by small numbers of macrophages, leukocytes, and plasma cells were present, with densely packed tumor cells lying at the periphery of these areas.

A different picture was presented by the specimens from CEH-treated rats. Extensive necrotic foci containing tumor cells with pronounced degenerative changes were seen in all specimens, surrounded and threaded in almost all of them by richly vascularized connective tissue structures. There was total or focal necrosis with infiltration by macrophages, lymphocytes, neutrophils, plasma cells, mast cells, fibroblasts, and fibrocytes.

In the CP-treated group, even rats with a clinically recorded tumor growth inhibition had developed a sarcomatous tumor that tended to invade the fatty and muscular tissues. Necrotic foci were not apparent in any of the specimens examined. All rats in this group were found to have intestinal necrosis at autopsy.

The results of this study demonstrate an antineoplastic action of CEH by a mechanism distinct from that of CP. As documented histologically, this embryonal preparation, unlike CP, not only inhibits tumor growth but also causes extensive tumor necrosis. Moreover, there was no evidence of toxicity in the form of intestinal necrosis such as was observed in all the CP-treated animals. The presence of lymphocytes and macrophages in necrotic areas may, in our view, be regarded as an indication that the embryonal homogenate predominantly stimulates cellular mechanisms of antitumor resistance.

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The Metastatic Potential of Tumors Depends on the pH of Host Tissues

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pH-Metric characteristics of Ehrlich's ascitic carcinoma and of a protein-induced tumor were compared in mice at different stages of the neoplastic process from tumor cell inoculation to the animal's death. On the acidographic curves, the time of change from acid to alkaline pH values coincided with that at which the tumors began to metastasize. It is suggested that this finding may be of use for the early diagnosis of tumor metastases.

Key Words: *metastases; pH; tumor strain; acidosis; alkalosis; mice*

Metastases from tumors are a top-priority clinical problem in oncology and are the subject of a voluminous literature. However, in none of the studies published so far has the metastatic process in tumor bearers been specifically considered in relation to the pH values of their tissues, although it would seem quite logical to look for the cause of metastases in the active response of these tissues.

The interest of researchers in the pH of tumor tissue is reflected in the literature only by data on single pH measurements in various tumors [2-4], and the authors of some publications have made an at-

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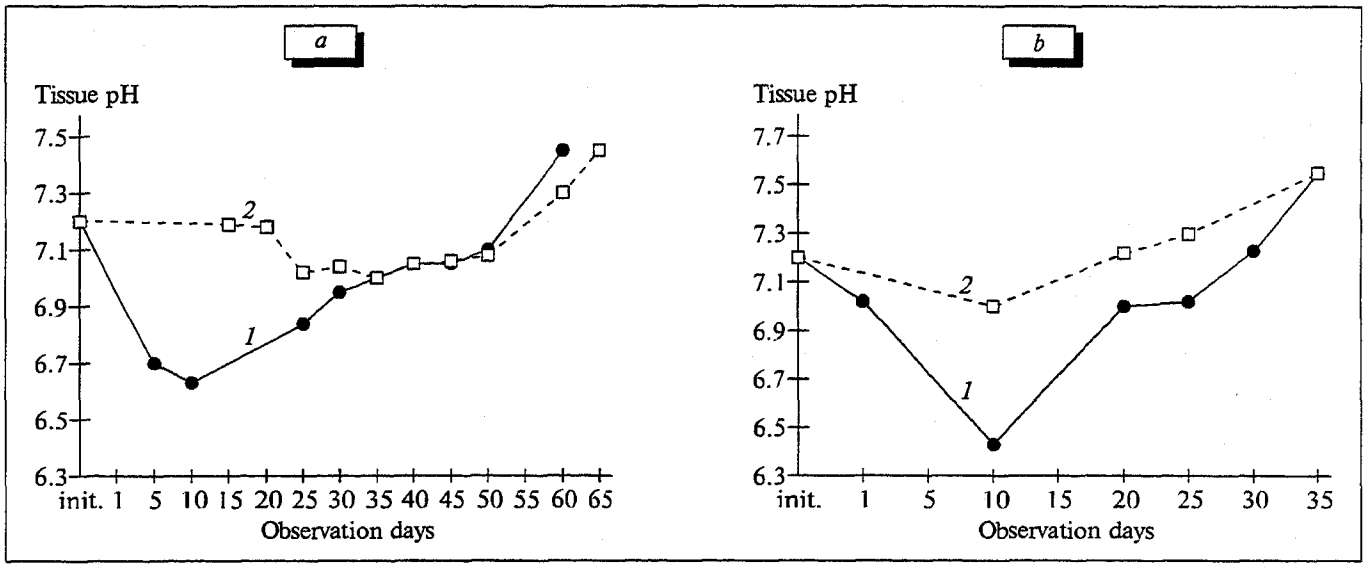

Fig. 1. Acidographic tracings obtained for tumor (*1*) and normal (*2*) tissues in mice with EAC (*a*) and PIT−I (*b*).

tempt to classify neoplasms in terms of their once-measured pH-metric characteristics [1].

Yet what we know about the biochemical transformations undergone by neoplastic tissues gives us good reason to believe that an active reaction of the medium in neoplasms of any type may, at different stages of their development, have pH values ranging widely from strongly acid to very basic. In view of this, the present study aimed to address three important questions: 1) how the stage of tumor growth relates to the pH of the tumor tissue; 2) how the pH of the primary tumor tissue affects that of normal tissue in the tumor bearer; and 3) what role the pH of tissues in the tumor bearer may play in the metastatic process.

MATERIALS AND METHODS

Two models of soft tissue tumors were used, differing in their malignant aggressivity as judged by the mean survival of mice bearing the respective tumor: Ehrlich's ascitic carcinoma (EAC, solid form), mean survival 2 months; and a protein-induced tumor (PIT-I, sarcoma), mean survival 1 month (this model was produced by the author).

The mice used were (CBA×C57Bl/6) F_1 males weighing 26-30 g. They were inoculated intraperitoneally into the right thigh with 10^6 EAC or PIT-I cells in a volume of 0.1 ml. The pH was recorded *in vivo* simultaneously in normal tissue of the left thigh and tumor tissue of the right thigh on day 1 after tumor cell inoculation and then at 5-day intervals throughout the period of tumor progression until the animal's death. The pH indicator was a hydrogen ion-selective glass electrode of our design specially adapted for measurements inside tissues. The potential from this electrode was delivered to the input of an ionometer. The reference electrode was a standard silver-chloride electrode electrolytically connected to the animal's body. Ten minutes before measurements, the mice were anesthetized with Nembutal in a dose of 50 mg/kg. Readings were taken 7-10 min after insertion of the working electrode into the tissues (i.e., when the pH level became stable).

RESULTS

Figure 1 shows curves of pH variations in the tumors and in normal tissues of the contralateral paw during the observation periods. The neoplastic process is at first seen to be accompanied by a precipitous decline of pH in the affected paw in both groups of mice, causing the tumor to be acidified to values far exceeding lethal ones. At a certain stage of tumor growth, the curves sharply change their direction toward alkalinity after the drop in pH has reached its maximum for the particular tumor (6.65 for EAC and 6.45 for PIT-I). The pH values of neoplastic tissue on the descending limb of each curve are repeated on the ascending limb. However, while these values on the former limb signify a true acidosis, their occurrence on the latter may represent a compensated acidosis that subsequently progresses to stable alkalosis, with the pH reaching high values (7.8 and more) in both groups. The active response of normal tissue, as judged by the course of the curves, is similar to that of tumor tissues except that the acidosis is less marked (7.00 for EAC and 6.98 for PIT-I) and most of their pH values do not go beyond the limits of adaptive changes.

Comparison of the curves in Fig. 1 shows that the acidification of normal tissues in EAC-bearing

mice began much later (on day 15) than in PIT-I bearers, in which it started on day 1. This difference appears to be mainly due to the greater aggressivity of the PIT-I strain.

In the last few days of the animals' life, the pH values recorded for normal and tumor tissues were almost equal in the two groups and indicative of persistent and rapidly progressing alkalosis.

A number of mice from both groups were sacrificed after some pH measurements to examine their internal organs. In the EAC-bearing group, lung tissue was seen to have occasional small dark dots on day 20 after tumor inoculation, i.e., on the 10th day after the start of tumor tissue alkalization. In the group of PIT-I bearers, such dots were first observed on day 12 after tumor inoculation, which corresponded to the 3rd day of alkalization. Subsequently, on days 35-40 in EAC bearers and on day 15 in PIT-I bearers, light-colored lumps the size of small or large lentils were seen in the lung tissue. These lesions progressed more rapidly in the PIT-I bearers to assume, by day 25 or later, the appearance of clusters composed of light lentiform lumps which histological examination established to be metastases.

The results of this study warrant the following conclusion. Underlying any pathological process, as consequences of specific metabolic transformations, are molecular changes in organs and tissues, whose rate and direction are mainly governed by the concentration of H^+ ions. From this standpoint, our curves of pH variation with time suggest that the changes in pH occur in stages corresponding to the biochemical stages in the development of neoplasms, and that these stages should be taken into consideration both in making the diagnosis and in selecting the most appropriate treatment.

Our comparative study of two tumor types has shown that the metastatic potential of the PIT-I strain depends directly on the degree of acidification undergone by tissues of tumor-bearing mice, confirming the greater aggressivity of this tumor strain.

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