relations between the various phases even out. Even at that time, however, there still persist differences from the picture presented by the mice of the first group to be killed. The number of mitoses in the outer zone of the corneal epithelium is still perceptibly lowered while at the same time it in all cases exceeds the initial value in the central zone. Changes in the mitotic index are also seen when the mice are removed from a group one by one over a varying length of time. In series 8, in which the operation lasted for 20 minutes, we observed a gradual fall in the number of cell divisions. The first mouse to be removed, at 11:00hours, had a mitotic index of 352, which fell to 347, 342, 305, 297,275, 183 for the succeeding ones. The regularity of this phenomenon is further shown by the observation that inhibition of mitosis was initiated by a diminution in the relative and absolute number of cells in prophase. As in the preceding series, this effect was most pronounced in the outer zone of the cornea. In the first 3 mice to be removed, 27% of mitotic cells were in prophase, as compared with only 16.5% in the last three. I. A. Utkin has previously described a similar observation.

Taken as a whole, the above experimental results permit of no doubt that the effects described are related only to the factor of change in the numerical composition of the groups of mice, and are not related to the time of day or of year. Mitotic division of cells is most sensitive to what would seem to be barely perceptible changes in the environment.

The experimental results described in this paper can be comprehended only from the standpoint of the decisive importance of reflex mechanisms in regulating cell division. This viewpoint is confirmed by the incontestably proven high sensitivity of metabolic processes to environmental factors. Changes in metabolic activity of members of groups of animals in response to changes in the nature and size of the group have been established for a number of mammalian species, including rodents [1, 2].

Our experimental results show how cautiously one should approach the planning and execution of experiments on mitotic activity. The results indicate the necessity of a critical revision of the experimental material presented by certain research workers on the subject of the dynamics of cell division, unless they took into account the effects of changes in the numerical composition of their groups of experimental animals.

LITERATURE CITED

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