THE INTERACTION BETWEEN AUXIN AND ETHYLENE AND ITS ROLE IN PLANT GROWTH*

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Auxin induces ethylene formation in several plants[1, 2] and in segments cut from roots, stems, and fruits.[3] If ethylene alters the metabolism of auxin, as has been suggested,[2, 4, 5] then this auxin-induced ethylene formation might interfere with studies of the uptake, transport, and destruction of the applied growth hormone. Moreover, the newly synthesized ethylene would mask or modify a response to applied gas and thus complicate any investigation of the effect of ethylene on auxin metabolism. It will be shown that all of these problems arise under the conditions commonly used to study auxin metabolism, and the significance of this finding will be discussed with special reference to a postulated mechanism of ethylene action.

Materials.—Pea seeds (*Pisum sativum var. Alaska*) were soaked for 5 hr and planted in vermiculite if the seedlings were to be etiolated, or in potting soil if light-grown. The plants were kept at 23 ± 3°C either in darkness with occasional dim red light for 7 days, or on a 16-hr light cycle under Gro-lights for 14 days. Sections were then cut from the apical part of the uppermost internode. Other seedlings were planted in the same manner as etiolated pea plants, except that corn (*Zea mays var. Barbecue*) was sterilized in 10% Clorox for 10 min and rinsed for 3 hr before it was sown. Oat (*Avena sativa L. var. Victory*) and corn coleoptiles were harvested at 80 hr, sunflower (*Helianthus annus L.*) after 5 days, and sections were cut 2–3 mm below the cotyledons or tip.

Methods.—Straight growth test: Groups of ten 10-mm sections were placed in 10 ml of solution contained in 125-ml flasks. The medium typically consisted of 10^{-6} *M* indole-3-acetate (IAA), 2% sucrose, 5×10^{-2} *M* potassium phosphate buffer (pH 6.8), and 5×10^{-6} *M* $CoCl_2$ in glass-distilled water. To add ethylene, the flasks were sealed with vaccine caps and a known quantity of a calibrated ethylene-air mixture was injected. The flasks were gently shaken in the dark at 23°C, and at various times the fresh weight and length of the sections were determined. In some studies the tissue was shadowgraphed with green light, and stem curvature measured from the film with a protractor. Ethylene production was estimated by removing samples of air from the flasks and gas-chromatographing them on an alumina column.[6]

Transport and destruction of C^{14}-IAA: Polar transport through 7-mm stem sections was determined by applying apical donor blocks containing carboxyl-labeled C^{14}-IAA (8mC/mM) in 3% agar, and 1.5% agar receiver blocks. After 2 hr the receiver blocks were remelted, dried on a grooved planchette, and counted with a 30% efficient automatic gas-flow counter; the radioactivity remaining in the stem segments was extracted by crushing them on a planchette with 1 ml of 50% ethanol. Plating a homogeneous suspension of duplicate tissue at less than 2 mg/cm^2 resulted in the same count, so it was concluded that self-absorption was not a complication. All transport experiments were carried out in sealed desiccators at 100% relative humidity, and enough ethylene was added to bring the concentration to 100 ppm. Polar transport was also studied indirectly by incubating 15-mm pea stem sections in media containing 10^{-6} *M* C^{14}-IAA (8mC/mM). At various time intervals the sections were rinsed, cut into 5 equal lengths, and the radioactivity per mg dry weight was determined for each. The destruction of auxin which occurred during the incubation was appraised by counting the $C^{14}O_2$ released. Air samples removed from the flasks were passed through a dry-ice freezing trap into an evacuated 250-ml Cary-Tolbert ionization chamber, and radioactivity was determined by the rate of charge method using a Cary electrometer. When tissue was omitted from the flasks, 98% less volatile radioactivity was produced in 24 hr, and when samples of air from around the tissue were pretreated with 5% KOH, all counts were removed, indicating that they were due solely to $C^{14}O_2$ evolved by the stem sections. For lateral transport studies ten 15-mm corn coleoptile sections were mounted horizontally with their vascular bundles oriented in a vertical plane, a donor block containing C^{14}-IAA

was applied apically, and a receiver block at the base.[7] After 135 min each section was marked on its upper side, the apical 5 mm discarded, and the next 5 mm split into upper and lower halves whose radioactivity per mg dry weight was determined. The receiver block was also counted. The method for pea tissue was similar, except that twenty 7-mm sections were used, the apical 2 mm discarded, and usually the segments were split into upper, middle, and lower zones (representing 25.1 ± 2.6, 49.3 ± 6.3, and 25.5 ± 2.4% of the dry weight, respectively) instead of upper and lower halves. Curvature was determined with a protractor after shadowgraphing 15-mm sections at the end of the same transport period.

Results and Discussion.—Auxin-induced ethylene formation in pea stem sections: Pea stem sections elongate best when they are supplied with 10^{-6} *M* IAA, whereas their fresh weight continues to increase until ten times that level of growth hormone is applied (*8*, Fig. 1). Those concentrations of IAA which inhibit elongation also

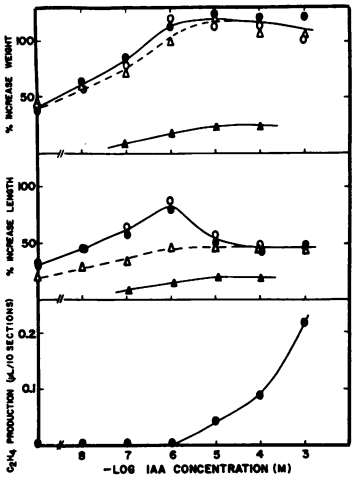

FIG. 1.—*Upper:* Effects of IAA and C_2H_4 on the increase in fresh weight of etiolated pea stem sections. After 3 hr, control in sealed flasks (▲—▲). After 18 hr, control in Petri dishes (○—○); control in sealed flasks (●—●); and treated with 10 ppm C_2H_4 (△---△). *Middle:* Effect of IAA and C_2H_4 on elongation. Symbols same as above. *Lower:* C_2H_4 production during 18 hr. All flasks contained 2% sucrose, 5×10^{-2} *M* potassium phosphate buffer (pH 6.8), and 5×10^{-2} *M* $CoCl_2$.

stimulate the tissue to produce ethylene (Figs. 1 and 2), and since there is a close correlation between the intensity of ethylene production and the extent of the inhibition, it is natural to wonder whether ethylene might be contributing to the auxin response. To answer this question, sections were incubated in various concentrations of auxin in the presence and absence of applied ethylene, so that the effects of auxin and ethylene could be compared. Using 10^{-6} *M* IAA (the highest concentration which does not stimulate ethylene formation), it was found that elongation is markedly inhibited by application of ethylene, whereas the increase in fresh weight is only slightly retarded (Fig. 3). These changes are caused by an isodiametric expansion of cells in the cortical region of gassed tissue in contrast to their normal longitudinal mode of expansion.[9] High concentrations of auxin cause the same effect, however (Fig. 1), and the resultant tissue is morphologically and cytologically indistinguishable from that gassed with ethylene.[5] This suggests that the ethylene might be the cause of the swelling and inhibition of extension growth heretofore attributed to high levels of auxin, and in order to ex-

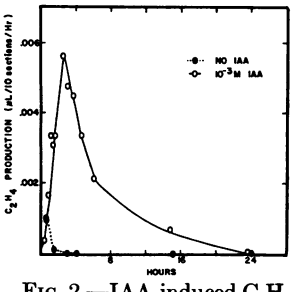

FIG. 2.—IAA-induced C_2H_4 formation in etiolated pea stem sections. Medium is described in legend to Fig. 1. A detectable acceleration in ethylene formation also occurs within a few hours after 10^{-4} or 10^{-5} *M* IAA is applied.

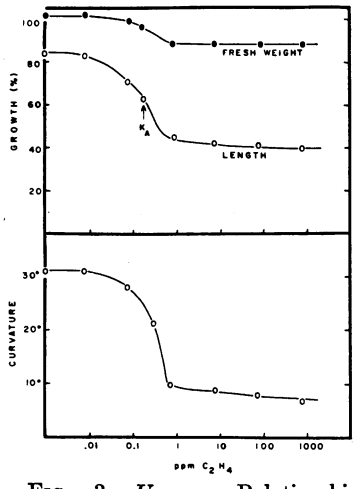

FIG. 3.—*Upper:* Relationship between % increase in fresh weight, % elongation, and the concentration of C_2H_4 present during an 18-hr experiment with etiolated pea stem sections. Flasks contained 10^{-6} *M* IAA and the medium described in the legend to Fig. 1. K_A is the concentration of C_2H_4 causing a half-maximal effect. *Lower:* Curvature in the same stem segments after 2.5 hr have elapsed. The initial random curvature of controls was 8°.

amine this possibility three types of experiments were devised in which the effect of endogenous ethylene was minimized or eliminated. Each showed that auxin alone does not inhibit the growth of pea tissue: (1) Because ethylene has very little effect on the final fresh weight of growing pea stem sections (Fig. 3), if the fresh weight were to decrease at a high IAA concentration, this would have to be due to auxin action *per se*. No such decrease occurs (*8*, Fig. 1); instead, the fresh weight reaches its maximum value at 10^{-5} *M* IAA and is little changed at higher auxin levels. (2) After auxin application, endogenous ethylene cannot affect the growth of pea stem sections for at least 4 hr because ethylene formation is not stimulated for 1 hr (Fig. 2) and the tissue does not respond to applied gas for 3 hr (Fig. 4). Measurements taken at 3 hr (Fig. 1) show that the rate of growth, evaluated either as increase in fresh weight or length, approaches a maximum at 10^{-5} *M* IAA with no decline at higher levels of auxin. (3) When pea stem segments are treated with 10 ppm ethylene, their rate of elongation is reduced by 50 per cent, and additional ethylene has no further effect (Fig. 3). Under these conditions, ethylene produced in response to auxin would also be without effect; hence, an inhibition due to auxin could be readily discerned. No such inhibition occurs when the IAA concentration is raised; instead, extension growth saturates at 10^{-5} *M* IAA (Fig. 1) just as in the two previous examples. Thus, three different approaches yield the same conclusion; only when auxin induces ethylene formation and the gas has had sufficient time to act, does IAA lead indirectly to an inhibition of elongation. It is important to note that ethylene does not have to accumulate in the ambient atmosphere for tissue to be inhibited by the gas which it produces. This is proved by the fact that the dependence of growth upon IAA concentration is the same regardless of whether pea stem sections are incubated in sealed flasks or in Petri dishes from which ethylene readily escapes (cf. open vs. closed circles, Fig. 1).

Does ethylene mediate the entire response to a high concentration of auxin? If so, the ethylene present within the tissue at each concentration of auxin should quantitatively account for the inhibition of elongation which results. Stem segments containing more than 1 ppm ethylene cannot be affected by added ethylene because they must already have responded maximally to their endogenous gas (Fig. 3). Therefore, 1 ppm ethylene must be present within sections growing in just over 10^{-5} *M* IAA, for this is the lowest concentration of auxin which renders tissue completely unresponsive to applied gas (Fig. 1). But a concentration of 1 ppm ethylene ought to bring about a 50 per cent reduction in the rate of elongation, and if this is exactly the change caused by application of 10^{-5} *M* or more auxin, it may be concluded that ethylene is responsible for the entire inhibition heretofore

ascribed to auxin. Application of $>10^{-5}$ M IAA does cause a 50 per cent decline in the rate of elongation (Fig. 1): when the auxin concentration is increased to 2×10^{-6} M or higher, elongation is progressively retarded until a maximum inhibition of 50 per cent results at an IAA concentration just in excess of 10^{-5} M (Fig. 1). Since the final length of pea stem sections would have been almost the same at 10^{-6} M IAA and all higher concentrations if there were no effect from endogenous ethylene (see results at 3 hr, Fig. 1), it is clear that *ethylene alone* is responsible for the inhibition of elongation which occurs at "supraoptimal" concentrations of auxin. By an analogous argument it can be shown that the swelling which accompanies this inhibition is also due solely to ethylene. At low IAA concentrations the ratio between per cent increase in fresh weight and per cent increase in length is constant at an average value of 1.3, whereas at auxin concentrations $>10^{-5}$ M the tissue swells and the ratio changes to 2.6. Regardless of the IAA concentration, when 1 ppm ethylene is applied, the ratio is always 2.6, and therefore it is clear that ethylene, not auxin, causes the swelling.

Auxin-induced ethylene formation in other tissues: The entire growth inhibition in etiolated *sunflower* stem sections at a high concentration of IAA is due to auxin-induced ethylene formation. The argument which proves this is similar to that advanced for peas, except that 10 ppm ethylene is required to inhibit the growth of sunflower maximally (about 30%), and as a result this tissue does not become insensitive to applied gas until 10^{-3} M IAA is present and ethylene evolved at a relatively high rate. Added ethylene inhibits elongation and the increase in fresh weight to almost the same extent, and this explains why high concentrations of auxin inhibit both weight and length in sunflower. The growth of *light-grown pea plants* does not saturate at any applicable concentration of IAA.[8] In part, this is because they are considerably less responsive to ethylene than etiolated seedlings and produce the gas at a slower rate in the presence of 10^{-5} or 10^{-4} M IAA. As a result, even though green tissue swells when it responds to its own ethylene, the inhibition of elongation due to auxin-induced ethylene formation is never sufficient to offset the auxin-induced increase in length. Auxin stimulates *corn* and *oat* coleoptiles to evolve ethylene, but the production does not continually increase as the IAA concentration is raised. Instead, ethylene formation and growth reach their highest rates at 10^{-5} M IAA, and then both decline as the auxin concentration is increased further. Only 1 ppm ethylene is needed to inhibit the growth of corn and oat coleoptiles maximally, and in both cases length and weight decline in unison by about 40 per cent. Since corn produces large quantities of ethylene and becomes insensitive to applied gas when the concentration of IAA is raised to 10^{-6} M, the tissue must respond completely to its endogenous ethylene at that and higher auxin levels. However, the growth inhibition at high auxin levels is not due to ethylene action, for ethylene production *decreases* as this inhibition sets in, and the response to applied ethylene is not large enough to account for the precipitous decline in growth which occurs at $>10^{-4}$ M IAA. The situation is similar in *Avena*. In fact it is unlikely that *Avena* coleoptiles ever respond substantially to the ethylene they produce, for even when 10^{-5} M IAA is present and ethylene formed most rapidly, the rate of evolution is so low that the tissue still reacts to applied gas. It must be concluded that auxin-induced growth inhibitions occur for basically different reasons in coleoptiles and seedlings such as sunflower and peas.

Effect of ethylene on auxin synthesis: Within a few hours after ethylene is applied to *Vicia faba*,[4] pea,[5] and *Avena* seedlings,[4, 5, 10] the amount of diffusible auxin declines. Three explanations for this phenomenon may be envisioned: ethylene might enhance the destruction of auxin, inhibit its polar transport, and/or reduce its rate of synthesis. Ethylene did not stimulate pea sections to release more $C^{14}O_2$ from carboxyl-labeled IAA at any time during a 24-hr period. These studies were carried out using a medium containing 10^{-6} *M* IAA since greater amounts of auxin stimulate ethylene formation and make it impossible to determine what the rate of destruction would have been in the absence of the gas. When 15-mm pea stem segments were incubated in this medium, addition of ethylene did not alter the total uptake of auxin or its distribution along the segments. Since radioactivity entered through both ends and distributed as though it were being pumped from apex to base, it would appear that ethylene has no effect on polar auxin transport. Studies using donor and receiver blocks substantiate this conclusion by showing that application of ethylene does not change auxin uptake or the velocity and capacity of the polar transport system in etiolated pea, corn, or sunflower stem sections during a 3-hr period. Similar results have been reported for *Avena* and pea tissue.[4, 5, 10] Since ethylene has no effect on either the destruction or polar transport of IAA in pea stem sections, it must be concluded that less diffusible auxin is present in gassed tissue because the rate of auxin synthesis is lowered. This result, coupled with the studies on auxin-induced ethylene formation, suggests the possibility of a feedback mechanism in which each hormone controls the other's rate of synthesis. (See *Note added in proof.*)

Effect of ethylene on tropisms and lateral auxin transport: When pea seedlings are placed on their sides, the stem starts to curve upward within 2 hr and the roots downward within 3–4 hr, whereas if ethylene is present, both continue to grow in a horizontal direction. Many plants react similarly,[11, 12] but ethylene does not prevent geotropic curving of *Avena* or corn coleoptiles. Pea stem segments afford a convenient system in which to study the influence of ethylene on geotropic sensitivity. Not only does the gas prevent them from curving in response to gravity (Fig. 5), but in addition it inhibits a spontaneous curvature from developing when the sections are incubated in liquid medium (Figs. 3 and 6). If 10^{-6} *M* IAA is present, pea tissue begins to curve immediately, even though the flasks are con-

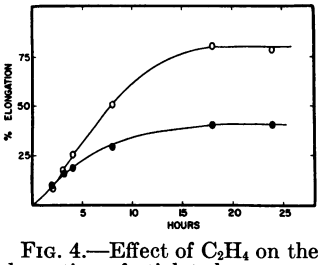

FIG. 4.—Effect of C_2H_4 on the elongation of etiolated pea stem segments. Control (○—○) and 5 ppm C_2H_4 (●—●). Flasks contained 10^{-6} *M* IAA and the medium described in the legend to Fig. 1.

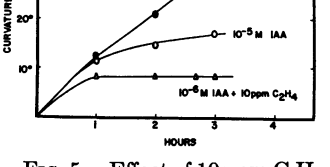

FIG. 5.—Effect of 10 ppm C_2H_4 on the geotropic curvature of 15-mm etiolated pea stem sections. Tissue was mounted horizontally with an apical donor block containing IAA, and a basal receiver block. Similar results were obtained with sunflower.

tinuously shaken; the curvature reaches a maximum within 3 hr, and thereafter straightening gradually occurs (Fig. 6). If IAA is omitted (or growth inhibited by azide, iodoacetate, etc.), the curvature develops more slowly, and when ethylene is present, the bending never takes place. This apparently spontaneous tropism may be analogous to a stem circumnutation which pea and other seedlings exhibit under certain conditions,[12, 13] and this also ceases shortly after ethylene is applied.[12] Curvature development and elongation are inhibited in perfect unison as the ethylene concentration is raised (Fig. 3), suggesting that inhibition of both spontaneous and geotropic curvatures might be due to the effect of ethylene on elongation, but this cannot be the case because curvature is arrested by ethylene several hours before the gas begins to slow the rate of elongation (cf. Figs. 4, 5, and 6). For this reason the effect of ethylene on lateral auxin transport was investigated. Using 10^{-6} *M* IAA in the donor block (Table 1), it was found that after 135 min the specific activity of the upper quarter of the sections was 28.4 per cent that of the lower quarter. (This method is preferable to the usual procedure of comparing upper and lower halves because, unlike coleoptiles, solid sections contain most of their tissue mass in the center of the stem where the auxin gradient must be very slight. As a result, after 135 min the specific activity of the upper half of pea sections was 37.5 ± 2.7 per cent that of the lower half, a much smaller differential than that obtained in experiments with quarters.) When ethylene is present, the uptake and polar transport of auxin are not affected, but lateral transport is almost completely abolished (Table 1), indicating that lateral and polar auxin transport are mediated by completely separate systems. This result agrees with that of van der Laan[4] who found that auxin did not redistribute laterally in the stems of *Vicia faba* seedlings which had been grown in the presence of ethylene until they experienced a horizontal nutation. It may be concluded that ethylene causes ageotropism and horizontal nutation by inhibiting lateral transport of auxin and, in addition, we should like to suggest (as did Borgström[14]) that all tropistic responses to ethylene are mediated by the same mechanism. Many of these responses to ethylene are

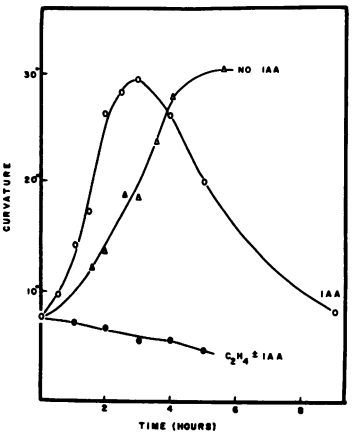

FIG. 6.—Effect of C_2H_4 on the development of a spontaneous curvature in 10-mm etiolated pea stem sections. Flasks contained the medium described in the legend to Fig. 1, supplemented as indicated with 10 ppm C_2H_4 and/or 10^{-6} *M* IAA. Tissue was continuously shaken. Similar results were obtained with sunflower stem sections.

TABLE 1

EFFECT OF ETHYLENE ON THE LATERAL TRANSPORT OF C^{14}-IAA IN PEA STEM SECTIONS

	10^{-6} *M* IAA	10^{-6} *M* IAA + 100 ppm C_2H_4	10^{-5} *M* IAA
Uptake (cpm/mg in intact segment)	24.6 ± 4.6	22.8 ± 6.4	191 ± 17
Relative specific activity*			
Upper	0.58 ± 0.07	0.91 ± 0.08	0.79 ± 0.13
Middle	0.99 ± 0.07	1.0 ± 0.07	1.05 ± 0.02
Lower	1.46 ± 0.10	1.11 ± 0.13	1.10 ± 0.02
Polar transport (cpm/10 sections)	6.1 ± 1.4	5.3 ± 1.0	60.0 ± 4.9

* Specific activity of each zone relative to that of the intact segment (average of 8 expts.).

duplicated by applying high concentrations of growth hormone. Auxin must accomplish this by stimulating ethylene formation, and if this is true, pea sections should not carry out lateral transport or curve in response to gravity if too much auxin is present in the donor block. When the concentration of IAA is raised from 10^{-6} to 10^{-5} M, uptake and polar transport are increased tenfold but, as expected, lateral transport and curvature are markedly inhibited (Table 1 and Fig. 5). An attempt to show that ethylene inhibits lateral auxin transport in corn coleoptiles gave negative results. Ethylene did not affect the uptake of auxin, using 10^{-6} or 10^{-5} M in the donor block, and it did not change the distribution of IAA in the tissue. It may be argued that these experiments are not conclusive because corn coleoptiles produce so much ethylene in the presence of 10^{-6} M IAA that they cannot respond to applied gas. This is a valid objection, but the studies with corn do show that ethylene could cause very little inhibition of lateral transport since a large auxin gradient arises in spite of the presence of the gas. Similarly, van der Laan[4] found that ethylene does not alter lateral auxin transport in the *Avena* coleoptile, and his experiment cannot be complicated by auxin-induced ethylene formation since *Avena* never produces so much ethylene that it fails to react to applied gas. These studies add further support to the proposal that all tropistic responses to ethylene are due to an inhibition of lateral auxin transport, for in corn and *Avena* coleoptiles where ethylene does not inhibit lateral transport, it does not produce any known tropistic effect.

Summary.—IAA induces ethylene formation in a variety of stem sections. The ethylene in turn causes the swelling and inhibition of extension growth in pea tissue, and the inhibition of growth in sunflower sections, which heretofore has been attributed directly to a high concentration of auxin. In pea stem sections ethylene leads to an immediate cessation of lateral auxin transport which is the basis for all tropistic responses to the gas.

Note added in proof: A paper in press [Morgan, P. W., and H. W. Gausman, *Plant Physiol.* (1966)] reports that polar auxin transport is markedly inhibited in stem sections cut from cotton or cowpea plants which have been fumigated with ethylene. We find that etiolated pea seedlings behave similarly; both the velocity and capacity of the polar transport system are reduced by 25% between the 4th and 5th hour after ethylene is applied to pea plants. When pregassing is continued for 18 hr, the inhibition of transport becomes almost complete, whereas auxin uptake is not affected. Since experiments cited in the present communication and elsewhere[4, 5, 10] have proved that ethylene is not a direct inhibitor of polar transport, the new findings indicate that the gas either prevents the development of the polar transport system in apical cells or else it causes a slow deterioration of the existing system. This might account for the loss of diffusible auxin which occurs within a few hours after seedlings are treated with ethylene,[4, 5, 10] and if so, it would not be necessary to postulate an effect of ethylene on auxin synthesis.

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*MUTATION WITHOUT SEGREGATION IN BACTERIA WITH REDUCED DARK REPAIR ABILITY**

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In a series of experiments in which acridine orange was added to glucose-limited continuous (chemostat) cultures of *Escherichia coli* B/r/1,try$^-$ and the cultures were then exposed so briefly to visible light that there was little or no cell death, newly formed mutant cells failed to show a segregational division.[1] The cells in these cultures appeared to be uninucleate, with the minimal value of DNA expected for a replicating genome. By analogy with the results of Ritchie, who used a similar acridine, proflavine,[2] photodynamic mutations induced in the presence of acridine orange appear to be of the base-analogue type, i.e., affecting only one base of a pair. Thus, the absence of segregational division in newly formed mutants led to the suggestion that mutation of a single base in the double-stranded DNA of a parental cell is sufficient to produce completely mutant progeny.[1]

Objections to this view have been based on the possibility of the presence of "repair" enzymes that could transfer the information from the mutated locus to the complementary strand before cell division. If such repair processes occur, the apparent absence of segregation would be the result of mutation of both strands. However, others have reported that lesions induced with acridine orange and light have consistently failed to be repaired under conditions where repair was possible for lesions induced with other agents.[3, 4] Our earlier studies also support the absence of repair in cells in which mutation to T5-resistance was induced with any of several different kinds of mutagens in glucose-limited cultures of *E. coli;* within experimental error, all of the progeny of each latent mutant expressed the mutant character.[5, 6]