Gibberellin-like Substances in the Developing Apricot Fruit

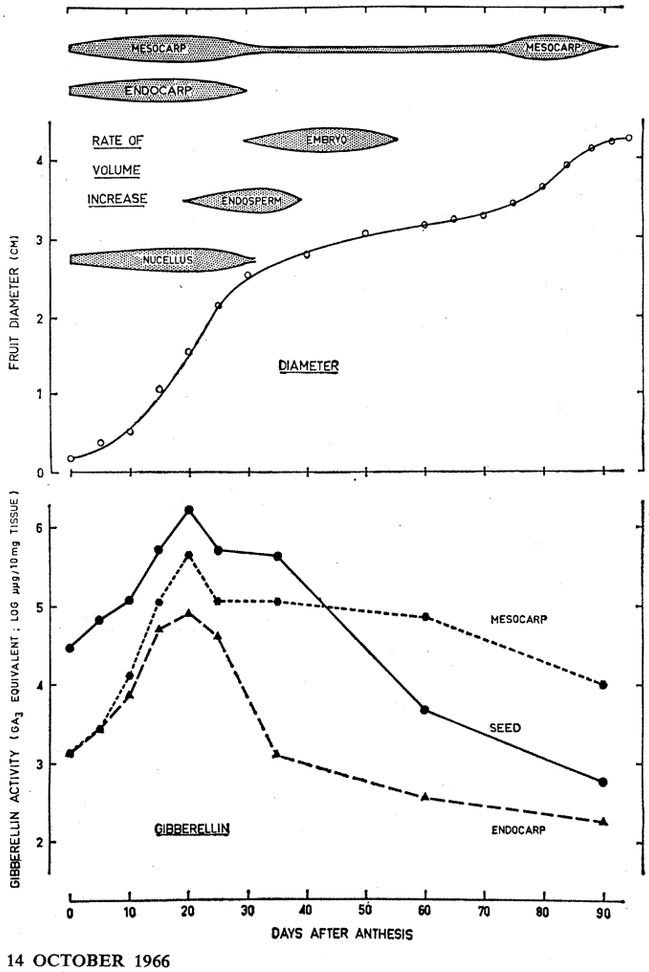
Abstract. *The concentrations of gibberellin-like substances in the seed, endocarp, and mesocarp of apricots correlates well with growth rates in these tissues between anthesis and maturity. The active substances are present in high concentration, are more polar than the known gibberellins, and are active in both the barley endosperm and dwarf maize bioassays.*

Gibberellins are important hormones for the growth of plants, yet there are few studies of the changes in concentration during development and growth of fruit tissues. We compare growth rates of the seed, endocarp (stone), and mesocarp (flesh) of apricot (*Prunus armeniaca* L.) between anthesis and fruit maturity with the concentrations of gibberellin-like substances extracted from these tissues, and describe some of the properties of these substances.

Apricots (cv. 'Moorpark') were sampled at intervals after anthesis from 10-year-old trees growing in the orchard of the Waite Institute. Samples for bioassay were immediately frozen with dry ice and later lyophilized. Tissue was separated into seed, endocarp, and mesocarp, ground to a powder, and 10-mg portions were shaken with 10 ml of ethyl acetate for 2 hours at room temperature. The liquid was decanted, and the residue was covered with another 10 ml of ethyl acetate for 22 hours at 23°C, and finally washed again with 10 ml. Extracts were combined, filtered, and evaporated under reduced pressure at 30°C to dryness. The solid residue on the filter paper was then extracted in the same way with absolute methanol. The evaporated extracts were dissolved in water, and 1-ml portions were tested for their gibberellin activity with the barley endosperm bioassay (*1*).

Methanol extracts of apricot seed and pericarp were ten times more active than ethyl acetate extracts. The results obtained with methanol extracts (Fig. 1) show that in all three parts of the fruit, gibberellin activity increased after anthesis and reached a maximum 20 days after anthesis. The seed had the greatest gibberellin activity and the endocarp the least. Thereafter concentrations of gibberellin-like substances decreased in all three tissues, the decrease being most rapid in the endocarp and slowest in the mesocarp.

For comparison, the growth rates of these parts of the apricot are included in Fig. 1. The concentration of gibberellin-like substances in the three tissues correlated well with their growth rate for the first 60 days after anthesis. The time at which gibberellin activity was greatest coincided with the time of greatest growth rate in the mesocarp, endocarp, and nucellus. Activity in the endocarp declined to a low level concurrently with cessation of growth and the sclerification of the tissue, 30 to 45 days after anthesis. Activity in the seed remained high and did not decline until the interval 35 to 60 days after anthesis, during which seed growth rate declined and stopped. The only tissue which grew



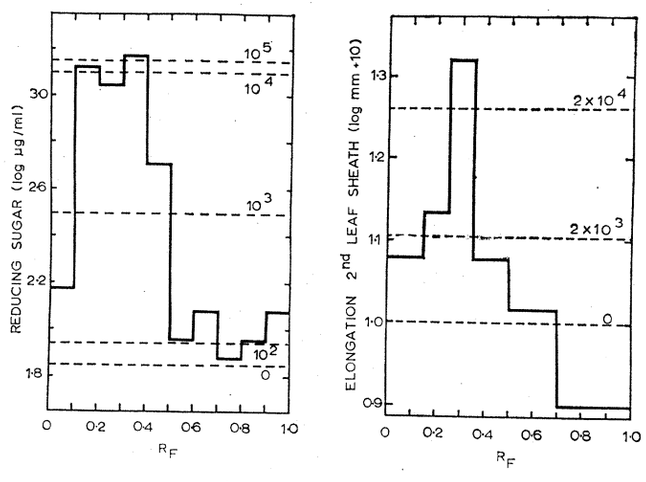


Fig. 1 (left). Concentrations of gibberellin-like substances in methanol extracts of seed, endocarp, and mesocarp of apricots compared with their growth rates during development. Nucellus, endosperm, and embryo are the principal tissues in the seed.

Fig. 2 (above). Paper chromatograms (isopropanol : ammonium hydroxide ascending) of methanol extracts of 15-day-old apricot seed (10 mg) bioassayed with barley endosperm (left) and d-5 dwarf maize (right).

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.