THE INFLUENCE OF LOW TEMPERATURES UPON ISOLATED EPITHELIAL TISSUES OF WHITE MICE AND FROGS

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In the works of E. N. Gulinova [2], O. I. Romanenko [9], E. K. Zhukov [3], E. Ya. Graevsky and G. S. Strelin [1] it has been demonstrated that low temperatures provoke in living tissues the same complex of non-specific responses (parabiosis, paranecrosis) which can be produced by irritants of the most varying type. This gives us the right to consider low temperatures in the general category of noxious agents – along with high temperatures, narcotics, the most diverse chemicals, electric currents, high pressures, etc.

The above-mentioned workers used highly specialized, conductive tissues – nerve and muscle.

In this experimental series we observed the effects of low temperatures on nonconducting, epithelial tissues. The purpose of our experiments was to determine the threshold low temperatures affecting isolated epithelial tissues, i. e., to determine the precise low temperatures having a demonstrable noxious effect. This type of work is also of undoubted clinical interest.

We thought it would be of interest to observe the effect of low temperatures, on the one hand, upon tissues coming into contact with the outside, and, on the other hand, with the internal tissues of the organism. As representative of the first group, we used horny epithelium and skin from the ear, as representative of the second group – epithelium from the urinary bladder and the intestine. These experiments were performed upon white mice and frogs both in the winter and spring seasons.

We observed the time of paranecrosis, i. e., the minimal time of action at the lowered temperature of interest to us which would produce a demonstrable parabiosis and paranecrosis of the cells, this being determined with the aid of vital, neutral red staining. As is well known, the picture produced by vital red staining in cells which are in a state of paranecrosis is sharply different from the picture seen in usual resting cells; diffuse staining of the nucleus and protoplasm and lack of granular staining characterizes the state of paranecrosis, while at the same time the uninjured cells are characterized by granular staining and absence of diffuse staining of the nucleus and protoplams [8].

We studied the effect of the following temperatures: $-20°$, $-10°$, $-3.5°$, $0°$ and $+18°$ (the lower temperatures were attained through the use of cryohydrate).

EXPERIMENTAL METHODS

The following experimental scheme was employed. The teased tissues were immersed for an hour in Ringer's solution at room temperatures, after which they were placed in the bottom of a test tube in a drop of Ringer solution; the test tube was immersed in a mixture of ice and salt in a Dewar flask. After a predetermined time interval, the test tubes were taken out and thawed at room temperatures (no special measures were taken

to thaw the tissues at an accelerated rate). After the thawing of the tissues, they were stained with neutral red (0.01%) for 30 minutes, and then were placed for 30 minutes in Ringer solution. The mouse tissues were stained at a temperature of 37°, the frog tissues at room temperature. The stained tissues were studied under the microscope.

EXPERIMENTAL RESULTS

The results obtained are shown in the table.

Time of Paranecrosis of Epithelial Tissues of White Mice and Frogs at Varying Temperatures

Animal	Type Tissue	Temperature in degrees				
		+17	0	−3.5	−10	−20
		appearance of paranecrosis (in hours)				
Mouse	Horny epithelium	96	192	72	24	24
	Skin epithelium	96	192	72	24	24
	Urinary bladder epithelium	72	192	72	2	0.5
	Intestinal epithelium	48	96	48	2	0.5
Frog	Horny epithelium	96	480	96	12	12
	Urinary bladder epithelium	96	480	96	6	0.5
	Intestinal epithelium	96	480	96	0.5	0.5

As can be seen from the table, almost all the epithelial tissues observed by us at room temperatures maintained their granular staining reaction for three days; on the fourth day paranecrosis made its appearance. Only for the urinary bladder epithelium and especially for the intestine of the mice did we observe a marked shortening of the time for the appearance of paranecrosis. An indication of this shorter survival of intestinal tissues is seen in the work of S. R. Muchnika [7], who explained it on the basis of an intensification of autolytic processes due to a high content of fermentative complexes.

The time for the appearance of necrobiosis at 0° (see table) increases as compared with room temperatures for all tissues, especially those of the frog; some of the cells retain granular staining for as long as 20 days and only by the 21st day do all the cells enter a state of paranecrosis.

At a temperature of −3.5°, which as a rule in our experiments did not cause freezing of Ringer's solution, the time for paranecrosis is markedly shortened (by 2-5 times). Therefore, we consider the temperature range between 0° and −3.5° to be the threshold range.

Upon further depression of the temperature to −10° which caused a rapid freezing of the solution,* the time for the appearance of paranecrosis was shortened still more, in different measure however, for the internal and external tissues. For example, a 2 hour stay at −10° caused paranecrosis of the intestinal epithelium and of the bladder epithelium, while at the same time the squamous epithelium from horn and skin had only a marked increase in granule formation (Fig. 1); with a 12 hour action one half of these cells underwent parabiosis, and only after a whole day's stay at such a temperature did paranecrosis occur in these cells. Accordingly, at −10° there is required a rather lengthy stay (24 hours), during which time these cells had continued to maintain their life property (granule formation).

The same difference between internal and external tissues is manifested also in their behavior at −20°. A 30 minute stay at this temperature caused paranecrosis of the cells from the intestine and urinary bladder, while the horny and skin epithelium cells survived to maintain their granule formation reaction for almost 24 hours (true, in only a small number of cells). The greatest resistance was manifested by the most superficial cells in the horny layer of the mouse; for up to several days (5) some of these cells retained their capacity for granule staining.

* Whether the tissues also froze we are unable to say, as we performed no special studies for elucidation of this point.

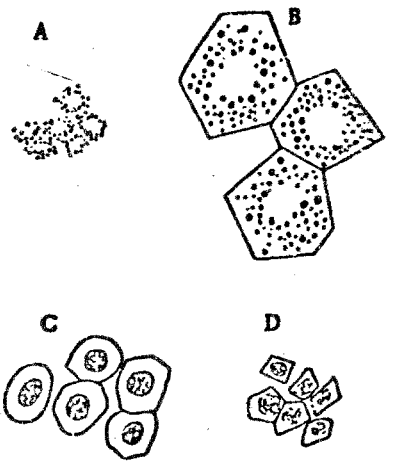

Fig. 1. Epithelium of white mouse after two-hour action of a temperature of −10°. A) Horny epithelium; B) skin epithelium; C) urinary bladder epithelium; D) intestinal epithelium. Neutral red stain 0.01%. Magnification 7 x 90, the sketching apparatus at level of table holding microscope.

The great differences between the capacities of the internal and external cells are clear from an examination of Fig. 2. As can be seen from the curves, all the tissues have a rather similar behavior more or less in the neighborhood of temperature from 17 down to −3.5°. Upon the action of temperatures of −10 and −20° the time for paranecrosis falls precipitately for intestinal and urinary bladder epithelium and much less markedly for the horny and skin epithelium.

We may surmise that the greater durability of the external tissues as compared with the internal, manifesting itself as shown by us under the action of low temperatures, is an expression of an overall greater stability of external tissues in general to general noxious agents. In favor of this are the findings obtained by I. E. Kamnev [5], who showed that external tissues placed for an hour in an isotonic sugar solution, do not change, while in the same time tissues from the internal parts of the organism go into a state of paranecrosis. For a final determination of this question there are needed further researches with various inadequate irritants.

The fact of the retention of living properties by cells from the external epithelium under conditions of a low temperature is in conformity with the findings in the literature which establish the presence of an exchange of materials

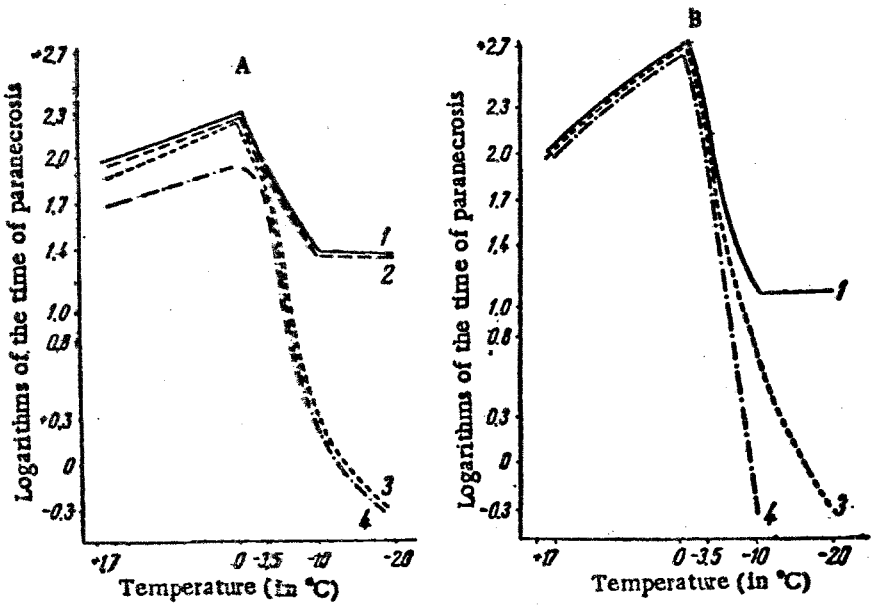

Fig. 2. Time of paranecrosis of epithelial tissues of a white mouse (A) and frog (B) at the indicated low temperature. 1) Horny layer; 2) skin; 3) urinary bladder; 4) intestine.

at temperatures below 0°. Thus, N. L. Kalabukhov [4] in his experiments demonstrated the presence of breathing in the meal worm (Tenebrio molitor), under conditions of cooling to −10°. The same was shown by P. V. Kozhanchikov [6] for the cucumber moth. In the work of Klinke [10] are given direct data showing that certain cancerous and normal cells survive freezing to −253°.

On the basis of the obtained data it can be deduced that lowering temperature to 0° increases the time of survival of isolated tissues; further lowering of the temperatures shortens the length of life of the tissues as compared with room temperatures. The threshold region of the action of low temperatures upon epithelial tissues must be considered as being in the region 0° to −3,5°.

Epithelial tissues which border on the outside environment are more capable of withstanding low temperatures, than are epithelial tissues lying within the organism.

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