2. M. M. Kalashnikova, "Changes in the ultrastructures of parenchymatous cells of the rat liver after local x-ray irradiation and mechanical injury," Candidate's Dissertation, Moscow (1965).
3. M. M. Kalashnikova, Arkh. Patol., No. 10, 39 (1971).
4. A. A. Manina, Arkh. Anat., No. 3, 77 (1967).
5. N. D. Ozernyuk, Growth and Reproduction of Mitochondria [in Russian], Moscow (1978).
6. D. B. Roodyn and D. Wilkie, The Biogenesis of Mitochondria, Methuen, London (1968).
7. D. S. Sarkisov, Essays on the Structural Basis of Homeostasis [in Russian], Moscow (1977).
8. V. Aschenbrenner, R. Druyan, R. Albin, et al., Biochem. J., 119, 157 (1970).
9. W. Bernhard and C. Rouiller, J. Biophys. Biochem. Cytol., 2, 73 (1956).
10. H. Braun, Exp. Cell Res., 20, 267 (1960).
11. G. Brunner and W. Neupert, FEBS Letters, 1, 153 (1968).
12. H. Gansler and C. Rouiller, Schweiz. Z. Path., 19, 217 (1956).
13. M. M. K. Nass and K. Nass, J. Cell Biol., 19, 593 (1963).
14. R. W. Swick, A. K. Rexroth, and J. L. Stange, J. Biol. Chem., 243, 3581 (1968).

EFFECT OF REPEATED COOLING ON THE STATE OF THE ADRENALS AND PROLIFERATION OF THE CORNEAL EPITHELIUM IN ALBINO RATS

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The effect of repeated cooling on the state of the adrenals and on the mitotic cycle and number of DNA-synthesizing nuclei in the corneal epithelium was studied in albino rats. The animals were cooled by a contact method to a body temperature of 28°C and exposed at that temperature 1 h daily for 5 days. Marked activation of the adrenals was observed: The weight of the glands was doubled, their cholesterol concentration reduced by two-thirds, their blood 11-hydroxycorticosteroid level increased fourfold, and their adrenalin excretion stimulated. The mean number of mitoses in the cornea was reduced by half. The depression was not connected with any change in the rate of mitosis but was due to delay in interphases. There was no change in the level of pathological mitoses. Chronic exposure to stress was not accompanied by any change in the number of DNA-synthesizing nuclei or the intensity of DNA synthesis.

KEY WORDS: mitotic activity; stress; hypothermia; DNA synthesis; cornea.

In previous investigations the writers found that chronic exposure to stress (intravenous injection of pyrogenal daily for 5 days) causes prolonged depression of mitotic activity without changing the index of labeled nuclei [10].

The object of the present investigation was to test if this rule applies also to the effect of a different stressor, namely moderate hypothermia. The study of this problem is of applied importance: moderate hypothermia is widely used in clinical practice. However, the basic research into the effects of low temperatures on cell proliferation has been undertaken on tissue cultures [2, 8, 13]. The few studies of the action of cold on cell division which have been undertaken on homeothermic animals have given contradictory results and were carried out on specialized tissues which play an active part in adaptation to low temperature [3, 12].

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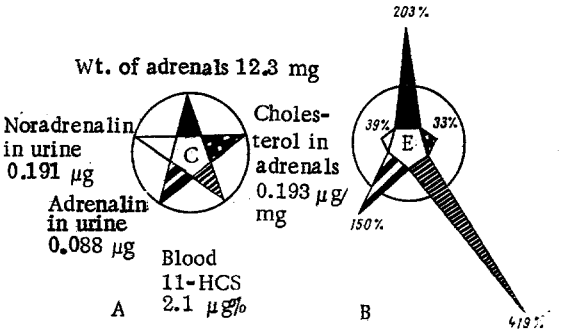

Fig. 1. Indices of the state of the adrenals of animals during repeated cooling. A) Control groups (C); B) experimental group (E) (indices expressed as percentages of control).

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighting 150-200 g. Contact cooling of the animals for 1 h was carried out by the method described previously [7], daily for 5 days from noon to 1 p.m. The animals were decapitated 6 h after the last (fifth) cooling. To prepare autoradiographs one cornea was incubated for 1 h in medium No. 199 with [^{3}H]thymidine in a dose of 2 μCi/ml, after which it was rinsed in medium No. 199 and fixed in Carnoy's fluid. Autoradiographs were prepared in the usual way. The number of DNA-synthesizing nuclei was counted in the stratum basale and stratum spinosum. After examination of 2500-3500 nuclei the index of labeled nuclei (ILN) was calculated in percent. The intensity of DNA synthesis was judged on the basis of counting grains of silver above 100 labeled nuclei in each preparation. The mitotic cycle was determined in total preparations of the corena fixed immediately after sacrifice. Mitotic activity was judged from the number of mitoses in 100 fields of vision. The level of pathological mitoses was determined in accordance with Alov's classification [1] and expressed in percentages of the total number of mitotic figures. To rule out any change in the rate of mitosis itself, parallel experiments were carried out with animals receiving an intraperitoneal injection of colchicine in a dose of 4 μg/g body weight 3 h before sacrifice. To achieve a complete stathmokinetic effect, simultaneously with the intraperitoneal injection of colchicine into the animals, 0.1 ml of 0.1% colchicine solution was instilled into both eyes. The adrenals of the rats were dissected, weighed on analytical scales, and their cholesterol content determined by Lifschutz' method [11]. Blood 11-hydroxycorticosteroids (11-HCS) were determined by the method of Pankov and Usvatova [6] and catecholamines in the 24-h sample of urine were determined by Matlina's method [4]. The numerical results were analyzed by Student's method. Altogether 56 animals were used in the experiments.

EXPERIMENTAL RESULTS

The experimental results show that the rats exposed to repeated cooling developed a marked reaction to the stressor (Fig. 1). The weight of the adrenals in the experimental animals doubled and their cholesterol content fell by two-thirds. The blood 11-HCS concentration increased fourfold. These results are in agreement with those obtained bo other workers who found activation of adrenal function during exposure to cold [9, 10, 14]. The changes in catecholamine excretion were opposite in character: Whereas the adrenalin content in the urine increased by 60%, noradrenalin excretion was significantly reduced. This observation agrees with the view that hormonal-mediator dissociation develops during exposure to stress [5].

Analysis of the mitotic cycle showed considerable inhibition of cell division (Table 1).

The mean number of mitoses in the cornea of the experimental rats was reduced by half. Changes in the level of pathological mitoses were not observed as a result of exposure of tissue cultures to low temperatures [2]. The experiments with colchicine confirmed that the decrease in the number of dividing cells during repeated stress does not depend on changes in the rate of mitosis but is the result of delay in interphase. The mean number of mitosis in the experimental rats was reduced almost by half as a result of treatment with colchicine. Autoradiographic analysis with [^{3}H]thymidine showed that repeated cooling is not accompanied

TABLE 1. Effect of Repeated Cooling on Some Indices of Proliferation in Corneal Epithelium of Albino Rats

Group of animals	Mean No. of mitoses	No. of pathological mitoses, %	Mean No. of mitoses after treatment with colchic.	ILN, %	Mean No. of grains above nucleus
Control	234,4±24,0	6	980,0±121,9	12,85±1,36	28,6±2,7
Experimental	118,1±12,33	6,5	542,1±81,6	12,51±0,74	34,7±2,5
P	<0,001	>0,5	<0,01	>0,5	0,1<*P*<0 25

by any change in the number of DNA-synthesizing nuclei: Whereas ILN for the control animals was 12.85%, for the experimental rats it was 12.51%. The intensity of DNA synthesis likewise showed no significant change. The mean number of grains of reduced silver above the corneal nuclei of the control animals was 28.6±2.7, compared with 34.7±2.5 for the experimental animals (0.1<P<0.25).

In the present investigation no attempt was made to differentiate the role of low temperature itself in the depression of cell division and the role of increased secretion of adrenal hormones in this process. In the present experiments it is difficult to rule out completely an antimitotic effect of low temperature, which has been repeatedly demonstrated on tissue cultures [2, 8, 13]. However, the authors' previous experiments on adrenalectomized animals, and also experiments using β-adrenoblockers, suggest that increased secretion of adrenal hormones during hypothermia is a determining factor of the depression of mitotic activity under these experimental conditions. Furthermore, rapid reversibility is characteristic of the antimitotic effect of a moderately low temperature: The mitotic index and the ratio between the phases were completely restored as early as 1 h after restoration of the normal temperature in tissue culture [2].

In the present experiments inhibition of cell division was observed not only 6 h, but also 12 h after cooling. In additional experiments the animals were decapitated 12 h after cooling for 5 days. In this series of experiments depression of mitotic activity also persisted: Whereas the mean number of mitoses in the control animals was 237.9±16.8, in the experimental rats it was 165.2±14.3 (P<0.01).

The facts described above suggest that adrenal hormones play the leading role in the depression of cell division during repeated moderate hypothermia in albino rats, just as during pyrogenal stress.

It can thus be concluded from the results of these investigations that depression of mitotic activity during chronic exposure to stress is not connected with changes in the number of DNA-synthesizing nuclei or with lengthening of the S period. Suggestions regarding the nature of the divergence between ILN and mitotic activity have been put forward by the writers previously [10]. The results of the cytophotometric and autoradiographic analyses of changes in the cell cycle of the corneal epithelium observed by the present writers in pyrogenal stress will be published in the near future.

LITERATURE CITED

1. I. A. Alov, The Cytophysiology and Pathology of Mitosis [in Russian], Moscow (1972).
2. M. N. Boltovskaya, Byull. Éksp. Biol. Med., No. 2, 216 (1977).
3. M. N. Maksimova and S. F. Sid'ko, in: Human Adaptation in the Far North [in Russian], Krasnoyarsk (1976), p. 35.
4. É. Sh. Matlina, Z. N. Kiseleva, and I. É. Sofieva, in: Methods of Investigation of Some Hormones and Mediators [in Russian], No. 3, Moscow (1965), p. 25.
5. É. Sh. Matlina and G. N. Kassil', Usp. Fiziol. Nauk, 7, No. 2, 13 (1976).
6. Yu. A. Pankov and I. Ya. Usvatova, in: Methods of Investigation of Some Hormones and Mediators [in Russian], No. 3, Moscow (1965), p. 137.
7. N. B. Simon and S. S. Timoshin, Byull. Éksp. Biol. Med., No. 11, 1371 (1976).
8. F. V. Sushkov and V. V. Portugalov, Byull. Éksp. Biol. Med., No. 12, 81 (1975).
9. E. M. Stabrovskii and V. I. Severovostokova, Fiziol. Zh. SSSR, No. 8, 1269 (1972).
10. S. S. Timoshin and N. B. Murzina, Byull. Éksp. Biol. Med., No. 8, 248 (1978).
11. M. Tulczinski (Editor), Laboratory Methods of Clinical Investigation [in Russian], Warsaw (1965).

12. B. Kaissling and O. Bucher, Z. Zellforsch., 146, 417 (1973).
13. H. Nagasawa and W. S. Devey, J. Cell Physiol., 80, 89 (1972).
14. S. J. Shepherd, M. J. Smith, and B. B. Longwell, Endocrinology, 50, 143 (1952).

INTERACTION BETWEEN MITOGENIC LECTINS AND PLASMA MEMBRANES OF LYMPHOCYTES

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Mitogenic signals from concanavalin A and phytohemagglutinin were shown to undergo summation in time under conditions when each mitogen separately, if the duration of contact with lymphocytes during the experiment was the same (14 or 20 h), did not induce mitogenesis. The results are discussed from the standpoint of cell–cell interaction between lymphocytes. It is suggested that the ability of these signals to undergo time summation lies at the basis of the nonspecific mechanism of protection against tolerance.

KEY WORDS: mitogens; lymphocytes; plasma membrane.

In order to understand the molecular mechanism of activation of lymphocytes and induction of tolerance, information is needed on the state of the receptor molecules on the surface of the plasma membrane of lymphocytes. A convenient model of interaction between lymphocytes and antigens is provided by the process of nonspecific activation by certain mitogens, mainly lectins of plant origin and of different carbohydrate specificity, which induce RNA, DNA, and protein synthesis and also blast transformation [6].

Because of the nonspecific nature of mitogenic stimuli, it might be expected that mitogens would be "mutually interchangeable," or in other words that signals from different receptors could undergo time summation.

In the investigation described below interaction between two mitogens — concanavalin A (conA) and phytohemagglutinin (PHA) — with lymphocytes from rat lymph nodes was studied.

EXPERIMENTAL METHOD

Purified suspensions of mesenteric lymph node cells from Wistar rats were used. The cells ($2 \cdot 10^6$) were incubated at 37°C in 2 ml of medium No. 199 to which 5% inactivated human blood group AB serum and antibiotics were added. Mitogenesis was induced by addition of conA (from Boehringer Mannheim) or PHA (from Difco) to the culture. The carbohydrate specificity of lectins has been studied sufficiently well, so that it was possible to remove cells binding conA selectively from the surface by washing in medium containing hapten carbohydrates: α-methylmannopyranoside (α-MM) or α-methylglucopyranoside (α-GP) (from Calbiochem). The cells were incubated with the haptens for 20 min at 37°C 14 or 20 h after addition of mitogenic concentrations of conA, after which they were washed three times with culture medium containing the hapten carbohydrate, PHA or conA was added, and culturing continued. Next, 48 h after the beginning of cultivation, 0.5 μCi [^{3}H]thymidine (specific activity 20.6 Ci/mmole) was added to the cultures. After 72 h the cells were treated with cold 5% TCA and the insoluble fraction (DNA) was harvested on membrane filters. The quantity of [^{3}H]thymidine incorporated into DNA was determined by means of the ZhS-106 scintillator on a Mark II counter. The quantitative data on incorporation of [^{3}H]thymidine into lymphocyte DNA consisted of the arithmetic means of measurements in the three to five parallel cultures, expressed as cpm/$2 \cdot 10^6$ cells.

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