Temperature-Dependent Histotypic Changes in Walker Carcinosarcoma in the Chorioallantois

Abstract. *Solid tumors grew in the chorioallantois of chick embryos after the topical inoculation of ascites tumor cells. The microscopic character of the growth varied depending on the temperature of incubation. At 32.5°C a sarcomatous appearance was prominent; at 37.5°C carcinomatous structure in several alveolar patterns predominated; at 42.5°C growth was that of a "giant cell" carcinoma.*

The effect of variation in temperature on the physiopathology of cancer has had very limited study. It has been examined in spontaneous and transplanted renal cell carcinoma of the frog by Lucké and Schlumberger (*1*). A few descriptions of the effect of temperature of incubation on the heterologous transplantation of mammalian cancer to the embryonated egg are also available (*2*). These latter studies are limited to descriptions of the effect of temperature on the weight of tumor growths after transplantation of tumor tissue either to the yolk sac or to the chorioallantoic membrane.

We were interested in the possible effects of temperature on the histopathologic appearance of heterologous tumor transplants in terms of the structure, organization, maturation, differentiation, and invasiveness of tumor tissue. We therefore studied the effects on the growth of the Walker carcinosarcoma 256 in chick embryos, of incubation at 32.5°, 37.5°, and 42.5°C, during the period between the 11th and 18th days of incubation. Preliminary studies had shown that the chick embryo can tolerate temperatures ranging from 32.5° ± 2.0°C to 42.5° ± 2.0°C, during the second half of its incubation period.

All the embryos were incubated at the normal temperature, 37.5°C, until the eleventh day of incubation. On either the 8th, 9th, or 10th day of incubation, the chorioallantoic membrane was inoculated with 0.1 ml of freshly drawn ascites tumor fluid (Walker carcinosarcoma 256). On the 11th day the eggs were examined by candling and those viable were divided into two or three groups and returned to incubators at different temperatures, as shown in Table 1. A few eggs were opened on the 11th day and the chorioallantoic membranes removed for histologic examination. On the 18th day of incubation, the chorioallantoic membranes were removed from all the viable eggs and fixed in formalin.

The tumors were studied grossly and histologically. Gross examination revealed that the tumors from eggs incubated at 37.5°C were the largest in size; those from eggs incubated at 32.5°C were smaller, while those grown at 42.5°C included the smallest tumors. Many of those grown at the extreme temperatures were similar in size.

The microscopic structure of the tumors from eggs incubated at 32.5°C and 42.5°C differed both from each other, and from those incubated at 37.5°C. Mitotic figures were found at all three temperatures. Tumors grown at 37.5°C frequently showed large groups of cells in an alveolar arrangement, with scanty stromal components lined by proliferating carcinoma cells in loose association with one another and with the stroma of the septae (Fig. 1*a*). This pattern sometimes varied, the alveolar spaces being filled with red blood cells, and lined by a thin layer of viable carcinoma cells. Occasional giant cells were found. The stromal cells in some of the carcinomatous areas appeared to be neoplastic, providing rare foci which merit the designation carcinosarcoma.

The alveolar arrangement of cells was absent in the tumors grown at 32.5°C.

Table 1. Summary of data on eggs inoculated with Walker carcinosarcoma 256.

Temperature (°C)	No. viable on 11th day	No. incubated	No. viable on 18th day	No. of eggs with tumors
Experiment No. 1. Fifty-six eggs inoculated on the 10th day				
32.5		12	8	8
37.5	56	20	19	19
42.5		12	9	0
Experiment No. 2. Eighty eggs inoculated on the 8th day				
32.5		19	11	10
37.5	62	19	14	14
42.5		19	13	12
Experiment No. 3. Eighty eggs inoculated on the 9th day				
32.5		14	13	13
37.5	36	14	4	4
42.5		0		
Experiment No. 4. Eighty eggs inoculated on the 9th day				
32.5		22	19	19
37.5	70	22	7	7
42.5		22	8	7

Instead, many areas consisted exclusively of large, neoplastic, spindle-shaped cells presenting a picture of sarcoma. Other areas consisted of carcinosarcoma—that is, a tumor in which both parenchyma and stroma are neoplastic. In these areas small nests and cords of epithelial tumor cells were found in a malignant stroma (Fig. 1*b*), the cells in these nests being tightly packed and the cell membranes indistinct.

The tumors from eggs incubated at

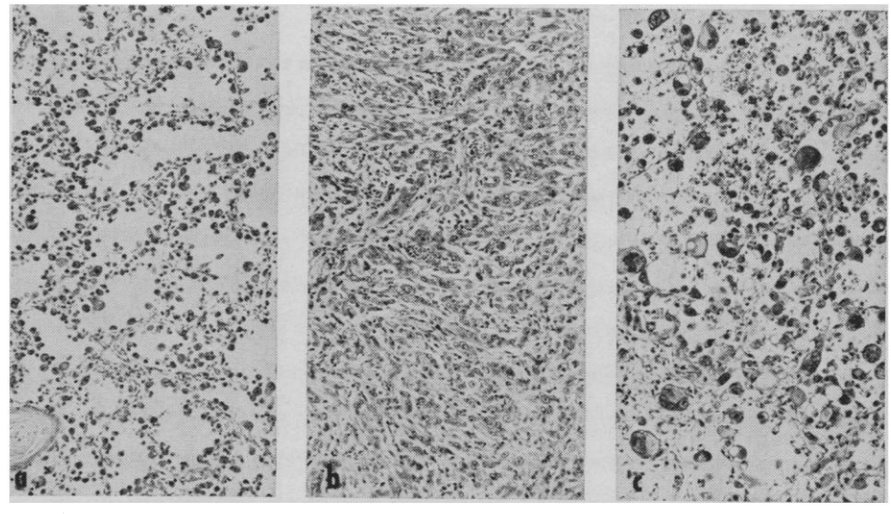

Fig. 1. Comparison of histologic patterns of cells in Walker carcinosarcoma 256 grown on the chorioallantoic membranes of chick embryos. (*a*) Section of tumor from egg incubated at 37.5°C, showing alveolar spaces with thin septae of connective tissue, lined by tumor cells of various sizes. The laminated structure in the lower left corner is a keratin pearl derived from the ectoderm of the chorioallantois. (*b*) Tumor grown at 32.5°C. The tissue is densely cellular, and many areas consist of neoplastic epithelial and connective tissue cells producing a carcinosarcomatous pattern. Only at this temperature can areas be found that are exclusively sarcomatous. (*c*) Tumor grown at 42.5°C. The tissue has an alveolar character, and there are many bizzare giant cells. (Hematoxylin and eosin; about × 90)

42.5°C, showed cells in an alveolar arrangement similar to that in the tumors grown at 37.5°C, but there was a complete absence of any sarcomatous component, and the character of individual tumor cells differed (Fig. 1*c*). There were many giant cells at 42.5°C, with bizarre nuclei in which mitotic figures could be found in some instances.

Evidently, when the eggs were incubated at 32.5°C, the sarcomatous potentialities of the tumor became dominant. The differing patterns of tumor growth may be the result of a cytogenetic selection in which certain cells, either with sarcomatous or with carcinomatous potentialities, are selected favorably at different temperatures. Alternatively, differing characters of growth may be examples of modulation. The same stem cells may, under one set of circumstances, grow predominantly in a spindle-shaped pattern, and under another, in an epithelial pattern (*3*).

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References and Notes

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Chromatographic Heterogeneity of Rabbit Serum Amylase

Abstract. *A chromatograph of normal rabbit serum shows three major peaks of amylase activity. Material associated with each peak differed in composition of serum protein, electrophoretic distribution of amylolytic activity, optimum* p*H for activity and Michaelis-Menten constants for starch hydrolysis. These data suggest but do not prove conclusively that normal rabbit serum contains at least three molecular forms of amylase.*

At least two amylases have thus far been distinguished in the serum of man and a variety of experimental animals by electrophoretic (*1–3*) or immunologic techniques (*4*). These observations suggest that the amylase activity of normal serum represents the net action of a group of amylolytic enzymes which may be elaborated by a number of different tissues. To elucidate the heterogeneity of serum amylase and the physico-chemical properties of these amylases, we have chromatographically separated fractions of normal rabbit serum and characterized the separate fractions in terms of electrophoretic behavior and enzyme activities.

Samples (approximately 3.0 ml) of serum from five normal rabbits were treated with Sephadex G-25 to remove the ionizing salts. The samples were then applied to DEAE-cellulose (diethylaminoethyl) columns and eluted with a chloride gradient solution at a flow rate of 20 to 25 ml per hour. The effluent showed three major peaks of amylase activity (Fig. 1). Peak 1 (tubes 10 to 20) was neither bound by DEAE-cellulose, nor associated with appreciable quantities of serum protein. With increasing chloride, two additional well-defined maxima of saccharogenic activity were observed in effluent tubes 30 to 45 (peak 2) and 46 to 80 (peak 3). Tubes 30 to 80 also contained protein. The chromatographic behavior of peak 1 suggests that the material is less negatively charged than that of peaks 2 or 3. Differences in charge may also account for the appreciably greater quantities of protein in the effluents from peaks 2 and 3.

The mean percentage distribution of amylase activity in peaks 1, 2, and 3 of the five rabbit sera were 26.4, 31.5, and 20.4 percent of total activity, respectively. The total recovery of amylase activity in no case exceeded that of unfractionated rabbit serum. Similar results were noted in electrophoretically separated fractions of rat serum as compared with whole rat serum (*5*). By contrast, the amylase activity recovered from normal human serum subjected to paper electrophoresis totaled more than the activity recorded in the unpartitioned serum (*1*, *2*). This discrepancy may be due to an amylase inhibitor which is active in whole serum (*1*).

Collections of effluent from each peak were pooled, dialyzed for 24 hours against distilled water, lyophilized, and reconstituted in 0.5 ml distilled water. Samples of the concentrate (20 μl) were placed on Whatman 3MM filter paper and subjected to electrophoresis for 16 hours in a horizontal chamber at a constant potential of 80 volts in veronal buffer (*p*H 8.6; ionic strength, 0.075). When the strips were stained with bromphenol blue, the protein in

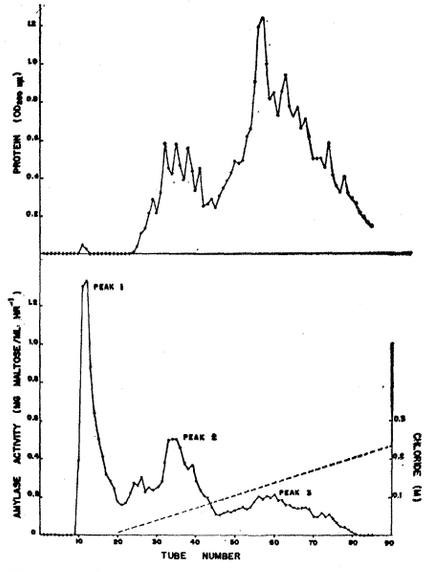

Fig. 1. Distribution of protein estimated spectrophotometrically (*9*) (top), and saccharogenic activity (*10*) (bottom) of normal rabbit serum fractionated by means of a chloride gradient on a DEAE-cellulose column (30 × 1.5 cm, Whatman paper DE-50 (*11*).

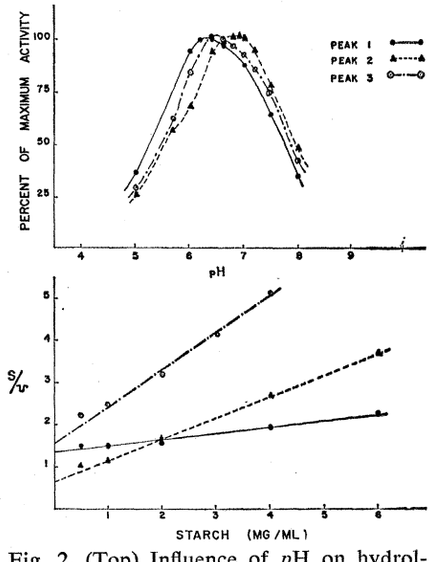

Fig. 2. (Top) Influence of *p*H on hydrolysis of starch by chromatographic fractions of normal rabbit serum. (Bottom) Relationships between starch substrate (*s*) concentrations and reaction speeds (*v*) used to calculate *km* values of 4.4, 0.6, and 0.9 mg/ml for peak 1, 2, and 3 concentrates, respectively.