INDUCTION OF METASTASIS OF FROG CARCINOMA BY INCREASE OF ENVIRONMENTAL TEMPERATURE*

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Plates 10 to 12

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The pathways by which cancer spreads are well known, but information concerning the factors that subsequently lead to the formation of secondary tumors is very scanty (1). The present paper deals with one such factor, namely temperature. Neoplasia, like normal biological processes (2), is greatly influenced by it, as shown in studies on the growth of intraocular transplants of frog carcinoma (3). Whether temperature also affects the complex process of metastasis is the principal question with which this paper is concerned.

For studying the effects of temperature on metastasis the carcinoma arising in the kidneys of the leopard frog (Rana pipiens) has certain advantages: (a) It is representative of the commonest morphologic type of cancer in man and warm blooded vertebrates generally; i.e., the adenocarcinoma. It has the same structure, exhibits the same invasiveness of adjacent tissues, and possesses the same potentiality to form secondary tumors by metastasis (4). But unlike its counterpart in man and warm blooded vertebrates, this potentiality tends to remain unexpressed. (In this respect the frog carcinoma resembles many cancers of other cold blooded vertebrates.) The relatively low incidence of metastasis under natural conditions is favorable for experiments designed to induce or augment the process.

(b) The frog carcinoma, originating in the kidney, often attains a size sufficient to allow its ready recognition in the living animal by palpation. The tumor may then be outlined more precisely by roentgenograms. Thus any experimentally induced change in size can be made apparent by periodic examinations.

(c) Most important for the present experiments is the close dependence of the frog's internal temperature on that of the environment, and its ability to withstand relatively great changes for considerable periods. Thus an opportunity

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is afforded to investigate the effects of temperature on a cancer over a far wider range than is possible in warm-blooded vertebrates.

Evidence will be presented that, under the conditions of the experiments, temperature exerts little effect on growth of the primary tumor, whereas it profoundly affects the process of metastasis.

**Materials and Methods**

Ninety-four leopard frogs having palpable kidney tumors were assembled, and divided into four groups. The frogs in group A served as controls. They were kept under as natural conditions as possible in a cool room designed as an amphibian vivarium; here, the temperature during the course of the experiments was held close to 18°C. The frogs in groups B and C were placed in constant temperature rooms at 7°C and 28°C, respectively. The animals were maintained in individual aquarium jars on thick pads of cotton thoroughly saturated with water and frequently changed. Under these conditions the rectal temperature of the frogs was the same as that of the environment, as we repeatedly demonstrated by thermocouple measurements. The temperatures to which the frogs were exposed, 7°C and 28°C, were chosen because they represent the extremes in which frogs can be maintained satisfactorily for weeks. It is probable that under natural conditions frogs exist for considerable periods at a temperature near 7°C. It is doubtful, however, whether they are exposed to 28°C for more than short times; thereafter they will seek cooler water. The latter temperature may be considered artificial at least for the species used in the experiments.

Most of the animals in the three groups were in an indifferent state of nutrition at the beginning of the experiments. They probably had not received food for several weeks or even months when they arrived at the laboratory. Moreover, since the conditions of the experiments did not permit feeding them, the majority, during the prolonged periods of exposure to the several temperatures, averaging from 7 to 17 weeks, showed considerable wasting. In the interpretation of the experimental results the poor nutritional state had to be considered (5, 6). Hence, a new group of tumor-bearing frogs was brought together, all of which were well nourished. Most of them were placed in the thermostat room at 28°C, as a second series exposed to this temperature (group D). The remaining frogs, twelve in number, were sacrificed soon after arrival to serve as additional controls on the incidence of metastasis under natural conditions.

The size of the kidney tumors in the frogs of groups B and C was recorded at intervals of approximately 20 days by means of roentgenograms. This was easily accomplished, for in frogs which have not been fed the empty and collapsed gut tract is readily displaced upward by injecting air into the celomic cavity; the outline of the tumors may then be clearly seen in the films (Figs. 1 and 2). The frogs were anesthetized with ether, and air was introduced into the lower portion of the body cavity by means of a needle attached to a large syringe; two roentgenograms were made of each animal, one in the anteroposterior, the other in the lateral position. The need for rapid exposure of the films necessitated the use of intensifying screens; the technical factors used were 1/15 second at 45 kv. and 50 ma. at a target-film distance of 36 inches.¹

The frogs in the control groups and in the well nourished group exposed to 28°C were not subjected to Roentgen ray examination because of the possibility that injection of air under some pressure and the other procedures necessary for the examination might favor dissemination of tumor cells.

¹ We wish to thank Dr. Rosalind S. Thorner, formerly Fellow in Radiology, Hospital of the University of Pennsylvania, for her aid in making these roentgenograms.
RESULTS

First Series of Experiments.—In this series the effect of temperature was studied on tumor-bearing frogs that were kept at 7° and 28°C, respectively.

TABLE I
Incidence of Metastasis after Exposing Tumor-Bearing Frogs to 7°C. and to 28°C.

<table>
<thead>
<tr>
<th></th>
<th>7°</th>
<th>28°</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>days</td>
</tr>
<tr>
<td>109</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>109</td>
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<td>0</td>
<td>42</td>
</tr>
<tr>
<td>58</td>
<td>2 small metastases</td>
<td>30</td>
</tr>
<tr>
<td>58</td>
<td>0</td>
<td>56</td>
</tr>
<tr>
<td>94</td>
<td>0</td>
<td>29</td>
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<tr>
<td>76</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td>84</td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td>109</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
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<td>1 small metastasis</td>
<td>40</td>
</tr>
<tr>
<td>109</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>109</td>
<td>0</td>
<td>74</td>
</tr>
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<td>109</td>
<td>0</td>
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</tr>
<tr>
<td>109</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>109</td>
<td>0</td>
<td>84</td>
</tr>
</tbody>
</table>

The term "extensive metastases" indicates numerous secondary growths which usually involved more than one organ.

The results of exposure to 18°C. are not given in this table since they were quite similar to those for the group kept at 7°. Metastasis occurred in only one of the twenty-six tumor-bearing frogs.

(groups B and C). As stated above, the animals in these groups were indifferently or poorly nourished, and were periodically subjected to Roentgen ray examination. The length of exposure to 7° averaged 98 days, and to 28° approximately half this time, 47 days. The incidence of metastasis found in the two groups is shown in columns 1 and 2 of Table I. It is seen that at 7°
only two out of twenty one of the primary tumors formed secondary growths—a small nodule in the liver in one case, two small nodules in the liver in the other. The results in the group maintained at 28°C. are strikingly different. Here six out of eighteen tumors became disseminated, and the secondary growths were extensive. A representative example of such metastasis is shown in Figs. 9 to 11. The liver and lungs of the frog were so riddled with secondary tumors that the organs were almost completely replaced. It is noteworthy that in this as well as in other animals of the group the secondary tumors were of approximately uniform size, suggesting that they had become established at about the same time.

In twenty six control frogs maintained under fairly natural conditions at 18°C. for an average of 140 days only one secondary tumor in the liver was encountered.

TABLE II

<table>
<thead>
<tr>
<th>Temperature</th>
<th>No. of animals</th>
<th>Average duration of exposure</th>
<th>No. with metastatic tumors</th>
<th>Per cent with metastatic tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>7°C</td>
<td>21</td>
<td></td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>18°C</td>
<td>26</td>
<td>98</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>28°C (1st series)</td>
<td>18</td>
<td>47</td>
<td>6</td>
<td>54</td>
</tr>
<tr>
<td>28°C (2nd series)</td>
<td>17</td>
<td>35</td>
<td>19</td>
<td>54</td>
</tr>
</tbody>
</table>

Second Series of Experiments.—An even more pronounced effect of prolonged exposure to elevated temperature was found when well nourished frogs were used (group D). Their exposure averaged 50 days; i.e. approximately the same as in the first series. But as is shown in column 3 of Table I, a considerably greater proportion of tumors, thirteen out of seventeen, formed secondary growths, usually in several organs. Thus one frog had numerous metastatic tumors scattered through the liver, the lungs, pancreas, ovary, mesentery, lumbar plexus, and spleen (Figs. 12 to 14). Similarly in another frog there was widespread involvement of liver, lungs, mesentery, and urinary bladder (Figs. 15 to 18). Such extensive involvement has seldom been encountered by us in recently caught frogs or in frogs maintained under more natural conditions of environmental temperature. It seems reasonable to attribute the much higher incidence of metastasis in this over the first series to the healthier condition, and particularly the better state of nutrition, of the animals.

In the twelve well nourished control frogs, freshly acquired and sacrificed during the period of the experiment, none of the tumors had metastasized.

The results in the two series are summarized in Table II. Here we have
combined the groups living at temperatures to which they are accustomed in
nature, 7° and 18°, and the groups in which the frogs were exposed to 28°, to
which they are not accustomed for long periods. In this combination the
nutritional state of the animals has for the present been left out of considera-
tion. The table shows that at 7° and in the control group at 18° only 6 per cent
of the tumors have metastasized, whereas at 28° the percentage is increased to
nine times this figure, namely 54 per cent. But increase in the occurrence of
metastasis is not the only change: there is a much greater degree of dissemina-
tion. The organ distribution of the secondary tumors is graphed in Text-
fig. 1, which is based on the nineteen metastasizing tumors included in columns
2 and 3 of Table I. The liver and the lungs are most frequently involved;
and as already emphasized the secondary growths are usually multiple and
are frequently extraordinarily numerous. It may be concluded, then, that
prolonged elevation of temperature has in some manner facilitated the process
of metastasis.

Effect of Temperature on the Primary Tumors.—The mechanism by which
elevation of temperature induces metastasis of frog carcinoma is doubtlessly
complex; but we can analyze in these experiments at least one possible com-
ponent, namely the effect of temperature on the primary tumor. In previous experiments we had found that increase in temperature greatly accelerates the growth of transplants of frog carcinoma (3). Are primary tumors similarly affected? Is there any correlation between change in their size and the occurrence of secondary tumors?

The results obtained in two groups of frogs maintained at 7 ° and at 28 °, and subjected periodically to Roentgen ray examination, are collected in Table III. The findings were contrary to our expectations. Definite enlargement of the kidney tumors was noted in relatively few cases (an example of which is shown in Figs. 6 to 8); in the majority there was no significant change; in some tumors there was actually slight shrinkage (Figs. 3 to 5). Moreover, there was no correlation between change in size of the primary tumors and presence or absence of secondary tumors. Metastasis occurred, or failed to occur, irrespective of whether the primary tumors enlarged, remained stationary, or shrank.

**COMMENT**

At least three steps are involved in the formation of a metastatic tumor: initially, invasion of tissues (including vascular channels and body cavities); next, transport of detached cells or fragments of tumor, and their mechanical arrest; finally, establishment of intimate contact with a suitable tissue and acquisition of a vascular stroma. For a detailed discussion of these processes the comprehensive texts of Willis should be consulted (1). Here it must suffice to inquire whether there is any indication that temperature influences these sequential events in the frog carcinoma.

Turning first to invasiveness, we had previously observed that at 28 ° intraocular transplants of frog carcinoma more readily invade the usually
resistant corneas than they do at lower temperature (3). Recently Coman (7)
and McCutcheon, Coman, and Moore (8) have brought convincing evidence
that invasion of tissue by cancer depends principally upon two factors: (a)
decreased mutual adhesiveness of malignant cells which facilitates their
separation from each other so that they become detached units, and (b) ameboïd
movement of the detached cells, whereby they are enabled to wander into the
surrounding parts. Both of these factors are influenced by temperature.
Regarding mutual adhesion, Zeidman (9) has lately demonstrated that this
property is diminished in normal cells by an increase in temperature; although
his work has not as yet been extended to malignant cells, it seems fair to assume
that they are affected in like manner.

More directly bearing on the subject is the effect of temperature on growth
of frog carcinoma in tissue culture (10). This tumor, an adenocarcinoma,
exhibits two types of growth with regularity. The first is in the form of tubules
which extend out into the semisolid medium and retain their form as long as
they are completely enveloped. When, however, the tubules make contact
with an unyielding surface, they adhere to it, and the character of growth now
changes into a flat membrane. At the margin of the membrane, cells tend to
become detached from the growing mass; elevation of temperature not only
causes more rapid extension of the membranous growth, but more ready de-
tachment of its marginal cells (11).

The separated cells exhibit active locomotion by means of broad ruffle
pseudopodia. A photograph of such a migrating cell is shown in Fig. 12 of an
earlier paper (10); the rate of locomotion of these cancer cells as seen in cine-
matographic films is given in Figs. 13 to 20 of that paper. Thus, in frog
carcinoma invasiveness and the principal factors believed to be responsible for
it, are definitely affected by temperature.

After the cells have found their way into vascular channels, they are trans-
ported by the flowing current until they are finally trapped. The fate of such
emboli has been studied by numerous investigators, and there is general agree-
ment that the great majority of the arrested tumor cells do not develop into
metastases but are destroyed (12–17). In other words, tumor emboli are not
metastases; before they can become such they must survive for a sufficient
length of time to multiply and penetrate through the wall of the vessel in which
they are mechanically arrested; thereafter all save the most invasive growths
must induce the formation of a supporting vascular stroma by the adjacent
tissue(12, 18). Now it has been demonstrated that elevation of temperature
does favor vascularization of frog carcinoma implanted into alien soil (3).
When bits of this tumor are transplanted to the anterior chamber of the eye,
vascularization of the implants is invariably more prompt and more efficient
at 28° than at lower temperatures.

No doubt these are not the only factors concerned in the metastasis-inducing
effect of temperature on frog carcinoma. It seems quite possible, however, that those mentioned as well as others can be subjected to analysis by further experiments.

SUMMARY

Metastasis of the kidney carcinoma of leopard frogs (*Rana pipiens*) has been induced by exposing tumor-bearing animals for approximately 50 days to a constant temperature of 28°C. Under these conditions 54 per cent of the frogs developed secondary growths, whereas in groups kept at 18° or at 7° metastatic dissemination was found in only 6 per cent. Moreover, at the elevated temperature the metastases were usually more numerous and more widely disseminated; they were also fairly uniform in size, suggesting that they had developed at nearly the same time.

Dissemination of the kidney tumors was influenced by the nutritional state of the frogs, occurring more readily in well nourished than in poorly nourished animals.

Periodic Roentgen ray examinations showed that the size of the primary tumors was not significantly or uniformly affected during the course of the experiments. No correlation was found between change in size of the kidney tumors and the incidence of their metastasis.

Although the mechanism by which temperature induces metastasis of frog carcinoma cannot as yet be elucidated, previous experiments with this tumor indicate that certain factors at least may be involved: Elevation of temperature has been found to cause more ready detachment of cells of frog carcinoma in tissue culture; to bring about increased velocity of locomotion of the detached cells; to lead more promptly and efficiently to vascularization of transplants; and to effect their greater invasiveness.

BIBLIOGRAPHY

11. Lucké, B., unpublished experiments.
EXPLANATION OF PLATES

PLATE 10

Fig. 1. Roentgen ray film showing the appearance of bilateral kidney tumors in a frog whose body cavity had previously been injected with air. (The markings along the margin of the film are at intervals of 1 cm.)

Fig. 2. The kidney tumors as they appear at postmortem examination 1 day after the Roentgen ray film was made. Comparison of Figs. 1 and 2 shows that the size and shape of the tumors can fairly well be determined by Roentgen ray examination after intracelomic injection with air.

Figs. 3 to 5. A series of Roentgen ray photographs of kidney tumors in a frog which had been kept at 7°C. for a period of 94 days. The three films were made on January 21, March 10, and April 19, respectively; they show moderate and progressive reduction in size of the tumors. The decrease in size of the tumors is associated with nutritional wasting, as can be seen by comparing the outlines of the thighs.

Figs. 6 and 7. Roentgen ray films of a kidney tumor as seen from the side, made on January 21 and on March 10, respectively. The frog had been kept at a temperature of 28°C. for 42 days.

Comparison of these films shows a considerable increase in size of the primary tumor, and a metastatic spread upward to the liver and anterior to the parietal peritoneum.

Fig. 8. Postmortem appearance of the same frog 2 days after the last Roentgen ray examination. The figure shows the primary kidney tumor, and the multiple metastases in the liver and on the parietal peritoneum.
(Lucké and Schlumberger: Metastasis induced by increase of temperature)
A frog which had been kept for 24 days at 28°C. Roentgen films taken before the animal was placed at this temperature revealed no evidence of metastasis. At autopsy the liver and lungs were found riddled with metastatic tumors (see Figs. 10 and 11).

**Fig. 9.** Representative section of lung from frog shown in Fig. 9. The organ is almost completely replaced by small tumors of fairly uniform size. × 30.

**Fig. 10.** Section of liver from same frog showing numerous metastases. × 30.
(Lucké and Schlumberger: Metastasis induced by increase of temperature)
PLATE 12

FIG. 12. Extensive metastasis in frog which had been exposed to 28°C. for 45 days. The photograph shows massive bilateral primary tumors of kidneys, filling nearly the lower half of the celomic cavity. Numerous metastatic tumors are scattered through the liver, involving particularly the right lobe. To the left of the midline, and touching the lower margin of the liver is a metastatic tumor in the ovary (the eggs appear as black dots over the surface of the tumor). Additional metastatic tumors are present in the spleen (Fig. 13), in a lumbar nerve (Fig. 14), in the pancreas, the mesentery, and in the lungs.

Fig. 13. Section of a metastatic tumor in spleen. × 30.

Fig. 14. Metastatic tumor involving and partially destroying a large nerve from the lumbar plexus. × 8.

Figs. 15 to 18. Multiple metastatic tumors from a frog exposed for 39 days to 28°C.

Fig. 15. Metastatic tumors in the urinary bladder. × 3.

Fig. 16. Metastatic tumors in both lungs. × 3.

Fig. 17. Metastatic tumors in mesentery. × 3.

Fig. 18. Metastatic tumors in liver. × 3.
(Lucké and Schlumberger: Metastasis induced by increase of temperature)