

THE EFFECT OF TEMPERATURE ON THE GROWTH OF VIRUS-INDUCED FROG CARCINOMA

II. THE TEMPERATURE COEFFICIENT OF GROWTH IN VITRO*

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The quantitative relation between temperature and rate of growth can be expressed by the coefficient of van't Hoff, Q_{10} . Values of this coefficient for various forms of normal growth are remarkably similar, Q_{10} usually ranging between 2 and 3; that is to say, an increase in temperature of 10°C. doubles or trebles the rate of growth (1). There are no comparable data for cancer. Since the growth of cancer differs so radically in a number of ways from all kinds of normal growth, experiments were designed to find a temperature coefficient for cancer. It was hoped thereby to gain information on the basic processes that underlie growth of cancer.

For studying effects of temperature on rate of growth (or on biological processes in general) cold blooded animals are more suitable than animals having thermoregulatory mechanisms. Hence, in the present experiments, we have used the carcinoma that commonly occurs in the kidney of the leopard frog, as result of the action of an organ-specific virus (2). The action of the virus is ordinarily attended by the formation of inclusion bodies, but in tissue cultures the tumor shows no such bodies (3) though in other respects the proliferating renal cells have the typical neoplastic character. As the first step in evaluating the temperature coefficient, we have investigated the rate of growth of this cancer in tissue cultures. This procedure has the advantage of simplicity; factors such as the nutritional state of the hosts and the blood supply of the tumors are eliminated, and accurate measurements can be made over a wide range of temperatures.

Method

Details of the method for growing frog carcinoma *in vitro* are given in a previous paper (3). In the present experiments modified Carrel culture flasks were used. Traces (approximately 0.3 mg.) of either crystalline potassium penicillin G or streptomycin sulfate were added to

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the supernatant nutrient medium. Several explants removed directly from kidney tumors—8 in all—were placed in each flask. Care was taken to have the explants of as nearly the same size as possible; their mean surface areas ranged from 0.67 to 0.74 mm.², excepting those maintained at 35°C., which were slightly larger. The explants in each flask showing the most vigorous growth at the end of the 1st day were chosen for measurement. The outgrowths, although tubular so long as they are completely embedded in the plasma medium, become membranous as soon as contact is made with the surface of the glass, that is to say within 24 to 36 hours (3). Each explant is then surrounded by a colony of compactly coherent but discrete cells growing in the form of a thin flat membrane. The edges of the membranes are sharply outlined and, in their outer parts, consist of a single layer of cells; in their inner and older portion, *i.e.* near the explant, they may be two or more cells in thickness. In most cul-

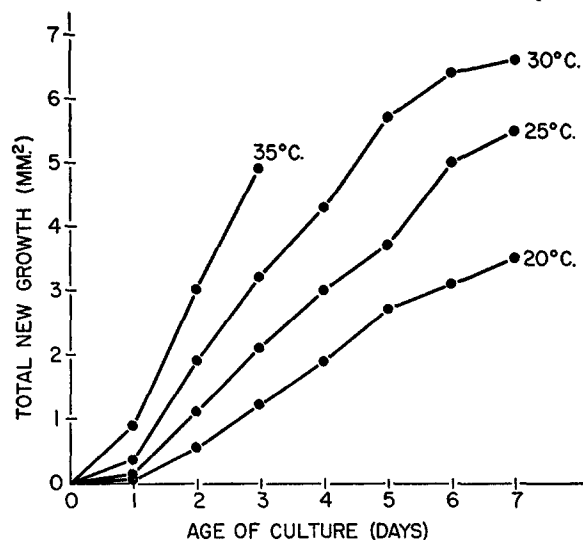


FIG. 1. Curves showing the course of growth of colonies of frog carcinoma cells at four different temperatures.

tures, stroma cells are entirely absent and macrophages inconspicuous; the membranes consist almost exclusively of cancer cells.

The tissue cultures were maintained at four different temperatures: 20°, 25°, 30°, and 35°C. \pm 1°C. Measurements of the colonies were made daily for 7 days, excepting the cultures growing at 35°C., in which liquefaction of the plasma clots by the cancer cells made observation impracticable after the 3rd day. The growth of the colonies was measured by Ebeling's method (4). The surface areas of the outgrowths from the explants were obtained by projecting on paper by a drawing ocular, at appropriate magnification, the outlines of the colonies. The drawings were then measured at leisure by a planimeter; and, by calibration, the measurements were converted into square millimeters. Despite the limitation of this method, which does not take into account possible variation in thickness of the membranes, such measurements give significant results (5).

The Temperature Coefficient of Cancerous Growth

The effect of temperature on rate of growth of frog carcinoma *in vitro* is graphically shown in Fig. 1. Here the means of surface areas of colonies are

plotted at daily intervals for four different temperatures. The resulting graphs are typical of curves of growth. It is evident that the rate of growth of the colonies is markedly influenced by temperature. Numerical details of the data from which the graphs were constructed are given in Table I. In this table the figures represent the means of the entire amount of new growths around the explants, together with the standard error of the means and the number of different colonies measured.

TABLE I
Increase in Surface Area of Colonies of Frog Carcinoma Cells on Successive Days at Different Temperatures

The figures represent mean values of total surface areas of colonies in mm.², ± the standard error of the means and, in parentheses, the number of colonies measured at the stated age and temperature.

Days	20°C.	25°C.	30°C.	35°C.
1	0.07 ± 0.01 (56)	0.14 ± 0.01 (56)	0.38 ± 0.04 (48)	0.9 ± 0.11 (16)
2	0.56 ± 0.06 (56)	1.1 ± 0.04 (56)	1.9 ± 0.18 (47)	3.0 ± 0.44 (15)
3	1.2 ± 0.11 (56)	2.1 ± 0.16 (56)	3.2 ± 0.31 (43)	4.9 ± 0.77 (8)
4	1.9 ± 0.17 (54)	3.0 ± 0.24 (53)	4.3 ± 0.45 (25)	
5	2.7 ± 0.22 (52)	3.8 ± 0.36 (45)	5.7 ± 0.67 (16)	
6	3.1 ± 0.29 (44)	5.0 ± 0.60 (27)	6.4 ± 1.0 (10)	
7	3.5 ± 0.35 (41)	5.5 ± 0.80 (21)	6.6 ± 1.1 (10)	

From the data given above, the temperature coefficient of growth can now be computed by the equation:

$$\log Q_{10} = \frac{(\log K_1 - \log K_2)}{\Delta t} \times 10$$

in which K_1 is the rate of growth at the higher, and K_2 at the lower temperature, and Δt the difference in the temperatures at which the two measurements are made. The Q_{10} values were computed for all six possible intervals of temperature in order to obtain the most reliable averages. These values for successive days of growth are given in Table II. Two main facts are brought out: first, the average values of the temperature coefficient for the entire periods of growth (shown in the bottom line) are much alike; and, second, the averages of Q_{10} for successive days of growth (shown in the last column) steadily decrease. This is precisely what happens in normal growth. Thus the Q_{10} values collected by Sir D'Arcy Thompson for growth of such varied forms of life as yeast cells, rootlets of corn, of lupine, and of pea, of early stages of various echinoderms, of drosophila, and of tadpoles all closely agree (1). Their average value of 2.8 is nearly the same as the over-all average of growth of frog carcinoma *in vitro*; namely, 2.5. Also, as was first pointed out by Peters, the temperature coefficient alters with age, usually though not always declin-

ing as growth advances (6). We may therefore infer that there is no difference between the general effect of temperature on rate of growth of frog carcinoma *in vitro* and of varied forms of normal growth, or, in other words, that the temperature coefficient of van't Hoff holds equally for neoplastic and for normal growth.

COMMENT

The results obtained with tissue cultures of frog carcinoma obviously cannot, without experimental evidence, be applied to cancer in general. At present there are no data in the literature on the temperature coefficient for growth of any cancer in the living animal, nor is there any such information for growth of tissues *in vitro*, whether neoplastic or normal.

TABLE II
Values of Q_{10} at Successive Days for Different Intervals of Temperature

Day	20-25°C.	20-30°C.	20-35°C.	25-30°C.	25-35°C.	30-35°C.	Average
2nd	3.9	3.4	3.1	3.0	2.7	2.5	3.10
3rd	3.1	2.7	2.6	2.3	2.3	2.3	2.55
4th	2.5	2.3		2.1			2.30
5th	1.9	2.1		2.4			2.13
6th	2.6	2.1		1.6			2.10
7th	2.5	1.9		1.4			1.93
Average	2.75	2.41	2.85	2.13	2.50	2.40	2.50

In complex processes such as growth, many chemical reactions are involved, and any temperature coefficient obtained is perhaps something of an average. As stated in the introduction, frog carcinoma is a transmissible disease due to an agent which induces intranuclear inclusions and which has other attributes indicating that it is a virus. There is as yet no information as to whether continued growth of the cancer cells *in vitro* depends upon the continued presence of this agent within them. As already mentioned, no inclusion bodies are observed under such circumstances. If the agent persists two processes would seem to be involved in the present experiments: effect of temperature on rate of growth (*a*) of the virus, and (*b*) of the neoplastic cells. And if the virus is the driving force in the multiplication of the malignant cells, then the temperature coefficient obtained in these experiments is primarily that of the agent and only secondarily that of the cells with which it is associated.

It is well known that identical values of this coefficient do not at once signify identity of processes. The closely similar values of the temperature coefficient for growth of one particular kind of cancer *in vitro* and for various

forms of normal growth is a matter of interest. It suggests these questions: Is the action of temperature on growth of cancer primarily exerted on those chemical processes that cancer has in common with all forms of growth? Is it possible, by means of temperature effects, to distinguish between two basically different characteristics of cancer—those that cancer shares with growth in general, and those malignant attributes that set cancer apart from any other kind of growth?

It seems possible that these questions can be answered by further experiments.

SUMMARY

The temperature coefficient of van't Hoff, Q_{10} , for growth of frog carcinoma *in vitro* over a range of 20–35°C, averages 2.5. This value is closely similar to those obtained for various forms of normal growth.

The values of the temperature coefficient slightly but progressively decrease with advancing age of the cancer colonies. A similar relation obtains in many but not in all forms of normal growth.

Thus, the law of van't Hoff is found to hold equally for growth of the malignant tissue now under discussion and for normal tissue.

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