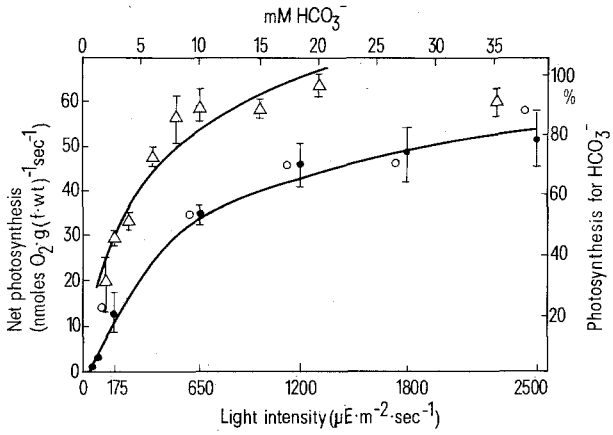

The response of net photosynthesis (△) to 1. varying HCO_3^- concentrations at pH 7.6 and a light intensity of 2000 $\mu E \cdot m^{-2} \cdot sec^{-1}$ or 2. varying light intensity at 20 mM HCO_3^-. Rates are expressed on a fresh weight (●) or chlorophyll (○) basis. Vertical bars represent SE. Regression equations ($p < 0.001$) are: $y = -63.5 + 15.0 \ln x$ ($n = 18$) and $y = -64.5 + 15.4 \ln x$ for fresh weight and chlorophyll bases respectively. Chlorophyll values are slightly offset to the left. The CO_2 regression equation is: $y = 18 + 27 \ln x$ ($p < 0.001$) omitting the highest concentration.

saturated in the range 750–1400 $\mu E \cdot m^{-2} \cdot sec^{-1}$[5-7]. From the fitted curves, the light compensation point was calculated as about 65 $\mu E \cdot m^{-2} \cdot sec^{-1}$, which is the same as that based on other methods[6].

$^{14}CO_2$ fixation versus O_2 evolution: There was no difference in the photosynthetic rate measured by $^{14}CO_2$ fixation compared with that measured as O_2 evolution (table). The $^{14}CO_2$ fixation values were adjusted by subtracting the rate of $^{14}CO_2$ fixed by leaf slices kept in the dark to account for dark CO_2 fixation and possible adsorption of $H^{14}CO_3^-$ (or $^{14}CO_2$) in such places as cell walls and intercellular air spaces of the slices. No attempt was made to partition the dark fixation rate of 6.4 ng $CO_2 \cdot cm^{-2} \cdot sec^{-1}$ into these components.

The net photosynthetic rate of 49 ng $CO_2 \cdot cm^{-2} \cdot sec^{-1}$ (table) is the same as that for Wayne soybean seedlings estimated by infrared gas analysis[5]. The cut surface area of the slices should not be considered when calculating photosynthetic rate, as it is not the main area of CO_2 diffusion[4]. Thus, in these leaf slices, gaseous exchange is through the stomata.

Using leaf slices in an oxygen electrode we find that rates of O_2 evolution and CO_2 fixation expressed on a fresh weight, chlorophyll or leaf area basis are the same as those for whole leaves using other methods.

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Anchorage-dependent culture of line 10 guinea-pig hepatoma cells

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Summary. A simple method for the anchorage-dependent culture of line 10 guinea-pig hepatoma cells is described.

The transplantable cell line commonly known as 'line-10 guinea-pig hepatoma' is an hepatocellular carcinoma converted to ascites form from primary hepatomas in strain-2 guinea-pigs originally induced by feeding diethylnitrosamine[3]. Intradermal (i.d.) inoculation of 10^6 ascites line-10 tumor cells leads to progressive local growth and metastases to the regional lymph nodes[4,5]. The animals die about 60 days following i.d. inoculation[6]. This neoplasm has been extensively investigated and multidirectional information concerning the antigenic and biologic properties of the transplanted tumors has led to its recognition as one of the most valuable animal tumor models used in cancer research today[7]. Immunotherapy of this tumor system has been very successful and predictions from this research have been extrapolated for use in the immunotherapy of naturally-occurring tumors in allogeneic species[8,9]. New approaches to studies of the mechanism of these maneuvers would be possible if continuous anchorage-dependent cultures derived from this tumor system were available; hitherto only suspension cultures have been attainable. Therefore, we now report a simple method for the propagation of guinea-pig line-10 hepatoma cells in an anchorage-dependent fashion.

Methods and materials. Plastic culture flasks (25 cm^2) were pretreated with human fibronectin (Collaborative Research Inc., Waltham, Mass., USA) at a concentration of 2.5 $\mu g/cm^2$ of growth area before addition of line-10 ascites cells. Fibronectin is a plasma protein isolated and purified from human fibrinogen which enhances and facilitates the culture of anchorage-dependent cells, and in this case, appears to induce and promote anchorage-dependency within many cells of the seeded population. Following pretreatment with fibronectin, the culture flasks were seeded with 4×10^6 line-10 hepatoma cells. The cells were obtained in ascites form, washed 2 times with MEM (Microbiological Associates, Bethesda, MD, USA) and numbers adjusted with MEM to seeding density. The culture medium consisted of MEM, 20% fetal bovine serum (Sterile Systems Inc., Logan, Utah USA), 100 units/ml penicillin and 100 μg/ml streptomycin. The cultures were allowed to incubate undisturbed for 4 days at 37 °C. Phase microscopy revealed the presence of many settled and anchorage-dependent islands of cells at that time (figure 1). Unattached cells were decanted and the cultures washed gently with MEM. Medium (MEM/FBS) was then added and the cultures allowed to propagate until maximum density was attained (figure 2). The viability of the cultured cells was tested by trypan blue exclusion and the induction of malignant metastatic lesions following the i.d. inoculation of the cultured cells (figure 3).

Discussion. The line-10 guinea-pig hepatoma transplant-

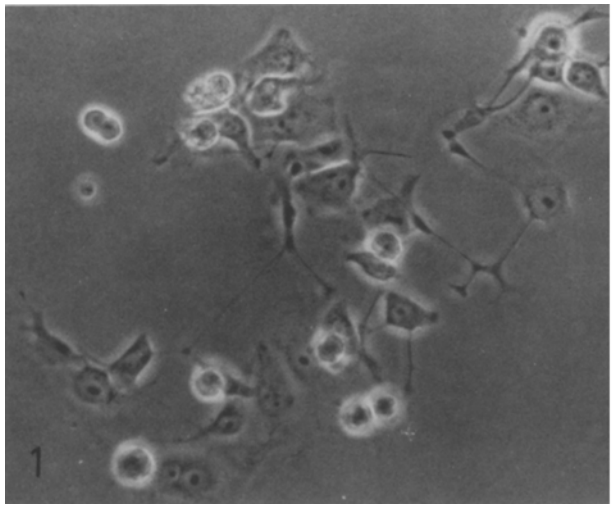

Fig. 1. Islands of line-10 guinea-pig hepatoma cells in culture 4 days after seeding. Phase contrast. × 480.

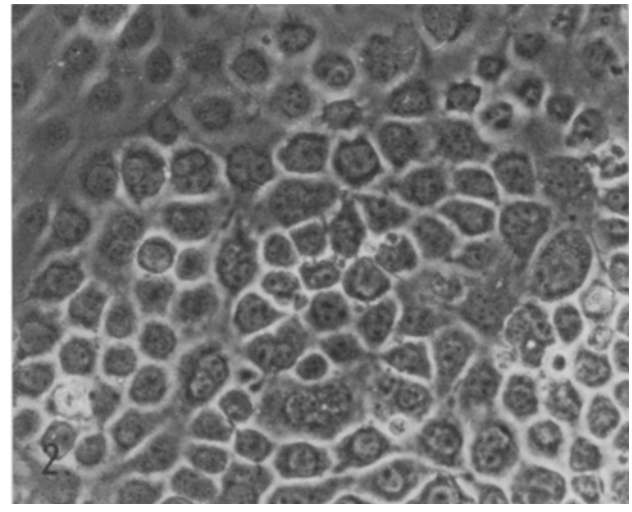

Fig. 2. Maximum density of cells in figure 1 culture 14 days after seeding. Phase contrast. × 480.

Fig. 3. Primary and metastatic lesions in strain-2 guinea-pig following i.d. injection of 1×10^6 line-10 hepatoma cells grown in vitro and described herein.

able tumor has been a valuable animal model tumor system in the development of cancer immunotherapeutic agents and delivery systems. Several aspects of the mechanisms of successful therapy have not been investigated due to the lack of anchorage-dependent cells used for in vitro immunologic assays. Hopefully, line-10 hepatoma cells, cultured as anchorage-dependent and described above, may provide appropriate targets for use in studies on the mechanism of killing of cancer cells by components of the immune system.

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