Studies on the Effect of Hypothermia

III. The Effect of a Single Short Period of Hypothermia on the Brown-Pearce Rabbit Epithelioma*

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Since hypothermia, or cryotherapy, has been advocated and is being utilized in human cancer therapy by Smith and Fay (12), it seems advisable to study the in vivo effect of induced hypothermia on transplantable tumors in laboratory animals under controlled conditions in order, if possible, to throw some light upon the changes brought about in such tumors by the period of reduced temperatures.

The effect of in vitro freezing on the takes of transplantable tumors has been studied by a number of investigators (2–6, 8–11) upon various types of growths. A pronounced variation is described in the response of different types, and even to a large extent of the same tumor. Viability also depends upon other factors, such as the way in which the tumor is frozen, en masse or in a saline suspension of cells; the speed of freezing, its duration (6, 8), and the number of times frozen. It appears that neoplastic cells resist intense cold suddenly applied better than do normal ones (6). Furthermore different normal tissues may vary considerably in their resistance to cold.

The reduction of body temperature to the levels of room temperature (22–26° C.) is in no way related to the conditions under which truly frozen cells have been studied. In no sense has the human patient (12, 13) or the laboratory animal been “frozen,” even though ice has been used to reduce the body temperature. Thus the short experiments reported here are not comparable with the technic used for human patients, where the reduced temperature may be maintained for days. They do, however, yield evidence of a sort in this regard, since they offer data on what might occur in the early periods of prolonged exposure to low temperature, and on what effects short exposures to low temperatures may have upon the growth rate, survival period, and metastatic rate of the tumor. It should be emphasized that these data are restricted to a study of only a small part of the effects of reduced body temperature and to only three exposure or dosage levels.

METHOD

Young, susceptible, white male rabbits of the New Zealand strain were inoculated in each testicle with 0.2 cc. of a freshly prepared suspension of the Brown-Pearce rabbit epithelioma from a rapidly growing stock tumor. The resulting diffuse nodules appeared promptly and usually grew rapidly in the controls until the limits of the testicle had been reached. This time varied between 1 and 8 weeks due to variations in distribution of the suspension in the tissue. Such a procedure is not as satisfactory for precise growth studies as is the fragment method, but it was thought advisable to use a suspension in order to obtain a tumor with a minimum amount of necrosis as quickly as possible and before the animal became cachectic. In some cases, particularly in experiment 3, the growth was so rapid at first as to make the growth curves after treatment of little comparative value.

The body temperature of the rabbit was reduced in the ice bath and maintained at a given level in the refrigerator for various times. Six to 10 animals were treated at once. This technic and the physiological alterations it produced are described in another paper of this series (1). Seventeen tumor-bearing rabbits were used in the preliminary experiment to establish the technic and proper low temperature level for maintenance.

Three experiments, totaling 48 rabbits, are analyzed to observe the effect of the 6, 8, and 24 hour exposure.

In addition, 10 animals were sacrificed for histological study of the tumor at varying times up to 14 days following hypothermia. Some rabbits succumbed during treatment, or from 1 to 3 days following hypothermia, for no apparent reason, and sections of their tumors were studied microscopically. These rabbits that succumbed during or immediately following hypothermia or were sacrificed for morphological study are not included in the charts illustrating the
growth characteristics of the tumor, nor in the data on survival time after inoculation, since it was thought that intercurrent infection or other factors not directly concerned with the tumor problem were involved. Histological study was also carried out on the primary tumors from animals that succumbed to metastasis at varying periods from 2 weeks to more than 3 months following hypothermia, to observe any morphological alteration in the tumor from the hypothermia.

DATA

EXPERIMENT 1

Twelve and 14 days after inoculation, when the tumors averaged 5.5 square cm., i.e. length x width (0.2 sq. cm. to 8.5 sq. cm.) 6 rabbits bearing 11 actively growing tumors were subjected to reduced body temperature of 18°C. (rectal) for 6 hours. The induction period usually consumed 2 to 4 hours and the temperature returned to normal spontaneously after removal of the animal from the cooling cabinet within 2 to 8 hours. Three of the tumors grew slowly from the start and were little affected by the treatment (Table I and Fig. 1). In 4 rapidly growing tumors, the treatment slowed the growth rate temporarily and then they grew at almost the same rate as before. Two rapidly growing tumors regressed quickly, one almost disappearing and then slowly recovering while the other continued shrinking slowly until the animal died of metastases in the third week. Two rapidly growing tumors remained almost stationary for over 2 weeks and then grew faster than before the treatment.

When the product of the lengths and widths of the tumors was averaged the average increase in size per week for the week before treatment was found to be 5.2 sq. cm.; that for the week after treatment averaged only 1.2 sq. cm., and then the average rapidity of growth increased so that by the fourth week after treatment it was 4.5 sq. cm. per week (Table I). The average length of life was 44 days (30 to 54 days) and all the animals died with widely disseminated metastases. The average length of life for stock controls was nearly 100 days, with a metastatic rate of nearly 50 per cent. The rate of growth of the stock controls was much more uniform, none of them reaching the tremendous size of the 3 treated tumors (27 to 35 sq. cm.). In general both tumors in each animal responded in the same way.

EXPERIMENT 2

Fifteen rabbits with 29 tumors had their body temperatures reduced to 20°C. (rectal) for 8 hours on the fourth to the 12th days after inoculation, when the tumors were barely palpable (2 to 3 mm. in diameter) as small, diffuse masses. Nine animals of the same group with 17 tumors were kept as controls.

The growth rate of the tumors averaged 2.37 sq. cm. per week for the week previous to treatment and then the average size became smaller for 2 weeks. This value was biased by the tremendous shrinkage of 2 large tumors and the latency of 9 others during this period. Four tumors actually seemed to grow faster after treatment. The extreme spread of the growth curves, i.e. 6 very rapidly growing tumors and 4 slowly growing ones, was much greater than that for the controls at the seventh week, yet the averages (Fig. 3) were nearly the same for the treated and the controls (Fig. 2). In general the trend was similar except for the 2 week period after treatment (Fig. 2).

<table>
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<th>Table I: Brown-Pearce Rabbit Epithelioma</th>
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<td><strong>Average Change in Growth per Week in Square Centimeters</strong></td>
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Fig. 1.—Experiment 1. Six rabbits, 11 tumors. Six hours at 18°C between 12th and 14th days. In Figs. 1 to 6, AV = average.
EXPERIMENT 3

Twelve animals bearing 23 tumors were subjected to a state of hypothermia 3 to 5 days after inoculation. They were maintained at an average rectal temperature of 30°C for 24 hours (Fig. 5). Their growth curves were observed for 30 days, by which time half had succumbed to metastases. Of 6 animals in the same group, bearing 12 tumors and maintained as controls, only one, or 17 per cent, died of metastases within the 30 day period (Fig. 4). This tumor grew so rapidly that the testicular structure apparently could not expand in keeping with it, and the growth curves are therefore flattened out in the second week. In 3 of the controls the tumors reached 8 to 9 sq. cm. and then shrank rapidly. Only 2 per cent of the control tumors continued to grow in the second week. This has biased the average curves, the outcome that they re-

produce looking more like a treatment effect than anything seen in the treated group. This is one of the handicaps of the suspension method of inoculation.

In the treated group there were only 3 that regressed; 4 remained stationary; 6 showed no change in rate; the remainder grew somewhat less rapidly, though actively, during the week after treatment. Following this, the growth behavior was very much more erratic than that of the controls. None shrank enough to indicate that it was going to disappear completely.
we have seen developed in the group receiving hypothermia. This may be due to chance, yet it seemed to occur in each experimental group.

It is difficult to determine what effect, if any, hypothermia may have upon the morphology of the Brown-Pearce rabbit epithelioma. The tumor normally presented areas of necrosis in the central portions of large rapidly growing masses as early as 5 days following inoculation, and no definite increase of necrosis in the treated tumors was observed on gross examination. Thus the abundant necrotic areas observed in sections studied after hypothermia may well have been an accompaniment of the usual growth of the tumors and in no way related to the hypothermia. A few hours after onset of the treatment the scrotum became dark and congested and autopsy at

**DISCUSSION**

The data presented here demonstrate that reducing the general temperature, including the tumor temperature, of rabbits for short periods of time (18°C. maintained for 6 hours, 20°C. maintained for 8 hours, and 30°C. maintained for 24 hours) has no definite lasting inhibiting effect upon the growth characteristics of the tumor.

The definite reduction in the survival period of animals treated by hypothermia, disregarding any that may succumb during or immediately following the treatment, may be related to the widespread metastases that quickly develop in treated animals (clearly in excess of the controls). This suggests that hypothermia of this duration may be instrumental in enhancing the dissemination of tumor fragments and

**Fig. 6.—Average growth curves of controls and tumors subjected to hypothermia.**

this period showed the cut tumor surface to be rather soft, red, and hemorrhagic with decided engorgement of all the superficial vessels. Specimens examined up to 5 to 7 days later sometimes contained small masses of dark red hemorrhagic tumor lying within normal looking, or slightly edematous and friable, tumor.

Microscopic examination usually revealed considerable dilatation and congestion of all vessels. Occasionally a rupture in a small vessel was seen, with the extravasation of blood cells into disorganized tumor (Fig. 7). Five to 7 days after hypothermia there were many small foci of large actively growing tumor cells arranged as a collar about the blood vessels spread throughout the extensive areas of hyalin necrosis and disintegrating tumor cells (Fig. 8). Microscopically the distribution of the necrosis suggested a zonal and partially focal disturbance related to interference with the blood supply, such as is found in old, large, regressing tumors. There was nothing resembling the cell degeneration and scarring noted after roentgen radiation. Favoring the development of metastases. We suggest that the uniform finding of diffuse hemorrhages in the treated tumors may offer the explanation for this, namely that the ruptured vessels present multiple portals of entry for dislodged cells (7).

These findings differ from those described by Smith and Fay (12). They report a decrease in size of tumor during the first 24 to 48 hours of hypothermia, retardation of recurrences, diminished rate of growth during recurrences, and a definite histopathological series of events characterized by reduction of blood supply with few blood vessels discernible in section, followed a few days later by necrosis and frank disintegration of the cells, polynuclear cellular infiltration, liquefaction, and the absorption of necrotic tumor. These alterations, however, occur after prolonged exposures (5 days), and it should be emphasized that our experiments, while conducted at much lower temperatures, fell far short of such prolonged treatment and that our findings are therefore not strictly comparable.

The engorgement of the blood supply to the tumor with extensive necrosis but no prolonged inhibition
The tumors were 14 days old and all were approximately 2.0 \times 3.0 \text{ cm.}, at the time the rabbit's temperature was lowered. The immediate reaction was swelling of the tumor and darkening of the scrotum. This was followed shortly by a definite shrinking, and one week later by progressive growth in other tumors in this group (Fig. 3).

(A) Three hours after cooling; extensive edema and a few focal hemorrhagic areas in the low power view. Most of the cells are devoid of cytoplasm and show contracted dense nuclei. A few shrunken cells (the darker concentrations) near blood vessels remain intact but show little nuclear detail. Old necrosis is also visible.

(B) High power view of one of the foci of shrunken, dense cells near small blood vessels. The periphery of the area is disrupted by edema.

(C) At 72 hours (low power view) the edema is less pronounced, the necrosis is more widespread, and the collections of dark nuclei show more clearly.

(D) In the high power view of one of these dense nuclear collections, masses of disintegrating cells are seen at the periphery of "lobules" whereas shrunken pyknotic and abnormal appearing cells are distributed around the vessels. Some of these must be viable.
(and possibly even enhancement) of its growth characteristics, together with the increased number of metastases and consequent shorter survival period, would indicate that while the short hypothermic state may have caused considerable damage to the tumor this has in no way benefited the host. While 24 hours of hypothermia is a long time in the life of the rabbit, it is probably not comparable to the 4 and 5 day period in the human subject. Great prolongation of hypothermia in the rabbit, in our experience, would be very difficult to achieve because 24 hours was found to be near the limit of endurance. In retrospect, it appears that grafts would have yielded better growth data than the suspension employed, but circumstances did not allow a repetition of our experiments. The data show a temporary delay in the growth of the tumor, and at the time we did not see any better experimental procedure for emphasizing this fact.

These experiments indicate that a certain amount of damage may be done to the tumor by this procedure, and perhaps by combining its effects with other procedures (roentgen radiation, heat, etc.) a much greater destructive effect could be obtained. Hypothermia, per se, enhanced rather than inhibited metastasis in the experiments here reported.

CONCLUSIONS

1. Short periods of hypothermia (6 hours at 18° C., 8 hours at 20° C., 24 hours at 30° C.) caused a variety of changes in the growth curves of the Brown-Pearce rabbit epithelioma. The rate of growth was increased in a few, unchanged in approximately one-third, static in one-third, and reversed in the remainder during the week following treatment.

2. An analysis of the averages of the growth curves indicates a definite slowing of growth during the week following treatment.

3. After the second week following treatment, however, the growth rate is essentially normal (based upon the averages) although the extremes in size of tumor are greater than those for the controls.

4. The number of animals succumbing from metastases was decidedly increased and the survival period thus decreased following short periods of hypothermia.
5. Numerous tears in the vascular system of the tumor resulting from the treatment probably were responsible for the dissemination of the tumor.

6. The only definite and immediate histological alteration produced by hypothermia was a notable congestion of all vessels, with focal hemorrhage. A week later diffuse necrosis with islands of actively growing tumor cells around the blood vessels gave evidence of prompt recurrence.

7. No definite "cures" were obtained by the exposure periods and technics used.

REFERENCES


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