EFFECT OF EXTREMAL FACTORS ON GROWTH OF KIDNEY TISSUE IN CULTURE

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UDC 612.46-085.23-06 : 616-092.9

Changes in the number of mouse kidney cells in culture were studied after exposure of the animals to various conditions: hypothermia, physical exertion, overcrowding in the cage, hunger, subcutaneous inflammation, ether, and medinal anesthesia. A single exposure of the animals to the various factors was followed by an increase in the number of kidney cells in the culture compared with the control. After repeated and prolonged cooling, physical exertion, and overcrowding of the mice, depression of tissue growth was observed. It is postulated that changes in growth of kidney tissue after exposure of the animals to extremal factors are a manifestation of the general adaptation syndrome.

The tissue culture method has been widely applied to the investigation of the effects of various chemical and physical factors on cells [8-10]. This method has been used to a lesser degree to study changes in the properties of cells in various pathological states [1, 2, 5, 7].

No information could be found in the literature regarding the use of tissue cultures to study the state of cells in the body during the stress reaction.

In the investigation described below, changes in the properties of kidney cells from adult animals exposed to certain extremal factors and detected in tissue culture were studied.

EXPERIMENTAL METHOD

Experiments were carried out on 625 male and female albino mice weighing 20-30 g. The animals were sacrificed by decapitation. Under sterile conditions the kidneys were extracted, longitudinal incision was made in them, and they were washed twice with a solution of medium containing antibiotics (penicillin and streptomycin, 1000 units/ml of each). The kidneys were then cut into small pieces with scissors. Pieces measuring 0.3-0.6 mm^2 were selected for cultivation and were explanted on the wall of test tubes. The tubes were inclined at a small angle in a stationary stand and incubated at 37°.

The pieces of kidney were grown in plasma-free cultures [6, 10], using medium No. 199 with the addition of 10% bovine serum and antibiotics: penicillin and streptomycin, 100 units/ml of each. To activate growth of the cells, two drops (0.06-0.08 ml) of 1% cysteine solution for each milliliter of medium was added to the tube. Four or five pieces of tissue were placed into each tube. To assess growth of the cells, from 40-50 pieces were taken from one animal. The number of cells growing on the wall of the tubes was counted 72 h after the beginning of cultivation. By this time 70-80% of the cultivated pieces of tissue had formed growing colonies of cells.

In each case the same number of animals was used in the control and experimental groups. To obtain comparable results the conditions of cultivation in the control and experimental series were strictly iden-

Laboratory of Human Acclimatization of the Far North, Arkhangel'sk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 8, pp. 94-98, August, 1970. Original article submitted January 15, 1970.

TABLE 1. Changes in Growth of Kidney Tissue in Culture after Exposure of Animals to Extremal Factors

Character of stress factor	Statistical index	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment
Cooling in chamber		mice sacrificed immediately after cooling		sacrificed 24 h after cooling		sacrificed 72 h after cooling		repeated cooling of mice for 14 days	
	$\frac{n}{M \pm m}$	15 (600) 24,9±1,23	15 (600) 29,3±1,41	18 (900) 18,4±0,57	18 (900) 24,9±0,66	19 (760) 13,6±0,41	19 (760) 22,1±0,76	20 (800) 20,4±0,62	20 (800) 15,1±0,62
	P	<0,001		<0,001		<0,001		<0,001	
	%	+17,6		+35,3		+62,5		—26,0	
Physical exertion		mice swam for 2-3 h and sacrificed immediately after		mice swam for 1 h on 3 days		mice swam for 1 h on 10 days		mice swam for 30 min on 16 days	
	$\frac{n}{M \pm m}$	16 (640) 22,9±1,08	16 (640) 30,3±1,01	15 (600) 22,2±0,64	15 (600) 22,4±0,79	15 (600) 30,1±1,48	15 (600) 26,4±0,84	15 (600) 23,9±0,65	15 (600) 21,7±0,76
	P	0,001		0,1		0,001		0,001	
	%	+32,2		—		—12,3		—9,0	
Subcutaneous inflammation		mice sacrificed after 3 h		mice sacrificed after 24 h		mice sacrificed after 72 h		mice sacrificed after 9 days	
	$\frac{n}{M \pm m}$	15 (600) 23,1±0,89	15 (600) 29,8±1,06	14 (560) 19,2±0,77	14 (560) 24,1±1,02	14 (560) 21,8±1,14	14 (560) 35,7±1,96	12 (480) 20,6±0,51	12 (480) 23,6±0,91
	P	0,001		<0,001		0,001		0,001	
	%	+29,0		+25,5		+63,7		+14,6	

Legend: n) number of animals; M ± m) mean number of cells per explant and error of mean; +) increase in number of cells in experiment relative to control; −) decrease in number of cells in experiment.

Note. Total number of cultures given in parentheses.

tical. The components of the nutrient medium were also constant within each series. The final result was obtained by calculating the total number of cells in a zone of growth for one piece of tissue in culture. The results were subjected to statistical analysis.

To study growth of the tissue in culture in states of stress, the animals were exposed to certain procedures: cooling, physical exertion. Changes in the number of cells were also investigated when the animals were kept under overcrowded conditions, in hunger, or after subcutaneous inflammation and necrosis.

EXPERIMENTAL RESULTS

The principal experimental results are given in Table 1.

The mice were cooled in a chamber at a temperature of −10° until a state of definite cold narcosis developed. After cooling for 1.5-2 h, the number of kidney cells in the culture was increased by 17.6%. With more rapid cooling of moist animals under the same conditions for 15-30 min to a state physiologically similar to that in the preceding experiments, no changes in the number of cells were observed. When the mice were sacrificed 24 and 72 h after a single cooling to −10° for 1.5-2 h, the number of cells in the culture was increased by 35.3 and 62.5%, respectively, relative to the control. After repeated cooling of the mice for 14 days at −5° for 5 h daily, the number of kidney cells in the culture was reduced by 26%.

These changes in growth of kidney tissue after cooling of the animals were not associated with the direct action of cold on the cells. When pieces of kidney were cooled separately at temperatures of 20, 10, and 0° for 3 h, in all cases inhibition of cell division was observed. The mean number of cells in the control was 29.3 ± 1.9, while after cooling of pieces of kidney at 20° the number of cells was 19.0 ± 1.45, after cooling at 10° it was 14.7 ± 1.3, and after cooling at 0° the mean number of cells in the culture was 15.5 ± 1.49.

The physical exertion to which the mice were subjected was swimming in water at a temperature of 36°. After swimming once in water for 2-3 h until the animals were completely fatigued, the number of cells in the culture increased by 32% compared with the control. After repeated swimming for 1 h daily for 3 days, the differences between the number of cells in the control and experimental groups of mice were not statistically significant. When the same daily exertion was continued for 10 days, inhibition of growth of the cells by 12.3% was observed. In an analogous series of experiments with repeated exertion, when the animals swam for 30 min daily for 16 days, the inhibition of cell growth was 9% compared with the control.

If the animals were kept for long periods in the cage under unsatisfactory conditions (32 mice in the series), inhibition of tissue growth in the culture also was observed. In these experiments one group of animals was kept in cages measuring 30 × 25 × 30 cm, with 5 animals to each cage, while another group of mice (30 animals) were kept in a single cage of the same size. The animals were kept under these conditions for 14 days. When the mice were kept in cages in groups of 5, the mean number of cells per culture was 21.8 ± 0.80, while when all the animals were kept together the number was 16.1 ± 0.70. Inhibition of tissue growth in the second case was 26% relative to the first ($P < 0.001$).

When a group of animals (30 mice) was completely deprived of food for 72 h, an increase in the number of kidney cells in the culture was found. The mean number of cells in the control was 8.0 ± 0.54, and in the experiment 9.9 ± 0.65 ($P < 0.001$). The difference was 24%.

Inflammation was produced by subcutaneous injection of 0.1 ml turpentine on the lateral surface of both thighs. When the animals (30 mice) were sacrificed 3 h after injection of turpentine, a considerable increase in the number of kidney cells in the culture was observed. In the control group the mean number of cells was 17.0 ± 0.73, and in the experiment 23.6 ± 0.79 ($P < 0.001$). The difference was 38.8%. When animals (36 mice) were sacrificed 72 h after injection of turpentine, the increase in the number of kidney cells relative to the control was smaller: in the control there were 21.9 ± 0.76 cells, in the experiment 23.2 ± 0.74 cells ($P < 0.02$). The difference was 5.9%.

On the assumption that turpentine may have a toxic action on kidney cells, a series of experiments was carried out in which subcutaneous inflammation was produced by injection of hot physiological saline (Table 1). In this case an increase in the number of cells was observed in all experiments. It was particularly marked immediately after injection and also after 72 h. These experiments suggest that during anesthesia, when inhibition of functions of the central nervous system may take place to some degree, inhibition of tissue growth must be anticipated. The effect of ether and medinal anesthesia was accordingly

studied. When ether was used (30 mice) the duration of anesthesia was 2.5 h. The animals were sacrificed immediately after the end of this period. In this case the mean number of cells in the control was 17.3 ± 0.47, and in the experiment 16.3 ± 0.53. The decrease in the number of kidney cells was 6% ($P < 0.01$). In medinal anesthesia (30 mice) of the same duration, the inhibition of tissue growth was greater (29%). In the control group the number of cells was 19.8 ± 0.70, and in the experimental group 14.1 ± 0.50 ($P < 0.001$).

The results of these experiments showed that a single exposure of the animals to an extremal factor is accompanied by an increase in the number of kidney cells in the culture. Prolonged and repeated exposure of animals to cold, physical exertion, or overcrowding leads to a decrease in the number of cells. In the presence of inflammation, the stimulation of tissue growth persisted throughout the period of observation.

The differences between the character of growth of kidney tissue observed in these experiments after animals had been exposed to different factors are associated with changes in the state of the cells in the body and can be attributed to the successive stages of development of the general response to stress [11]. The results are in definite agreement with changes in the activity of bone marrow cells depending on the stage of the general adaptation syndrome, as demonstrated by Gorizontov and Rudakov [3, 4].

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