THE EFFECT OF THE RATE OF FREEZING AND THAWING OF TUMOR TISSUES ON THEIR GROWTH IN TRANSPLANTATION

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The data in the literature on the question of the influence of the rate of freezing and thawing on the viability of tumor tissue are very inconsistent. Thus, Breedis and co-workers[3, 4], Snell and Cloudman [13], Burmester [5], Warner and Gostling [14], Hauschka and co-workers [9], and others consider slow freezing the most favorable for tumor tissue. Other authors, on the other hand, emphasize the importance of rapid freezing [1, 10, 12]. In the experiments of Gye and co-workers [8] the rate of cooling did not influence the viability of the tumor material. Gabrielson, Syverton and Kirschbaum [7] arrived at the conclusion that a combination of slow freezing with slow thawing only leads to a certain lengthening of the latent period, while rapid freezing in combination with rapid thawing causes a complete loss of activity in leukemic cells of mice. At the same time, Cassel [6] did not observe notable differences in the viability of material undergoing rapid and slow freezing and thawing. A detailed analysis of the literature on this question can be found in the survey by Kredzhi [2].

Our experiments for studying the effect of the rate of freezing and thawing on the viability of tumor tissue were set up with two tumor strains—Walker's carcinosarcoma and Ehrlich's ascitic carcinoma. Both these strains are characterized by complete transplantability, rapid growth, and, according to our data, the ability to tolerate freezing well.

METHOD

From the Walker carcinosarcoma tissue we prepared a suspension in the dilution of 1:3, using isotonic (5.3%) glucose solution. The ascitic fluid of mice with Ehrlich's carcinoma was carefully mixed with an equal volume of the same solution. The material was poured into ampules, which were then sealed and placed in a refrigerator at 4° for 30 min. After this a portion of the material was injected into animals of the control group, while the rest was subjected to freezing in a mixture of acetone and dry ice.

Slow freezing was accomplished by immersion of the ampule containing the material in the mixture of acetone and dry ice, the temperature of which was initially 4° and was subsequently lowered to −72° over the course of 20 min as a result of adding small pieces of dry ice. In this fashion the average rate of cooling of the mixture was 3.8° per min.

For rapid freezing the ampule was submerged for 3 min in a suspension of acetone and dry ice at −72°.

Then the ampules were transferred to a container with dry ice, where they were maintained at a temperature of approximately −70° for 7 days.

TABLE 1

The Effect of the Rate of Freezing and Thawing on the Viability and Growth of Walker's Carcinosarcoma

Group No.	Rate of freezing and thawing	No. of animals in group	Viability, %	Duration of the latent period, days			Aver. survival of animals after transplantation, days	Aver. tumor diam. on 14th day after transplantation, mm
				min.	aver.	max.		
1	Control (tumor tissue not frozen)	10	100	2	2, 3	4	18,2±1,8	37,3±1,6
2	Slow freezing and rapid thawing	10	100	3	3,1	4	1 ,4±1,05	37,5±1,5
3	Slow freezing and slow thawing	10	100	3	3,6	4	18,6±1,6	29,6±2,5
4	Rapid freezing and rapid thawing	10	90	3	3,7	4	19,2±1,6	30,1±3,3
5	Rapid freezing and slow thawing	10	60	3	3,9	4	32,0±3,5	7,1±2,3

For slow thawing the ampules were placed in a refrigerator at 4° for 1 hr, after which they were held at room temperature (18-20°) for 30 min.

Rapid thawing was obtained by transferring the ampules to a water bath at 40° for 1 min and agitating. The material was injected into the animals soon after the thawing.

Walker's carcinosarcoma was transplanted to white male rats weighing an average of 80 grams. An ampule containing 0.5 ml of the material was injected subcutaneously into the left paw. The Ehrlich's ascitic carcinoma was transplanted to white male mice of unspecifiedbreed, 20 g in weight (on the average); the injection was made intraperitoneally, using 0.2 ml.

We studied the following combinations: 1) slow freezing and rapid thawing; 2) slow freezing and slow thawing; 3) rapid freezing and rapid thawing; 4) rapid freezing and slow thawing.

There were 10 animals in each group. A total of 50 rats and the same number of mice were used in the experiment.

In addition to the other observations we noted the time at which the tumors appeared and the duration of the animals' survival after the transplantation. The tumors in the rats were measured in all three dimensions every three days, and their average diameter was determined.

RESULTS

As can be seen from the data presented in Table 1, in the experiments with Walker's carcinosarcoma the indices of tumor growth from the tissues subjected to slow freezing and rapid thawing were the closest to the results obtained from inoculating the animals with the control material (which was not subjected to freezing). An appreciable, statistically significant ($P < 0.01$) reduction in the transplant viability and retardation of growth was observed only in one group, where the combination of rapid freezing and slow thawing was applied. In this group the transplantation survival rate was 60% in all (as compared with 90-100% in all the remaining groups). The average duration of life of the animals in this group was 32 days, i. e., approximately 1.5 times greater than the duration of life of the animals in the other groups. The marked growth retardation of the tumors in this group was also manifested in the average diameter, which was 4-5 times smaller than in the other groups on the 14th day following the transplantation.

Figure 1 shows the change in the average diameter of the tumors for the different groups as dependent upon the duration of time after the transplantation. It can be seen from Fig. 1 that the most rapid growth was seen in

the tumors which developed from material subjected to slow freezing and rapid thawing (the indices for this group are very close to those of the control). Tumors from the tissue subjected to rapid freezing and slow thawing developed most slowly. The combinations of slow freezing with slow thawing and rapid freezing with rapid thawing led to a certain retardation in tumor growth (approximately the same degree in both cases), but it was not as marked as with the combination of rapid freezing and slow thawing.

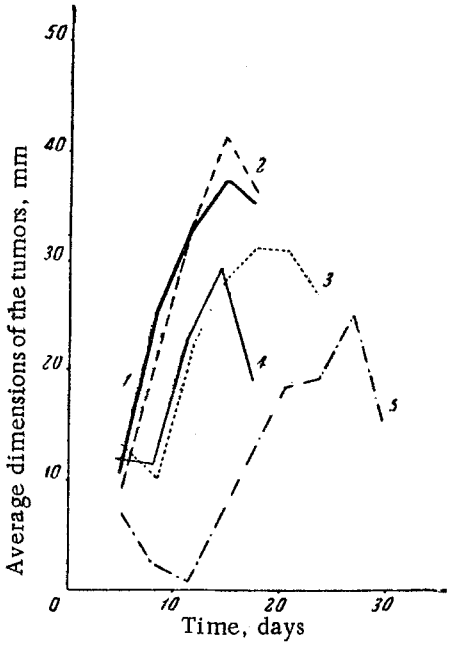

Fig. 1. The effect of the rate of freezing and thawing on the growth of Walker's carcinoma. 1—control; 2—slow freezing and rapid thawing; 3—slow freezing and slow thawing; 4—rapid freezing and rapid thawing; 5—rapid freezing and slow thawing.

TABLE 2

The Effect of the Rate of Freezing and Thawing on the Viability of Ehrlich's Ascitic Carcinoma and the Duration of Life of the Animals following the Tumor Transplantation

Groups	Rate of freezing and thawing	Viability (in %)	Aver. duration of animals life following transplantation (in days)
1	Control (ascitic fluid not frozen)	100	12.9±0.5
2	Slow freezing and rapid thawing	100	14.8±0.2
3	Slow freezing and slow thawing	100	16.8±1.2
4	Rapid freezing and rapid thawing	100	19.2±0.9
5	Rapid freezing and slow thawing	70	27.0±1.8

In the experiments with Ehrlich's ascitic carcinoma (Table 2) the application of rapid freezing and slow thawing led to a marked lengthening in the duration of life of the animals (up to 27 days as compared to 12.9 days in the control). The smallest deflection from the indices for the control, just as in the experiments with Walker's carcinosarcoma, took place in the group where slow freezing and rapid thawing were applied (average duration of life of the animals in this group was only 1.9 days greater than the duration of life of the animals in the control group). The combinations of rapid freezing and rapid thawing and slow freezing with slow thawing occupied an intermediate position between the two groups mentioned above (the duration of life of the animals in these groups was 19.2 and 16.8 days, respectively; the difference was not statistically significant).

The effect of the rate of freezing and thawing on the ascitic fluid transplanted to the mice on the duration of life of the animals is depicted graphically in Fig. 2.

The viability of Ehrlich's ascitic carcinoma was equal to 100% in all groups except the one in which rapid freezing and slow thawing was applied; in this latter group it was equal to 70%.

Thus, on the basis of our experiments with Walker's carcinosarcoma and Ehrlich's ascitic carcinoma, we can conclude that the least harmful combination for tumor cells is slow freezing and rapid thawing. This combination should obviously be considered the method of choice in the practical work involved with freezing of transplantable tumors. The application of the reverse combination—rapid freezing and slow thawing—leads to appreciable lowering of the viability of the tumor tissue.

Contemporary concepts of the mechanisms permitting the survival of tissues during deep freezing are still of a purely hypothetical nature. Thus, the most widespread, vitrification theory is based on the hypothesis that survival of protoplasm during deep freezing is a result of its freezing without the formation of crystals, but rather in an amorphous, so-called vitreous state. According to the opinion of Luyet [11], one of the most essential conditions for vitrification is an extremely high rate of cooling—in the order of several hundred degrees per sec; as a result of the tissue's rapid passage through the "dangerous" temperature zone (from –30 to –50°) crystallization cannot occur and vitrification takes place. According to this point of view the data

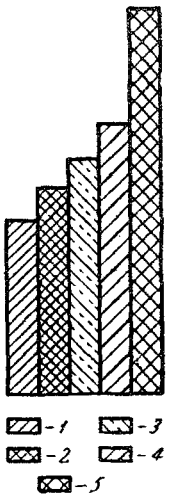

Fig. 2. The effect of the rate of freezing and thawing of Ehrlich's ascitic carcinoma on the duration of life of the mice following their inoculation with the frozen material. 1 – control; 2 – slow freezing and rapid thawing; 3 – slow freezing and slow thawing; 4 – rapid freezing and slow thawing; 5 – rapid freezing and rapid thawing.

showing more favorable results with slow freezing than rapid remains unexplained.

It is known, however, that slow freezing and supercooling lead to a significant elevation in the viscosity of the protoplasm, and thus resist the early advent of crystallization and inhibit it. In addition, in the process of slow freezing a marked dehydration of the cells occurs, due to a gradual loss of water from the extracellular formation of ice [2]; this fact also hinders crystallization.

Thus, vitrification is theoretically completely acceptable not only at super-high rates of freezing, but, on the other hand, at comparatively low rates as well. However, the final resolution of the question pertaining to the existence of physico-chemical changes in the protoplasm in the process of freezing-thawing will apparently belong to the methods of electron microscopy and structural analysis using roentgen techniques.

SUMMARY

A study was made of the effect of the rate of freezing and thawing on the transplantability and growth of Walker carcinosarcoma and Ehrich's ascitic carcinoma. The most favorable results in the take and growth of transplanted tumors was achieved by combining slow freezing with rapid thawing. Rapid freezing with slow thawing lead to statistically significant reduction of transplantability and delayed growth of the tumors.

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