

not in *f/f* mice. Total ALD activity per spleen increased 2.0-, 4.7-, and 8.5-fold in *f/f*, *f/+*, and *+/+* mice, respectively, which demonstrates an intermediate heterozygous expression of the gene *f* in adult splenic tissue under conditions of severe hematopoietic stress.

The data in Table 1 imply that, in times of severe red cell breakdown, extra hematopoiesis may be stimulated in the spleen, and suggest that the action of the gene *f* modifies this response. During the period of fetal development when the anemia of *f/f* fetuses is most pronounced, the fetal red cell mass enlarges very rapidly in both *+/+* and *f/f* fetuses (70- to 80-fold increases in cell number from the 12th to the 16th day of development) (2). This rapid increase in the number of red cells may constitute a severe stress to the fetal hematopoietic system. The appearance of hypochromic siderocytic anemia in *f/f* fetuses may be caused by an insufficiency of ALD, resulting from an inability of the hematopoietic system to respond adequately to the demand for this enzyme activity, an effect similar to the severely reduced ability of the spleens of phenylhydrazine-treated *f/f* adults to manifest increased ALD activity. Similarly, the absence of anemia in the heterozygous *f/+* fetuses may

indicate that the level of ALD activity in this genotype is sufficient to meet the demands of the presumed hematopoietic stress, an effect similar to the moderate increase of ALD activity in the spleens of phenylhydrazine-treated *f/+* adults.

The gene *f* thus appears not only to control the level of ALD activity but also to modify the extent of response of this enzyme activity to conditions of hematopoietic stress.

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## Gibberellic Acid: Action in Barley Endosperm Does Not Require Endogenous Auxin

**Abstract.** Endogenous auxin is not required for the increase in amylolytic activity induced by gibberellic acid in barley endosperm, as shown by the response of the system to anti-auxin. The action of gibberellic acid in this system is independent of the presence or absence of auxin.

Treatment of barley endosperm with gibberellic acid results in *de novo* synthesis of  $\alpha$ -amylase (1). This reaction is unique among the reactions induced by this acid, for auxin does not appear to be involved: exogenous auxin does not promote amylolytic activity in either the presence or absence of the acid (2). However, cereal seeds contain sizable amounts of endogenous auxin (3). The possibility exists that auxin is necessary for this reaction but that endogenous auxin satisfies the requirement. Such a situation exists in the elongation induced by gibberellic acid in *Avena* leaf sections (4). It is of

particular interest to determine whether endogenous auxin is required for this process because several current theories postulate an obligate interaction between auxin and gibberellic acid (5).

The response of a system to anti-auxin can be used to assess the role of endogenous auxin. An obligate role of auxin is indicated if the inhibitor action is at least partially reversed by subsequent addition of auxin. This technique has been successfully used to study the role of endogenous auxin in the elongation of several tissues (4, 6). We have now used it to examine the role of endogenous auxin in the in-

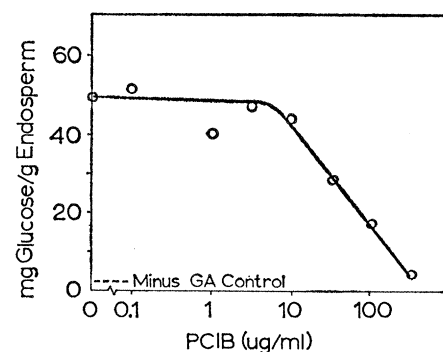


Fig. 1. Effect of the anti-auxin *p*-chlorophenoxyisobutyric acid (PCIB) on the increase in amylolytic activity induced by gibberellic acid (GA). Endosperm halves incubated in 3 ml of basic medium with varying concentrations of PCIB. Reducing sugars in medium determined after 22 hours at 30°C. In the absence of GA there were 1.7 mg of glucose equivalents per gram of endosperm.

crease in amylolytic activity in barley endosperm induced by gibberellic acid.

The induction of amylolytic activity has been followed by the technique of Paleg (7). Seeds of barley, variety Himalaya, were first heated for 1 hour at 70°C to reduce the amount of endogenous amylolytic activity. After sterilization seeds were bisected transversely and lots of four endosperm halves were incubated in petri dishes with 3 ml of basic medium. The medium contained potassium maleate buffer (2.5 mM, pH 4.8), streptomycin (500  $\mu$ g), gibberellic acid (1  $\mu$ g/ml), and, where required, the anti-auxin, *p*-chlorophenoxyisobutyric acid, and any one of

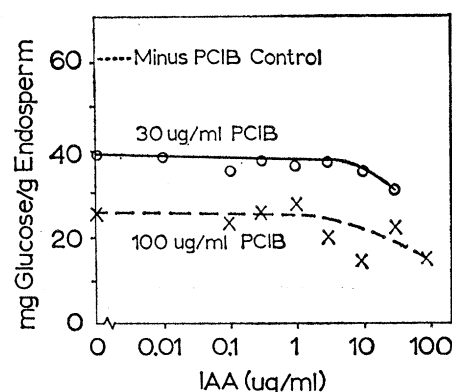


Fig. 2. Inability of the auxin indoleacetic acid (IAA) to reverse the PCIB-induced inhibition. Endosperms incubated in basal medium with 30  $\mu$ g of PCIB per milliliter (circle, solid line) or 100  $\mu$ g/ml (cross, broken line) and varying amounts of IAA. Reducing sugars determined after 22 hours at 30°C. In the absence of PCIB there were 60.2 mg of glucose equivalents per gram of endosperm.

the auxins indoleacetic acid, naphthaleneacetic acid, or 2,4-dichlorophenoxyacetic acid. The amount of reducing sugar in the incubation medium was assayed (Fig. 1) by the method of Somogyi (8).

Amylolytic activity was enhanced by concentrations of gibberellic acid in excess of 0.001  $\mu\text{g/ml}$ ; the greatest effect was obtained at 0.1  $\mu\text{g/ml}$ . Indoleacetic acid, at concentrations less than 10  $\mu\text{g/ml}$ , had no effect on this process in either the presence or absence of gibberellic acid; higher concentrations inhibited the induction of amylolytic activity. These results agree with those of Paleg (7) and show that our system is comparable to those studied previously.

The anti-auxin *p*-chlorophenoxyisobutyric acid had no effect on the induction of amylolytic activity at concentrations less than 10  $\mu\text{g/ml}$ , and at higher concentrations it was inhibitory (Fig. 1). Since the amounts of anti-auxin required for inhibition were greater than those required for inhibition of auxin action in other tissues (6), the possibility had to be considered that this inhibition might be due to a toxic effect on the cells rather than to a competitive effect on endogenous auxin. These two types of inhibition can be differentiated on the basis of the response of anti-auxin treated tissues to exogenous auxin. If the inhibition is due to competitive interaction between endogenous auxin and anti-auxin, additional auxin will partially reverse the inhibition; a nonspecific inhibition will not be affected by additional auxin.

Addition of auxin to endosperms that had been treated with anti-auxin was without effect whether the auxin was

indoleacetic acid (Fig. 2), naphthaleneacetic acid, or 2,4-dichlorophenoxyacetic acid (Table 1). In no case was any reversal of the inhibition obtained. Hence the effects of high concentrations of *p*-chlorophenoxyisobutyric acid are not due to antagonism between the anti-auxin and endogenous auxin. It appears that endogenous auxin is not involved in the increase in amylolytic activity induced by gibberellic acid in barley endosperm. In this system the action of gibberellic acid is independent of the presence or absence of auxin.

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## Systematic Relationships between Carbon and Oxygen Isotopes in Carbonates Deposited by Modern Corals and Algae

**Abstract.** Analyses of organic carbonates from Jamaican coral reefs show a positive correlation between the  $O^{18}:O^{16}$  ratio and the  $C^{13}:C^{12}$  ratio in some taxonomic groups of corals and algae, but essentially no correlation (nearly constant  $O^{18}$ ) in one suborder of reef-building corals. The strontium and magnesium contents apparently are controlled mainly by skeletal mineralogy and show no correlation with carbon or oxygen isotopic composition. The observed positive correlations between  $C^{13}$  and  $O^{18}$  content may be due to calcification processes utilizing carbon-oxygen compounds from two isotopically different sources or utilizing selected portions of a wide spectrum of carbon-oxygen compounds in which there is a positive correlation between  $C^{13}:C^{12}$  and  $O^{18}:O^{16}$  ratios. Coral and algal carbonates from Jamaican waters, with an annual temperature range of only about  $4^{\circ}\text{C}$ , exhibit a total  $\delta C^{13}$  range of more than 13 per mil and a  $\delta O^{18}$  range of more than 4 per mil. The wide isotopic variability resulting from vital effects of calcifying organisms must be taken into account in applying isotopic analysis to the study of sedimentary carbonate rocks which may include reef-derived carbonates.

The isotopic and elemental composition of organic marine carbonates is of interest in both biological and geological studies; compositional variations may provide indirect evidence regarding calcification processes and various geological problems, including the origin of limestones (1), ocean paleotemperatures, and some aspects of paleogeography, particularly regarding proximity to ancient shorelines (2) or coral reefs (3). Variations of carbonate isotopic composition due mainly to differences in external environment, from one marine biologic community to another, may be obscured by relatively large differences due to food selectivity and to metabolic isotope fractionation by various calcifying organisms living together in the same environment (see 4).

Organic carbonates which appear to

be deposited out of oxygen isotopic equilibrium with sea water, and whose compositions presumably are strongly affected by vital effects of the organisms, include those precipitated by coelenterates, echinoderms, and some algae (5, 6); they also exhibit wide variability of carbon isotope ratios in contrast with the isotopic composition of mollusk shells, for example, which is relatively constant within one biologic community (7).

The object of this investigation was to discover whether the isotopic and chemical compositions of organic reef carbonates exhibit any systematic relationship within and between taxonomic groups of calcifying organisms from the same general environment. Reef sites in Jamaica were selected for study because they have a wide variety of Caribbean corals and algae and because the shal-

Table 1. Inability of the auxins naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) to reverse inhibition induced by *p*-chlorophenoxyisobutyric acid (PCIB). Endosperms were incubated in basic medium with 100  $\mu\text{g}$  of PCIB per milliliter and concentrations of auxin as indicated. Reducing sugar content of medium, in milligrams of glucose equivalents per gram of endosperm, was determined after 22 hours at  $30^{\circ}\text{C}$ . The control, without PCIB, had a reducing sugar content of 60.2 mg/g.

Auxin concn. ( $\mu\text{g/ml}$ )	Reducing sugar (mg/g)	
	2,4-D	NAA
0	23.5	20.2
0.1	23.2	21.2
1	25.3	21.8
10	20.0	20.8
100	17.7	10.0