Glycerin prevents the 'heat-death' of Ehrlich ascites tumor cells

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Summary. When heated to 45 °C for 20 min Ehrlich ascites tumor and NK-lymphoma cells are irreversibly damaged, so that they lose transplantability. The presence of 7-8% glycerol during heat treatment protects EAT cells completely and NK-lymphoma cells partly against this injury.

Heat damage of Ehrlich ascites tumor (EAT) cells were first studied by Vollmar[1], who observed that, while a remarkable lesion of these tumor cells can be seen at 39-43 °C, cultures of hen heart fibroblasts and mouse spleen show a steady growth even when heated to 43-45 °C. Fekete et al.[2-4] found that EAT cells become untransplantable after heat treatment (45 °C), i.e. they perish due to 'heat-death'. Moreover, glycolysis, respiration, as well as glucose, fructose and mannose utilization decrease in these cells.

Ardenne et al.[5-19] found that the lowest temperature at which glycolysis and respiration of carcinoma cells become damaged is 42.3 °C. Using a complex therapeutic method, the authors noted that the cytotoxic effect of DL-glycerinaldehyde is enhanced by extreme hyperthermia under in vitro and in vivo conditions.

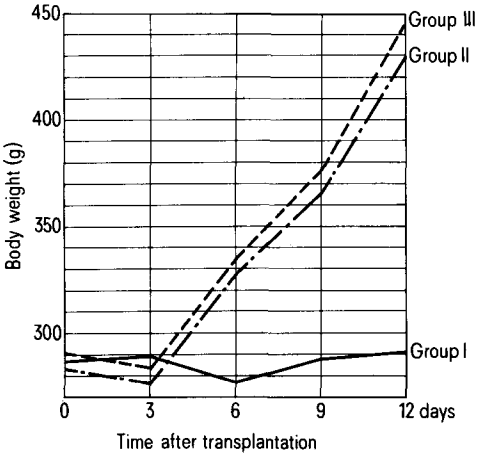
While we have numerous data on the heat damage of tumor cells, no data are available on the prevention of 'heat-death' in these cells. Now we report on our finding that the loss of transplantability of tumor cells following a heat treatment at 45 °C was prevented by glycerin solution.

Material and method. For the experiments, mice of both sexes from CFLP strain were used (28-30 g). The EAT and NK-lymphoma strains were received from the Research Institute of Oncopathology (Budapest). In each experimental group, 10 animals were included. The animals were injected (i.p.) with tumor cell suspensions pretreated in different ways and at different temperatures. The experiments were repeated several times using alternately male and female animals. The experiment was performed in the following steps: 10 ml of EAT collected from tumorous animals were measured, together with plasma, in 3 test-tubes. To the 1st tube 3 ml physiological saline, to the 2nd 2 ml saline + 1 ml glycerin, and to the 3rd 3 ml saline was added. After mixing well, the cell suspensions in the respective solutions in the 1st and 2nd tubes were placed in water-bath at 45 °C and incubated for 20 min. The 3rd tube was left at room temperature until transplantation. The heat-treated suspensions were than left at room temperature for 20 min. Transplantation was performed as follows: 3 groups each including 10 healthy mice were injected i.p. with 1 ml tumor cells suspensions in physiological saline heated at 45 °C (group I), in physiological saline + glycerin at 45 °C (group II), and in physiological saline at room temperature (group III). After transplantation the b.wt and the abdominal volume of the animals were determined every 3 days.

Results. The figure shows the curve of b.wt of mice treated in various ways. The curves stand for the mean values of 10 animals. As can be seen, the weight of animals of group I remained unchanged during the period of 12 days after transplantation. No tumor developed in these animals even after 6-8 weeks. Thus, the tumor cells perished at 45 °C. On the other hand, the animals in group II and III show a marked gain in weight due to the growth and accumulation of tumor cells. Beginning from the 12th day, the mice in group III (controls) died one after the other. Amounts of 15-20 ml of ascites fluid can be obtained from the abdominal cavity in such animals. The mice in group II died beginning from the 14th day, i.e. inhibition of tumor development seems less in these animals. If the animals of group II were killed 10-12 days after transplantation and the EAT cells were injected in healthy mice, a similar curve of b.wt was obtained as in group III. This shows that glycerin protects the tumor cells from heat damage so that they preserve their transplantability.

The effect of glycerin was also studied on NK-lymphoma cells incubated at 45 °C. In these cells, however, glycerin proved to be less effective in protecting against heat damage. In animals with NK-lymphoma, the first signs of tumor development were only observed on the 15th day after transplantation and only in about 60% of the animals.

When considering the effect of glycerin in protecting the tumor cells from heat damage, it should be mentioned that L-glycerin aldehyde has long been known as a compound inhibiting the glycolytic activity of carcinoma cells[20]. Warburg et al.[21] also found that EAT cells pretreated with D-, and L-glycerin aldehyde do not develop after transplantation. Riddick and Bessler[22] noted depressed glucose turnover, lactic acid and ATP production on D-, and L-glycerin aldehyde treatment of tumor cells. Wand et al.[23] observed that utilization of succinate, pyruvate and malate were inhibited in isolated tumor mitochondria, Drews[24] reported on decreased hexokinase activity due to the effect of D-, L-, and DL-glycerin aldehyde. Since in EAT cells incubated in physiological saline and in glycerin at 45 °C, respectively, the glycolysis and respiration and the activity of their enzymes decrease, it seems that other factors may be primarily involved in the 'heat-death' of tumor cells. Drews and Fölsch[25] found that D- and DL-glycerin aldehyde inhibit the incorporation of glycine-1^{14}C into the tumor protein. Oelkers et al.[26] stated that D- and L- glycerin aldehyde inhibit the synthesis of nucleic acids. It may thus be concluded that the damage in protein and nucleic acid synthesis produced by heat treatment is of more importance than the inhibition of glycolysis and respiration. Presumably, glycerin protects the enzymes that catalyze the synthesis of proteins and nucleic acid, from heat damage, coagulation and denaturation due to heat. Since from 7-8% glycerin the tumor cells may utilize only a negligible



The curve of body weight of mice treated in various ways.

quantity for their carbohydrate and fat metabolism, it seems probable that glycerin protects the highly sensitive cell membranes from the damaging effect of heat at 45 °C.

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Hepatic α-tocopherol binding protein in the rat: Absence of an effect of selenium deficiency on binding activity

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Summary. Selenium deficiency produces no effect on either the total content of or the binding properties of rat liver α-tocopherol binding protein.

The biochemical basis for the interactions of selenium and vitamin E, first noted at the nutritional level, are slowly being understood. Following the discovery of Rotruck et al.[1] that selenium is a necessary component of the enzyme glutathione peroxidase, Hoekstra[2] suggested that glutathione peroxidase reduces lipid hydroperoxides while vitamin E prevents their formation, a proposal consistent with an interaction of these nutrients in oxidant-induced disease. Other observations, however, suggest interactions not explained on this basis. For example, a number of laboratories have reported that at marginal vitamin E intakes, increased dietary selenium results in a decrease in plasma tocopherol levels in rats[3-5]. Other studies[6] have shown that at adequate vitamin E intakes the converse relationship exists, i.e. with increased dietary selenium there is an increased plasma tocopherol. Either situation suggests that selenium affects the absorption, transport or utilization of tocopherol.

We have recently described a highly specific α-tocopherol binding protein in experimental animals that is detectable only in the liver[7]. This localization, coupled with the fact that liver necrosis results from a combined selenium and vitamin E deficiency in rats, suggested that a possible interaction should be investigated. Accordingly, a study was undertaken to examine the effects of selenium deficiency on the binding of α-tocopherol to its hepatic binding protein.

Methods and materials. Female Fisher strain rats with 1-week-old litters were gradually introduced over a 4-day period to a selenium and vitamin E-deficient diet based on torula yeast as previously described[8]. The male pups were weaned at 21 days, caged individually, and divided into 3 dietary groups. The 1st group continued to receive the basal, doubly deficient diet; a 2nd group received basal diet supplemented with 0.1 ppm selenium as sodium selenite, and a 3rd group received a vitamin E-deficient, selenium-sufficient casein diet[9]. Rats were allowed free access to the diets and water.

At approximately 18 days post weaning, rats receiving the basal torula yeast diet began dying from liver necrosis. At this time 3 rats from each group were sacrificed and liver cytosol preparations made for assay of α-tocopherol binding protein as previously described[7]. Only the non-necrotic portions of the livers of rats receiving the doubly deficient diet were used. Protein content of the cytosol was measured by Biuret[10].

Results and discussion. Initial studies of the α-tocopherol binding protein were made with livers from rats fed the vitamin E-deficient, selenium-adequate casein diet. It was shown that supplemental dietary tocopherol reduced the

Effects of selenium deficiency on the specific activity of rat liver α-tocopherol binding protein

Diet	Body weight (g)	Liver weight (g)	Cytosol* protein (mg/ml)	Tocopherol bound pmoles/mg cytosol protein	pmoles/g liver
Torula yeast	50.3±0.6	2.6±0.1	7.8±0.6	3.3±0.2	126.7±16.6
Torula yeast +selenium	69.0±4.0	5.2±0.3	7.2±0.5	3.2±0.2	114.3± 8.3
Casein	77.0±2.0	4.9±0.2	12.9±0.8	3.1±0.1	207.3±22.2

Values are mean±SD. * Cytosol = 105,000×g supernatant. Tissues were homogenized in 4 volumes of buffer.