diversities than the effluents from unit 1, but the differences are significant only at the 95 percent confidence level.

All the waste water microcosms developed eutrophic conditions, with growths of blue-green algae well in excess of 1000 cell/ml, predominantly *Phormidium* accompanied by two to three other algal genera. The concentrations of algae in the lake water controls remained below 500 cell/ml with the green algae *Staurastrum* and *Ankistrodesmus* often predominating, accompanied by 7 to 12 other species.

On the basis of these results, it is evident that domestic waste water will produce eutrophic conditions in receiving waters. However, the data are not in support of the often stated position that the simple elimination of the phosphates from detergents will significantly decrease the rate of eutrophication caused by the resulting waste waters. Furthermore, the data show that the "ecologically safe," high-alkalinity, high-carbonate detergents offer no improvement.

Substitution of untested detergent formulations of this sort may appear to be an easy way out politically, but there is no indication that this technique will reduce eutrophication. Eutrophication may actually increase as the result of additional alkalinity, which would be still another factor added to our overall pollution problem. A much more effective idea would be the construction of facilities for the removal of all nutrients from waste waters in those areas where algal control is a problem.

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Asymmetry, Its Importance to the Action and Metabolism of Abscisic Acid

Abstract. *Unlabeled and ^{14}C-labeled enantiomorphs of abscisic acid (ABA) were obtained through acetylcellulose chromatography and tested as inducers of abscission, as inhibitors of seed germination, and as antagonists of gibberellic acid-induced synthesis and release of α-amylase. The activity of the* **R** *isomer was either equal to or less than that of the naturally occurring* **S** *form. Greatest differences were in the inhibition of root-related growth. In excised beam axes, although uptake of* **S**-[^{14}C]*ABA is faster, the internal concentration of* **R**-*ABA is higher because of faster conversion of* **S**-*ABA to inactive metabolic products. In axes a reversal in chirality is less important to the physiological action of ABA than to its metabolism.*

Abscisic acid (ABA) has been implicated in the regulation of a host of physiological responses in higher plants (*1*). Apparently the **S** isomer is the only naturally occurring form, but the other enantiomorph can be obtained by resolution of the synthetic racemate (*2*). This makes ABA the only optically active plant hormone for which both enantiomorphs are available. The only record of the relative effectiveness of these enantiomorphs consists of a statement by Milborrow to the effect that **R**- and **S**-ABA are equally active in inhibiting the coleoptile growth of dissected wheat embryos (*3*). We report a new procedure for the resolution of **RS**-ABA, the synthesis of **R**-[2-^{14}C]- and **S**-[2-^{14}C]ABA, and data on the relative effectiveness, uptake, and metabolism of these optically active substances.

RS-ABA can be separated from the accompanying *trans* analog obtained in the synthesis of Roberts *et al.* (*4*) by crystallization from ethyl acetate–toluene (1:3). Resolution is achieved in two steps; enantiomorphic enrichment on an optically active chromatographic column and selective solubilization of the optically active component with a hydrocarbon solvent containing a low percentage of a hydrogen-bonding compound (*5*). Chromatography of 80 mg of **RS**-ABA on a column of acetylcellulose (Woehlm) and elution with 3.3 percent ethanol in toluene in subdued light at 4°C leads to about 5 percent enantiomorphic enrichment. The **S** isomer is eluted first. Extraction of the combined, enriched fractions from several runs with 2.5 percent *n*-butanol in isooctane (by volume), thin-layer chromatography on silica gel GF_{254} with a benzene, ethyl acetate, acetic acid system (50:5:2), to remove accompanying *trans*-ABA, yields crystalline **S**- and **R**-ABA of optical purity comparable to that obtained by Cornforth *et al.* (*2*).

S-[2-^{14}C]ABA and **R**-[2-^{14}C]ABA were synthesized by the same procedure as that used by us for **RS**-[2-^{14}C]-ABA (*6*). **RS**-1-Hydroxy-4-keto-α-ionone (*4*) was partially resolved on acetylcellulose columns with 3 percent ethanol in toluene at 4°C, yielding enriched **S** isomer in the material eluted first. Extraction with 7 percent *n*-butanol in hexane (by volume) yielded oils that

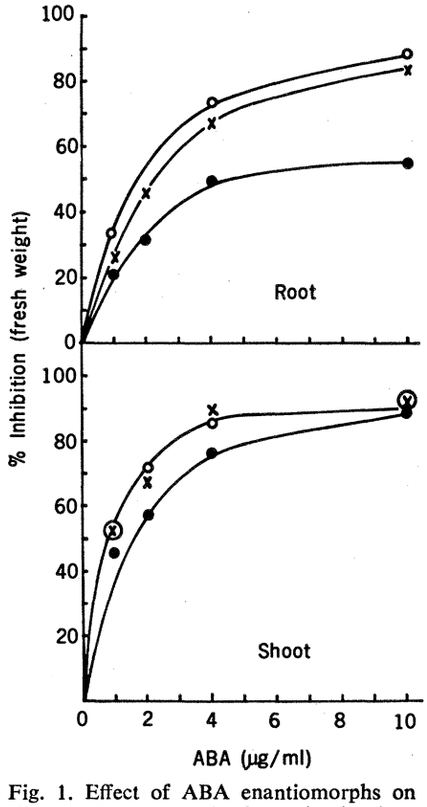

Fig. 1. Effect of ABA enantiomorphs on shoot and root growth of germinating barley seeds, *Hordeum vulgare* (var. Himalaya). The seeds were incubated in the dark at 26° to 27°C for 56 hours in 0.01*M* Hepes buffer *p*H 7.1 with 50 μg of chloramphenicol per milliliter. An average of 20 seeds was used per assay, and germination was close to 100 percent even in the presence of ABA. Shoots and roots were removed from the seeds, and their fresh weight was determined. ○, **S**-ABA; **X**, **RS**-ABA; ●, **R**-ABA.

crystallized after several weeks at 4°C. The **R** isomer, m.p. 107° to 109°C, had a molar extinction coefficient (ε_{238nm}) of 18,700. In the optical rotary dispersion (ORD) curve, the first peak was a minimum with a molecular rotation $[M]_{262nm}$ of $-59{,}000°$; at 249 nm $[M]$ was 0°; the second peak, a maximum, had $[M]_{227nm} = +223{,}000°$. For the **S** isomer $[M]_{263nm} = +53{,}700°$, $[M]_{248nm} = 0°$, and $[M]_{226nm} = -208{,}000°$.

In the germination of barley seeds, the growth of shoots and roots, measured as fresh weight increase, is inhibited more effectively by **S**- than by **R**-ABA (Fig. 1). Both isomers are more effective in the inhibition of shoot growth, but the enantiomorphic differences are greater in roots. Differences in the effectiveness are also observed in the germination of excised embryos of ash, *Fraxinus americana*, in which the first macroscopic sign of germination is stem curvature (*7*). At least three times more **R**-ABA than **S**-ABA is required to inhibit curvature in 50 percent of the embryos. This holds true for nondormant embryos as well as for dormant specimens in which germination was induced with gibberellic acid.

Both ABA enantiomorphs cause similar degrees of inhibition of fresh weight increase of excised bean axes at the end of 12 hours, but between the sixth and seventh hour **R**-ABA is somewhat less effective, Fig. 2. In an abscission assay with cotton explants (*8*) the **R**-ABA and **S**-ABA showed equal abscission-promoting ability in the range of 0.01 to 1.0 μg per petiole. Our resolved ABA samples had the same activity as **RS**-ABA, and **S**-ABA isolated from immature cotton fruit in this assay. No effects due to asymmetry were observed in the interference by ABA in the gibberellic acid-induced synthesis and release of α-amylase in embryo-free barley half seeds (*9*). At concentrations of 10^{-7} and 10^{-6} mole/liter, respectively, the enantiomorphs were tested

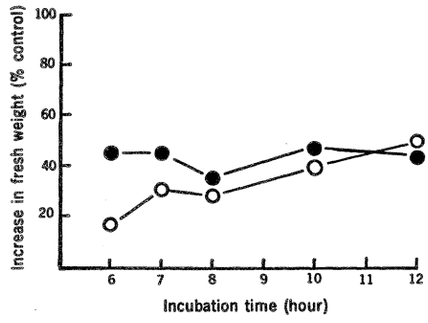

Fig. 2. Effect of ABA enantiomorphs on fresh weight increase of excised bean axes, *Phaseolus vulgaris* L. (var. White Marrowfat). Axes were incubated in a Dubnoff metabolic shaker at 26° to 27°C (*13*), with 100 μg (dry weight) per 2 ml of 0.01*M* Hepe's buffer *p*H 6.0 plus 100 μg of chloramphenicol. ○: 9 μg/2 ml of S-ABA; ●; 9 μg/2 ml of **R**-ABA.

in the presence of $10^{-8}M$ and $10^{-7}M$ gibberellic acid. Inhibitions were between 50 and 80 percent. We also tested the embryo-containing half seeds without exogenous gibberellins. Here too the ABA isomers were equally active. In the assay involving rapid closing of stomata of barley leaves (*9a*), the lag times for the two ABA isomers were similar.

Uptake and metabolism of exogenously added ABA enantiomorphs were studied to determine how they relate to the apparent physiological activity. Radioactive racemic ABA is rapidly metabolized by bean axes to two inactive compounds, designated M-1 and M-2 (*10*). M-1 is present only in very small amounts and is believed to be an intermediate between ABA and M-2. Our studies have now been extended to the radioactive ABA enantiomorphs. The latter were fed to bean axes; the radioactive compounds soluble in 70 percent ethanol were separated on TLC plates and eluted; the radioactivity was then counted. Although **S**-[^{14}C]-ABA leads to about the same growth inhibition as **R**-[^{14}C]ABA, its internal concentration is only about 20 to 25 percent that of **R**-[^{14}C]ABA (Table 1). The total internal radioactivity at 5 and 12 hours is higher when **S**-[^{14}C]ABA is fed. Because no external metabolism of ABA was detected, we assume that all of the radioactivity enters as ABA and conclude that the rate of entry into the axes is higher for **S**- than for **R**-[^{14}C]ABA. **S**-[^{14}C]ABA is also metabolized at a much faster rate. After 5 hours, when only 15 percent of the **R** isomer has been converted to M-2, **S**-[^{14}C]ABA is already 86 percent metabolized. If the two enantiomorphic samples were incompletely resolved, these variations must represent minimum values because the differences for optically pure samples would be even larger. Two explanations for a faster uptake rate of **S**-ABA can be suggested: (i) stereoselective uptake in favor of the **S** isomer or (ii) passive, nonstereoselective uptake favoring **S**-ABA because of its faster rate of conversion to M-2.

Since **RS**-ABA at high concentrations inhibits the fresh weight increase by 80 to 90 percent (*11*), the growth data in Table 1 cannot represent saturation values for both enantiomorphs. We first suspected that at low concentrations the physiological activity of the ABA enantiomorphs would be proportional to their internal concentrations and on this basis considered the **S** isomer to be the more effective substance. However, there are indications that even under nonsaturating conditions, growth inhibitions may not be directly related to the total internal amounts of ABA in excised bean axes. From Fig. 2 it is seen that the percent increase of inhibition of fresh weight shows little variation with time when **R**-ABA is fed and may even decrease with **S**-ABA. Yet the internal concentrations of both enantiomorphs increase during this interval (Table 1). Also, in experiments where the bean axes labeled with **RS**-[2-^{14}C]ABA were removed to buffer solution extremely rapid growth recovery rates were seen without concomitant depletion of internal ABA (*10*). This could mean that ABA is being stored, some of it being available and some not. We have also considered the possibility that the lack of correspondence between the percent of growth inhibition of bean axes and the internal ABA concentration is attributable to the formation of compounds that are antagonistic to ABA activity.

Table 1. Growth inhibitions and metabolism of **R**-[2-^{14}C]- and **S**-[2-^{14}C]ABA in bean axes. Axes (100-mg quantities) were incubated with 2 ml of 0.01*M* Hepes, *p*H 6.0, containing 100 μg of chloramphenicol, and [2-^{14}C]ABA (294,000 count/min) (calculated as 10 μg from a specific activity of 4 mc/mmole) at 26° to 27°C.

Form	Growth inhibition at 12 hours (%)	Internal concentration (count/min)					
		5 hours*			12 hours*		
		ABA	M-2	Total	ABA	M-2	Total
R	56	10,000	1,800	12,100	28,300	7,500	36,900
S	63	1,850	13,800	16,100	7,200	54,000	63,100

* M-1 accounted only for approximately 3 percent of the total radioactivity in the axes and was not determined in this experiment.

Since the ratio of S-[^{14}C]ABA to M-2 is constant between the fifth and twelfth hour, the latter compound would be a good candidate for such a role. However, purified M-2, obtained from axes fed RS-[^{14}C]ABA, although taken up by the tissues did not change the growth inhibition by RS-ABA when added to axes at six times the concentration of RS-ABA (*10*).

Participation of a racemase has not been ruled out. However, the fact that ABA asymmetry affects growth inhibition and metabolism to different extents decreases the probability that racemization is involved.

From our results it is seen that, depending on the assay system, either the overall effectiveness of the two ABA enantiomorphs is the same, or the R isomer is less active. Up to now the largest physiological differences were in the inhibition of root-related growth phenomena. It can also be concluded that certain other structural features, such as a *trans, trans* side chain (*1*), the absence of a free carboxylic acid or a 4′-keto group (*12*), are more deactivating than a change from the S configuration. We conclude that at the degree of resolution achieved here, a change in asymmetry has a more pronounced effect on the metabolic than on the hormonal reactivity of ABA.

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14. We thank F. T. Addicott for evaluating the ABA enantiomorphs in the abscission assay and for allowing us to include these results in this report. We thank W. R. Cummins for testing the effect of S-ABA and R-ABA on the closing of stomata. Supported in part by grants GB 27434 and 29428 from the National Science Foundation.

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Mutants with Abnormal Visual Pathways: An Explanation of Anomalous Geniculate Laminae

Abstract. *Rats with geniculate laminae that run perpendicular to the lines of projection are described. Earlier reports of laminae parallel to the lines of projection were based on mammalian mutants that may be relatively common. In these mutants, the chiasmatic course of axons arising in a patch of retinal ganglion cells is wrongly specified.*

The dorsal lateral geniculate nucleus in mammals receives an orderly projection from the retina (*1, 2*) and this projection is organized so that single points in the visual field can be represented as lines, the "lines of projection" (*3*), which pass more or less parallel to each other through the nucleus. In species that have a well-defined binocular part of the visual field, such as cat or macaque, the lateral geniculate nucleus is laminated and one set of laminae receives afferents from the ipsilateral eye, the other from the contralateral eye (*1, 2*). The laminae are arranged so that the two representations of the binocular portion of the visual field are in register, and the lines of projection thus pass perpendicular to the laminae (Fig. 1). In this way, the parts of the nucleus that receive afferents from homonymous points of the two retinae lie adjacent to each other, and significant binocular interaction can occur within the nucleus (*4*).

One might expect that in any species with binocular overlap and a laminated lateral geniculate nucleus the lines of projection would pass perpendicular to the laminae. Any other arrangement would suggest a patchy representation of the visual field in the nucleus and could not easily produce the known orderly representation of the visual field upon the cerebral cortex (*5*). However, as shown in Fig. 2, there is some evidence that in rats and rabbits the terminations of ipsilateral retinogeniculate axons form laminae parallel to the lines of projection. This is a puzzling situation. It appears to suggest either that some of the original observations are wrong or that the central visual pathways in these two species are arranged in an unusual and at present quite incomprehensible manner. The evidence summarized below suggests a third interpretation.

Lund (*6*) and Giolli and Guthrie (*7*) have shown that certain strains of albino rats and rabbits have significantly fewer ipsilateral retinogeniculate axons than do other, normally pigmented strains. However, the manner in which the reduced ipsilateral projection is organized has not been determined, so that it is not known whether the line of decussation lies further temporally in the retina or whether the reduction is produced in some other way. Siamese cats also have a reduced ipsilateral projection (*8*) and the pattern of this projection has been determined (*2*). In these cats there is a patch in the middle of the temporal hemiretina, and ganglion cells within this patch send their axons contralaterally instead of ipsilaterally as in normal cats. As a result of this chiasmatic misrouting of a limited group of axons, the laminae that receive an ipsilateral input are disrupted, and one sees discontinuous ipsilateral terminal zones in the place of a single continuous, normal lamina (Fig. 3).

We suggest that the reduced ipsilateral projection seen in some rats and rabbits is produced exactly as in