EFFECT OF EXTRACTS OF NORMAL AND BURNED SKIN ON CELL DIVISION IN THE CORNEAL EPITHELIUM

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Extracts from burned skin, unlike extracts from normal skin, do not stimulate cell division in the cornea. In addition, the property of inducing delay of commencement of mitosis is exhibited by extracts from burned skin. If the extracts are kept, their properties change.

The study of the toxemia of burns is of great clinical and theoretical importance [2, 14]. Recently biological methods have been used successfully for this purpose [6, 7, 9, 11, 12, 19].

An important aspect of the problem is to study changes in cell division in burns, for they are a highly sensitive biological parameter. In addition, the mitotic index can be used as a rest for burn toxemia. According to the literature, extracts from burned skin inhibit growth of cell cultures [17, 18].

The object of this investigation was to study changes in cell division after injection of extracts from burned skin into animals. Analogous extracts from normal skin served as the control. The test object was the surface epithelium of the cornea, for determination of the mitotic index of this tissue is widely used in the study of cell division during changes in the external and internal medium [1].

EXPERIMENTAL METHOD

Experiments were carried out on 129 noninbred mice weighing 18-20 g. The animals were distributed into three groups: 1) control animals receiving physiological saline, 2) animals receiving extract of normal skin (ENC), and 3) animals receiving extract of burned skin (EBC). The extracts were prepared as described in [10]. In the experiments of series I and II the animals were killed 45 and 90 min, and 6, 12, and 76 h after intravenous injection of the extracts (0.1 mg protein per mouse). In the experiments of series III the animals were killed 45, 60, and 90 min after application of EBC to the right cornea (1.9 μg protein), so that allowance could be made for the diurnal rhythm and factors inducing reactive inhibition of mitosis. The denucleated eyes were fixed in Carnoy's fluid. Dividing cells were counted in total preparations of the cornea in 100 fields of vision as described previously [4]. To select adequate methods of processing and analysis of the data, a special variance analysis was carried out. It has been shown [13] that the diurnal mitotic index (MI) in the liver does not obey the law of the normal Gaussian distribution and requires methods of statistical analysis different from the Student–Fisher t-criterion usually used [3]. However, the authors cited do not give the general principle for statistical analysis when studying cell division. Analysis of the results of the present experiments showed that cell division in the corneal epithelium obeys the law of the Poissonian distribution, but since MI is the arithmetic mean result of counting mitoses in different specimens, the values obtained no longer obey the Poissonian distribution, but approximate closer to the normal distribution in accordance with Lyapunov's central limiting theorem. It was therefore decided to

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TABLE 1. Mitotic Index in Corneal Epithelial Cells of Mice (per 100 fields of vision) after Injection of Extracts of Normal and Burned Skin at Various Periods of the Mitotic Cycle

Period of mitotic cycle	Time of sacrifice	Substance injected	MI				No. of corneas
			cornea	zone of cornea: peripheral	zone of cornea: intermediate	zone of cornea: central	
G_1	76 h	Physiological saline	29,2	15,3	32,1	39,0	17
		ENS	41,8†	23,4	39,0	60,0 †	15
		EBS	29,3	16,6	26,8	42,9	18
S*	12 h	Physiological saline	8,1	13,1	6,0	5,9	9
		ENS	8,2	12,6	9,3	3,5	10
		EBS	5,8	9,2	5,6	2,9‡	9
G_2	6 h	Physiological saline	38,4	26,9	41,2	47,3	10
		ENS "fresh"	40,3	26,1	38,9	53,3	10
		EBS "	41,5	30,4	42,4	48,9	10
G_2	6 h	Physiological saline	73,7	51,7	88,3	77,7	8
		ENS "old"	55,3‡	45,0	66,9‡	55,0‡	13
		EBS "	46,2†	25,2†	52,6†	60,2	8
Mitosis	45 min	Physiological saline	29,9	25,1	48,5	17,1	6
		ENS	56,9	34,6	59,6	77,0‡	6
Mitosis	45 min	Physiological saline	42,8	32,6	35,6	63,6	6
		EBS	35,5	21,0	25,0	58,3	6
Mitosis	90 min	Physiological saline	44,9	20,3	42,0	73,3	13
		ENS	44,3	26,9	43,4	64,5	12
		EBS	28,6‡	23,3	24,8	47,0†	16

*Animals sacrificed during the evening.
†$P < 0.01$.
‡$0.01 < P < 0.05$.

use nonparametric criteria of difference which are independent of the type of distribution. The summary table for selection of the criterion taken from the paper by Genkin and Gubler [3] was therefore used in conjunction with the Mann–Whitney U criterion of inversions to assess two sets with respect to the central tendency. The results were regarded as significant when $P \leq 0.01$. The standard error of the mean (M) is calculated for each type of distribution by means of special equations, and this calculation was not therefore carried out in the present investigation.

EXPERIMENTAL RESULTS

The effect of ENS and EBS at individual stages of interphase was studied in the experiments of series I. The times at which the animals were killed were chosen on the basis of data in the literature on the duration of the G_1, S, and G_2 periods in the corneal epithelial cells [8]. The results are given in Table 1.

ENS, if injected in the presynthetic G_1 period, induced a significant increase in MI in the central zone of the cornea. If injected in the S and G_2 periods, MI was the same as in the control.

If EBS was injected in the G_1 and G_2 periods, it had no significant effect on MI, while if injected during the period of synthesis (S) it had a tendency to reduce MI ($0.01 < P < 0.05$).

In the experiments of series II the effect of ENS and EPS was studied on the commencement of mitoses [i.e., on the possibility of blocking the progress of the cells from the G_2 period into mitosis (the G_2–M block described by Epiphanova [5])].

The number of divising cells 45 min after intravenous injection of ENS was increased from 17.1 to 77.0 (per 100 fields of vision). EBS caused no significant change in MI.

No significant changes in MI likewise were found 90 min after injection of ENS but EBS, on the contrary, induced a significant decrease in MI in the central zone of the cornea from 73.3 to 47.0.

The experiments of series III were carried out to study the action of EBS when applied locally.

They showed that EBS reduced MI in the central zone only during the first 45 min (control 54.8, experiment 22.0), after which its action ceases ($0.01 < P < 0.05$).

In the study of the effect of ENS and EBS on the postsynthetic G_2 period, the properties of extracts at +4°C for 2 months were studied at the same time. Table 1 shows that the action of the extracts changes on keeping, after which ENS has a slight inhibitory action while EBS significantly inhibits MI from 73.7 to 46.2.

On the basis of these results it is possible to evaluate the effects of ENS and EBS on the whole life cycle of the corneal epithelium cells. The G_1 period is known to be most sensitive to changes in the external environment [15]. The absence of any significant changes in MI during the action of extracts on the S and G_2 periods can therefore be explained by the small dose of the preparations used. The change in MI mainly in the central zone can be explained by the fact that at the time chosen for sacrifice, the highest value of MI is in fact observed in the central zone, and accordingly changes in MI were most marked in that zone. Since the processing taking place in interphase and mitosis are completely different [16], the action of EBS on these processes differed: EBS had no significant action on interphase but blocked the trigger mechanism of mitosis. The indefinite results for the blocking of passage of the cells from G_2 into M during the local action of EBS are evidently attributable to washing of the preparation off the cornea and the manifestation of reactive inhibition of mitosis.

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