after 60 days—the mesocarp—was the tissue which contained the highest gibberellin at that time. However, our data were inadequate to show whether the final period of rapid growth in this tissue was correlated with a higher gibberellin level, that is, whether activity in the mesocarp increased at 80 to 85 days.

The gibberellin in apricot seed and pericarp was purified by a partitioning procedure similar to that of Radley (*2*), together with ascending paper chromatography in a mixture of isopropanol, NH_4OH (density 0.88), and water (10 : 1 : 1). The results suggest the presence of nonbasic compounds which are more polar than gibberellic acid (GA_3). Little gibberellin-like material was extracted by ethyl acetate from an aqueous solution at *p*H 9. Even when the aqueous phase was *p*H 3, only about half the gibberellin-like material appeared in the ethyl acetate. Under the same conditions GA_3 was partitioned completely into ethyl acetate. Bioassays of strips of the paper chromatograms showed a single large area of active substance between R_F 0.1 and 0.5, with peak activity centered at 0.28. The center R_F of GA_3 was 0.40. Mobility relative to GA_3 (70 percent) is lower than that reported for most known gibberellins chromatographed under similar conditions (*3*).

The concentrations of gibberellin-like substances shown in Fig. 1 are very high compared with usual concentrations of hormones in plant tissues; the high values lie between 10 and 100 parts per million dry weight. Similarly high values for gibberellin have been found in floral apices of barley at the stamen initials stage and in seed of *Echinocystis macrocarpa* and bean (*4*). Comparisons must be carefully drawn, however, because gibberellins differ in activity in different bioassays. Paper chromatograms of methanol extracts of seed, 15 days after anthesis, were bioassayed by the barley-endosperm test and another test specific for gibberellins—the d-5 dwarf maize (*5*). The results (Fig. 2) are comparable; the centers of activity occurred at about the same R_F, and the amounts of activity were only slightly less when assayed with the dwarf maize.

The correlations between growth rate and gibberellin content in these tissues are better than correlations between fruit growth rate and auxins (*6*). However, we do not necessarily propose that the gibberellin-like substances extracted are the cause of growth in apricots. Other explanations are possible. They may be a product of growth or, as suggested by Dattaray and Mer with auxin in *Avena* coleoptile (*7*), they may represent the difference between production and utilization.

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Viral Neoplastic Transformation of Hamster Pineal Cells in vitro: Retention of Enzymatic Function

Abstract. *Cultures of hamster pineal tissue infected with certain oncogenic DNA viruses undergo neoplastic transformation and produce tumors when injected into homologous hosts. Hydroxyindole-O-methyl transferase, an enzyme found exclusively in the pineal gland, is present in low concentrations in transformed pineal cells in vitro and in larger amounts in tumors produced by the injected cells. This enzyme is not present in several nonpineal tissues similarly transformed.*

Oncogenic DNA viruses are able to alter the growth characteristics of certain mammalian tissues in vitro by a process referred to as transformation. There has been no previous evidence that such transformed tissues maintain specific biochemical characteristics of the original tissues.

The melatonin-forming enzyme, hydroxyindole-*O*-methyl transferase (HIOMT), is localized in the pineal glands of mammals (*1*). The unique tissue distribution of this enzyme suggested that it might be used as a marker of continued biochemical function of pineal cells transformed by oncogenic viruses.

Pineal glands obtained from 80 Syrian hamsters (3 to 4 weeks old) were pooled, minced into 1-mm explants, and used to establish five 2-oz (59-ml) prescription bottle cultures by methods described previously (*2*). When cells began to migrate from the explants on the 2nd to 3rd day, four culture bottles were infected with oncogenic DNA viruses. One culture was infected with $10^{6.0}$ $TCID_{50}$ (tissue culture infective dose, 50 percent effective) of polyoma virus, the second with $10^{8.5}$ $TCID_{50}$ of adenovirus 12, the third with $10^{7.9}$ $TCID_{50}$ of SV40, and the fourth with $10^{5.2}$ $TCID_{50}$ of the LLE46 strain of adenovirus 7 (the adenovirus 7–SV40 "hybrid"). Derivation of virus strains and methods of growth and titration have been described (*2*). One bottle was kept as a virus-free control.

Subsequently all cultures were kept in an incubator at 37°C and fed three times a week with a medium composed of medium No. 199 (80 percent) and fetal bovine serum (20 percent). Cultures were observed daily for evidence of transformation, characterized by increased acidity of growth media and by accelerated growth of cells in multiple layers and clumps; 10 to 14 days after infection there was evidence of transformation in the cultures infected with SV40 and LLE46. Transformation was not recognized in the culture infected with polyoma virus until the

Table 1. Hydroxyindole-*O*-methyl transferase (HIOMT) activity of transformed hamster cell cultures and of tumors produced by transformed cells from pineal gland, salivary gland, pituitary gland, and testis.

Tissue	Infecting virus	HIOMT activity ($\mu\mu$mole/mg)
	Normal	
Pineal gland	None	10–20
	Normal	
Pineal culture*	None	<0.1†
Transformed cell cultures		
Pineal‡	Polyoma	.96
Pineal‡	LLE46	.14
Salivary§	LLE46	$<.005$†
Salivary§	SV40	$<.005$†
Tumors produced by transformed cells		
Pineal	LLE46	.60–3.20
Pineal	SV40	.44
Salivary	SV40	$<.005$†
Pituitary	LLE46	$<.005$†
Pituitary	SV40	$<.005$†
Testis	LLE46	$<.005$†

* In vitro, 20 days. † HIOMT activity could not be demonstrated in these tissues. Values represent limits of sensitivity of assays. ‡ In vitro, 60 to 80 days. § In vitro, 180 to 200 days.

3rd week; and there was no evidence of transformation in the culture infected with adenovirus 12 and the control culture during the 7 weeks they were observed.

Twenty-eight days after infection all bottles were subcultured after trypsinization (*2*). Subsequently, cultures were trypsinized every 2 weeks and grown in 32-oz (940-ml) bottles. Fifty days after infection the transformed cells were scraped from the bottles, suspended in media, and injected subcutaneously into irradiated adult hamsters (approximately 10^8 cells in 1 ml of medium per hamster). All injected animals developed tumors within 20 to 40 days. Since we were unable to recover infectious SV40 or LLE46 from the transformed cells, even after growing the cells in direct contact with monolayers of primary African green monkey kidney cells and primary human embryo kidney cells, and since neither SV40 nor LLE46 have produced tumors in irradiated adult hamsters in 20 to 40 days, we feel certain that the tumors were produced by transformed cells rather than by any traces of infectious virus in the cultures.

Neoplasms that developed in hamsters injected with the SV40-transformed tissues were fibrosarcomas, while those that occurred in animals injected with LLE46-transformed cells were histologically bimorphic with both SV40-type fibrosarcoma and adenovirus-type components (Fig. 1). This characteristic histology has been described previously in tumors that developed in hamsters inoculated with LLE46 virus on the day of birth (*3*) and in those that developed in adult hamsters injected with LLE-46-transformed cells (*2*). No neoplasms have developed in animals injected with polyoma-transformed pineal cells during a 1-month period of observation.

HIOMT was analyzed by a modification of the method described by Axelrod *et al.* (*4*). Tissues were homogenized in dilute buffer (*p*H 7.9) and incubated with *N*-acetyl serotonin and C^{14}-*S*-adenosylmethionine. HIOMT catalyzed the formation of C^{14}-methylmelatonin; this was extracted from the incubation medium in chloroform and counted in a liquid scintillation spectrophotometer. The identity of the reaction product was confirmed by thin-layer chromatography with the use of a mixture of chloroform and methanol (93:7).

Normal hamster pineal tissue was found to synthesize 10 to 20 $\mu\mu$mole of C^{14}-melatonin per milligram of tissue per hour. This level of activity is of the same order of magnitude as in the rat (*5*). Uninfected hamster pineal tissue maintained in culture for 20 days grew poorly and had no HIOMT activity.

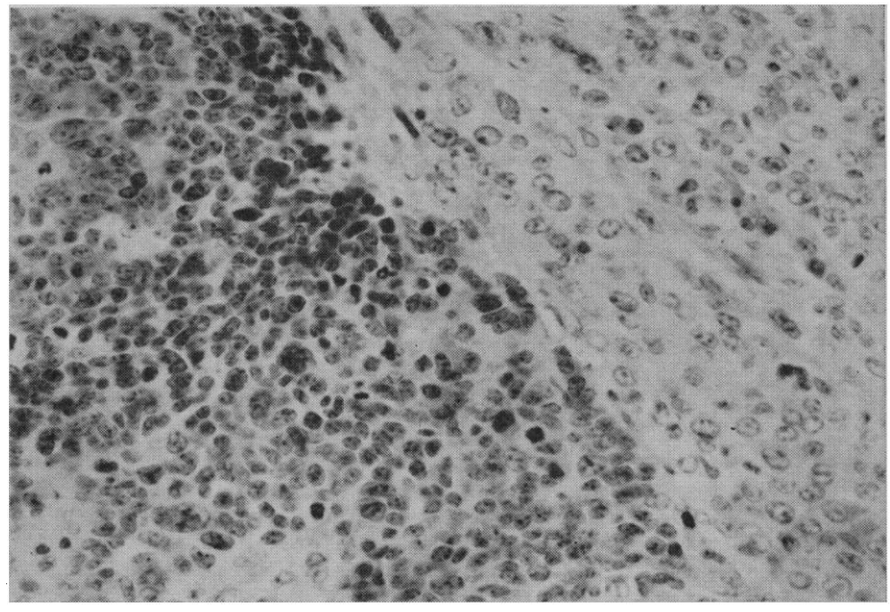

Fig. 1. Section of tumor produced in hamster by injection of LLE46-transformed hamster pineal cells. The tumor is bimorphic with areas resembling SV40 sarcomas (right) and those resembling adenovirus-induced tumors (left). Hematoxylin and eosin stain; × 400.

Our cultures of hamster pineal tissue that were transformed in vitro by polyoma and LLE46 viruses had moderate amounts of HIOMT activity 60 to 80 days after infection (Table 1). Tumors produced by the injection of pineal cells that had been transformed with SV40 and LLE46 viruses contained large amounts of HIOMT activity (2.2 to 16.0 percent of that of an equal weight of normal pineal tissue). These tumors, some of which weighed 8 to 10 g, contained up to 50,000 times as much HIOMT as a single normal pineal gland.

Cultures of nonpineal tissue (salivary gland, pituitary, and testis) that were transformed in vitro by SV40 and LLE46 (*2*, *6*) contained no HIOMT activity, nor did the tumors produced by injection of transformed cells from these tissues (Table 1).

Several other tissues obtained fresh from normal hamsters were examined for HIOMT activity. Liver and some regions of the brain synthesized minute amounts of C^{14}-melatonin (less than 1 percent that of pineal). This activity could be identified only because of the extreme sensitivity of the HIOMT assay and possibly represented nonspecific methylation by other enzymes in these tissues.

Although HIOMT was demonstrable in the transformed cells in vitro, significantly greater activity was present when the cells were growing as tumors in vivo. This difference could be due to some host factor acting on the tumor cells in the hamster; it also could result from selection in vivo of a population of transformed cells with greater enzymatic activity.

The retention of a specific biochemical function of pineal cells after transformation suggests that other endocrine tissues might also function, and perhaps produce characteristic hormones, after viral transformation.

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