ferent shapes also existed in the small group of dark-incubated embryos which were put into light 1 week before hatching. This indicates that the developmental defect was probably induced earlier. Further experiments are necessary to evaluate the effect of light on differentiation. Especially we need answers to questions like: which other parameters (color, size, direction, etc.) may be affected by the learning deficits? When is the critical period for light exposure during the incubation time?

1 Acknowledgments. We are grateful to Dr E. Pröve for his advice on the carrying out of our imprinting tests, to K. Weigel for help with the drawings and to Dr J.R. Wolff for helpful discussions.
2 Blakemore, C., and Mitchell, D.E., Nature, Lond. *241* (1974) 467.
3 Barlow, H.B., Nature, Lond. *258* (1975) 199.
4 Daniels, J.D., and Pettigrew, J.D., in: Studies on the Development of Behavior and the Nervous System, vol.3, p.195. Ed. G. Gottlieb. Academic Press, New York 1976.
5 Grobstein, P., and Chow, K.L., in: Studies on the Development of Behavior and the Nervous System, vol.3, p.155. Ed. G. Gottlieb. Academic Press, New York 1976.
6 Mistretta, C.M., and Bradley, R.M., in: Studies on the Development of Behavior and the Nervous System, vol.4, p.215. Ed. G. Gottlieb. Academic Press, New York 1978.
7 Bateson, P.P.G., and Wainwright, A.A.P., Behavior *42* (1972) 279.
8 Vince, M.A., in: Studies on the Development of Behavior and the Nervous System, vol.1, p.286. Ed. G. Gottlieb. Academic Press, New York 1973.
9 Gottlieb, G., ed., in: Studies on the Development of Behavior and the Nervous System, vol.3, p.25. Academic Press, New York 1976.
10 Impekoven, M., Adv. Study Behav. *7* (1976) 201.
11 Lorenz, K.Z., Ornithology *83* (1935) 137, 289.
12 Hess, E.H., Imprinting: early experience and the developmental psychobiology of attachment, p.472. Van Nostrand Reinhold Comp., New York 1973.
13 Bateson, P.P.G., Biol. Rev. *41* (1966) 177.
14 Riesen, A.H., ed., and Zilbert, D.E., in: The Developmental Neuropsychology of Sensory Deprivation, vol.3, p.211. Academic Press, New York 1976.

0014-4754/85/030400-03$1.50 + 0.20/0

Effects of retinoic acid on ascites cells of the TA3 mouse mammary carcinoma[1]

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Summary. Retinoic acid caused a decrease in adhesiveness but no growth change in the allotransplantable TA3-Ha cell and no change in adhesiveness or growth in the strain specific TA3-St cell.The retinoic acid binding protein was detected in the TA3-Ha, but not the TA3-St, cell.
Key words. Mouse mammary carcinoma; ascites cells; retinoic acid; cell adhesiveness; cell growth.

Correlation of the presence of a retinoic acid binding protein with altered adhesiveness has been found in 2 sublines of TA3 mouse mammary carcinoma ascites cells grown in culture. The 2 ascites cell lines, TA3-St and TA3-Ha, which were derived from the same spontaneous tumor in a female strain A mouse, have been studied extensively because of their different transplantability characteristics[4,5]. The TA3-St cell is capable of growth in ascites form only in the mouse of origin, strain A; whereas, the TA3-Ha ascites cell is able to grow in some foreign species, as well as in allogeneic mouse strains.

As shown in figure 1, the addition of retinoic acid (*β*-all-*trans*-retinoic acid, Hoffmann-La Roche, Nutley, NJ) to the culture medium showed a dose-dependent decrease in the adhesiveness[6] of the TA3-Ha cell (A). No effect upon the adhesiveness of the TA3-St cell could be observed (B). The specificity of this effect upon adhesiveness in the TA3-Ha cell was shown by the sharp increase in this property upon removal of retinoic acid from the TA3-Ha culture medium (fig. 1A). No effect upon the adhesiveness of the TA3-St cell could be observed as a result of retinoic acid removal (fig. 1B).

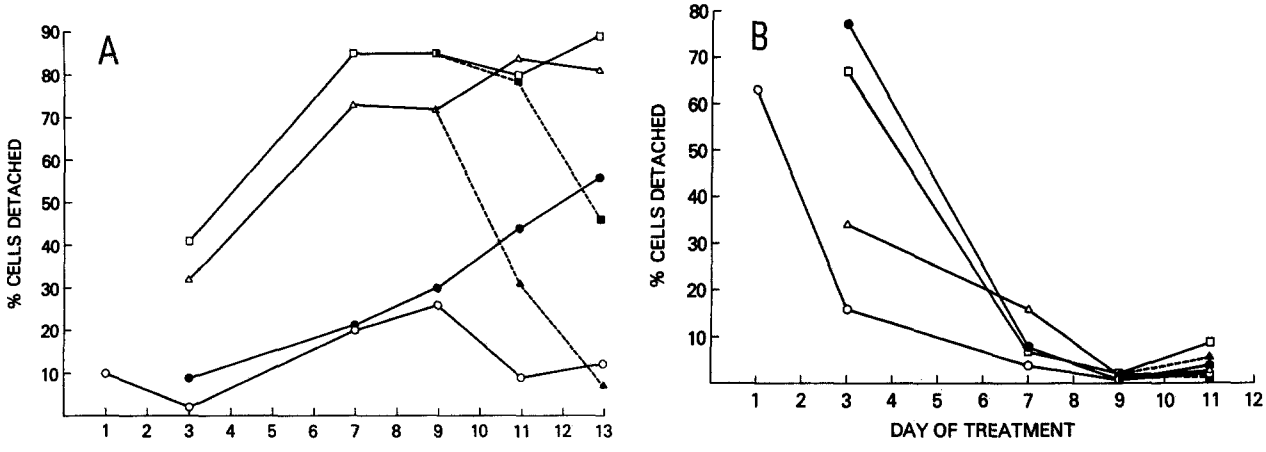

Figure 1. Time course of the effect of retinoic acid on the adhesion of TA3 cells. Cells were treated with control medium (○) or with control medium and Me_2SO (●); 1 μg (△) or 5 μg (□) of retinoic acid in Me_2SO per ml of medium. The time dependence of the effect on adhesion is shown. One half of the dishes treated with 1 μg (▲) or 5 μg (■) of retinoic acid were switched back to control medium at day 9 to show the reversibility of the effect. *A* TA3-Ha cells; *B* TA3-St cells.

No significant effect upon the growth rate of either the TA3-Ha (fig. 2A) or the TA3-St (fig. 2B) cell was observed. After removal of the retinoic acid from the culture media at day 9 no major change in the rate of growth of either cell was evident. In experiments to determine the growth rate and in those for measuring the adhesiveness, cells (5×10^5/dish) were grown in 10 ml of medium consisting of Dulbecco's Modified Eagle Medium (GIBCO, Grand Island, NY) with fetal bovine serum (M.A. Assoc., Bethesda, MD) (10%), penicillin (100 U/ml), and streptomycin (100 µg/ml) in 8-cm plastic tissue culture dishes (Falcon, Oxnard, CA) at 37°C in humidified 5% CO_2–95% air. Enough dishes were initially plated to supply at least 3 separate determinations at each time period. Retinoic acid in dimethylsulfoxide at concentrations required to give 1 and 5 µg/ml was added 24 h after plating the cells. Medium was replaced in all culture dishes on a daily basis. Total cells were determined by combining the unattached cells with those removed by incubation with 2.5 ml of PBS containing EDTA (0.02%) and trypsin (0.25 mg/ml) and counting in a hemocytometer.

The adhesion assay was performed according to Adamo et al.[6]. Plated cells were washed twice with PBS. Cells were removed from the dishes in 3 steps. a) After the addition of 2.5 ml of EDTA (0.02%) in PBS, the dishes were shaken at 110 rpm on a Thomas Rotating Apparatus at 22°C for 4 min. The detached cells were removed and counted in a hemocytometer. b) A solution of 2.5 ml of PBS containing trypsin (0.20 mg/ml) was added to each dish. After gently swirling the dishes for 30 sec, the detached cells were removed and counted. c) The remaining cells were detached by incubating at 37°C for 15 min in 2.5 ml of PBS containing EDTA (0.02%) and trypsin (0.25 mg/ml) and were counted. The percentage of cells removed in the assay was calculated according to the equation: $\% = \frac{a+b}{a+b+c} \cdot 100$.

The presence of a retinoic acid binding protein in the cytosol of the TA3-Ha line, grown in the absence of added retinoic acid, is illustrated by the sucrose density gradient centrifugation pattern shown in figure 3. No binding of [^{3}H] retinoic acid by the cytosol from the TA3-St cell was observed. The specificity of the binding of retinoic acid in the TA3-Ha cell was indicated by the displacement of the peak of radioactivity in the 2*S* region of the gradient by dilution of the labeled retinoic acid by a 200-fold excess of unlabeled retinoic acid. No effect was noted in the binding of retinoic acid in the cytosol of either cell by the addition of an equally large excess of retinol (not shown).

The procedure for the determination of the presence of retinoic acid-binding protein in the cytosol was that of Shidoji et al.[7]. The cytosolic fraction was incubated with all-*trans*(11,12-^{3}H)-retinoic acid (13 Ci/mmol, Hoffmann-La Roche) or all-

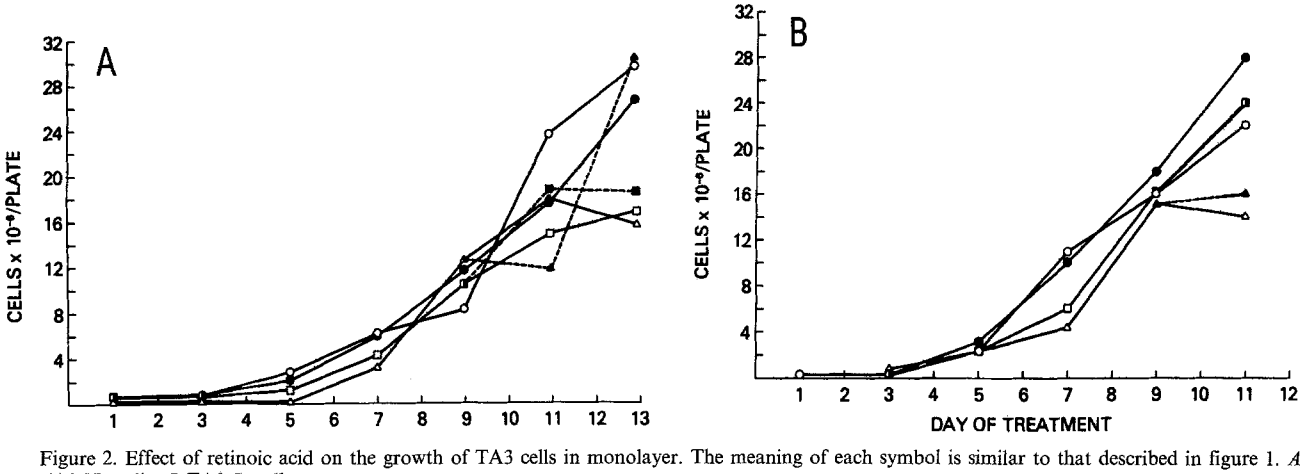

Figure 2. Effect of retinoic acid on the growth of TA3 cells in monolayer. The meaning of each symbol is similar to that described in figure 1. *A* TA3-Ha cells; *B* TA3-St cells.

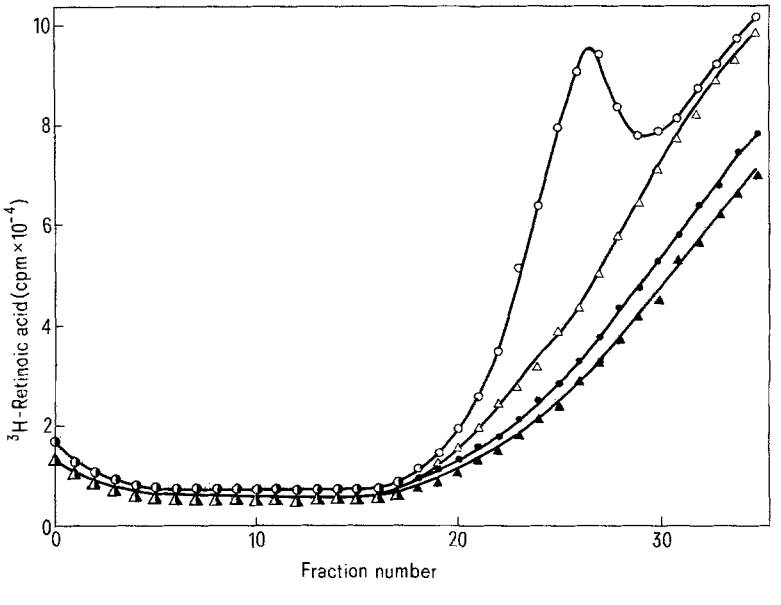

Figure 3. Sucrose density gradient analysis of cellular retinoic acid-binding protein from TA3 cells. (○), [^{3}H] Retinoic acid with cytosol from TA3-Ha cells; (●), [^{3}H] retinoic acid plus a 200-fold excess of retinoic acid with cytosol from TA3-Ha cells; (△), [^{3}H] retinoic acid with cytosol from TA3-St cells; (▲), [^{3}H] retinoic acid plus a 200-fold excess of retinoic acid with cytosol from TA3-St cells.

trans(15-^{3}H)retinol (2.7 Ci/mmol, New England Nuclear, Boston, MA). For competitive binding, the mixture contained either unlabeled all-*trans*-retinol (Hoffmann-La Roche) at a molar concentration 200-fold greater than that of the labeled materials. After an incubation of 16 h in the dark at 4 °C, each sample was centrifuged at 178,000 × g in a linear (5–20%) sucrose gradient for 21 h. Radioactivity was measured in Triton X-100 Omnifluor in a scintillation spectrometer.

The observation that a retinoic acid receptor is present in the allotransplantable TA3-Ha cell, but is absent in the strain specific TA3-St cell is consistent with the finding that retinoic acid added to the culture medium of the TA3-Ha cell produces an effect (a decrease in adhesiveness), whereas under similar conditions no effect was observed in the TA3-St cell. This contrasting result appears noteworthy, since both cell lines had similar origin, i.e., both were derived from the same tumor mass[4]. Different reported effects of added retinoic acid could be explained on the basis of different cell types and tissues of origin. These included the capacity to increase[6,8], decrease[9], or have no effect on[10], adhesion; to induce[11] or inhibit[12] differentation; to enhance[13] or to inhibit[14] cell proliferation; and to alter[15] or have little or no effect upon[10] morphology. Indeed, the response of the TA3-Ha cell itself cannot be considered as unique, since retinoic acid was reported by Shapiro and Poon[9] to exert a similar effect upon adhesion and growth rate in an established cell line derived from human intestinal epithelium. The present study suggests that the adhesive properties of tumor cell lines of similar epithelial origin, when grown in culture, may be affected in quite different ways by added retinoic acid. Response was observed only in one line, TA3-Ha, the cell found to possess the retinoic acid-binding protein. Whether or not the decreased adhesion of the TA3-Ha ascites cell resulting from added retinoic acid also alters its transplantation characteristics remains to be determined.

1 This study was supported in part by Public Health Service Grants CA-08418 and CA-18600 from the National Cancer Institute, National Institutes of Health.This is publication 955 of the Robert W. Lovett Group for the Study of Diseases Causing Deformities. We gratefully acknowledge the contributions of Drs Luigi M. De Luca and Anton M. Jetten of the Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institutes of Health, Bethesda, MD, without whose help this study would not have been possible.

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4 Hauschka, T.S., Weiss, L., Holdridge, B.A., Cudney, T.L., Zumpft, M., and Planinsek, J.A., J. natl Cancer Inst. *47* (1971) 343.
5 Codington, J.F., and Frim, D.M., Biomembranes *11* (1983) 207.
6 Adamo, S., De Luca, L.M., Akalovsky, I., and Bhat, P.V., J. natl Cancer Inst. *62* (1979) 1473.
7 Shidoji, Y., Hosoya, N., Analyt. Biochem. *104* (1980) 457.
8 Jetten, A.M., Jetten, M.E.R., Shapiro, S.S., and Poon, J.P., Exp. Cell Res. *119* 1979) 289.
9 Shapiro, S.S., and Poon, J.P., Exp. Cell Res. *119* (1979) 349.
10 Lacroix, A., Anderson, G.D.L., and Lippman, M.E., Exp. Cell Res. *130* (1980) 339.
11 Breitman, T.R., Selonick, S.E., and Collins, S.J., Proc. natl Acad. Sci. USA *77* (1980) 2936.
12 Takenaga, K., Honma, Y., Okabe-Kado, J., and Hozumi, M., Cancer Res. *41* (1981) 1948.
13 Schroder, E.W., Chou, I.-N., and Black, P.H., Cancer Res. *40* (1980) 3089.
14 Lotan, R., Biochim. biophys. Acta *605* (1980) 33.
15 Patt, L.M., Itaya, K., and Hakomori, S.-I., Nature *273* (1978) 379.

0014-4754/85/030402-03$1.50 + 0.20/0

Different biological behavior of AKR lymphoma cells from primary and metastatic tumors

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Summary. AKR lymphoma cells derived from primary s.c. tumors (PT) and cells from their metastases (MT) were inoculated into recipient mice in order to compare their malignant behavior. A higher malignant potential of MT compared to PT cells was found. The results support the hypothesis that metastasis is a process of selection of cells possessing a potential to metastasize, which preexist in the primary tumor. In the model used, both the selection of 'variants' of malignancy and the assay of malignancy were as close as possible to natural tumor progression.
Key words. Different malignancy; primary-metastatic cells.

Studies dealing with differences between primary and metastatic tumor cells are important for understanding the nature of malignancy, as well as for their therapeutic implications.

Many studies have tried to relate the degree of malignancy of tumor cells to various structural properties: karyotypic[2], biochemical[3,4], immunological[5] and the response to therapy[6]. Many of these studies used cells of different lineage from the same tissue, like the Morris hepatoma series[3]. More recently, variants of the same tumor, prepared by different in vitro[6], in vivo[7] or combined in vivo and in vitro manipulations[8] were studied. Although much important information was gained from studies of these variants, differences between primary and metastatic tumors of the same individuals are more directly relevant to natural neoplastic development.

Investigations devoted to the comparison of primary versus metastatic tumors of the same organism[9–14] showed a number of differences, including biochemical[9,10] and immunological features[11,12] and sensitivity to drugs[13,14]. These comparative studies were recently reviewed by Weiss[15]. Studies were also performed in order to find out whether cells deriving from metastatic tumors had a higher malignant potential than those deriving from the parent primary tumor[8,16]. The assay for malignancy was often performed by i.v. inoculation[7], a procedure which does not include the phase of invasion, or by surgical excision of the primary tumor[16], a procedure which enhances metastatic spread[17]. The two procedures are obviously incomplete models of natural tumor progression. However, very recently several successful attempts have been made to perform the assay of malignancy by s.c. inoculation[18–21].

Using the AKR lymphoma, we were able to obtain real spontaneous metastasis by injecting tumor cells s.c., permitting thereby the evolution of the natural process of metastasis includ-