our experience that diploids homozygous for mating type, arising from the segregation of triploids and tetraploids (*6*), fail to sporulate and would correspond quite well to this feature of the "illegitimate" diploid. Thus, part of the difference between the views of Winge and Roberts (*7*) and Lindegren (*5*) on "legitimate" and "illegitimate" diploids may be due to studies of fundamentally different material—namely, diploids that had arisen from mutation of mating type as opposed to diploids that had arisen through a mating of haploids of like mating type.

The present data provide direct evidence that mutation of mating type occurs.[3] With our material, the frequency of such spontaneous mutation appears to be too low to affect significantly segregation data.

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Manuscript received September 2, 1952.

A Naturally Occurring Antiauxin[1]

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Three crystalline substances have been separated from the extract obtained from flowering plants some five years ago (*1*). Two of these were isolated by high vacuum distillation at 125° C and fractional crystallization using ether and chloroform as solvents.[2] A third substance has been recovered from the distillation residue, with warm chloroform. This crystalline fraction has been found to have the characteristic of inhibiting callus formation induced by naturally occurring or applied auxin on wound surfaces. This callus-inhibiting property is demonstrated by placing ¼–½ mg of crystals of the substances in a longitudinal slit made in the third and fourth internodes from the tips of cocklebur plants (*Xanthium* sp. in Wisconsin). Callusing is encouraged by binding the slit stems with moist sphagnum moss. Callus tissue does not form when the crystalline material is present. Extracts from alfalfa, avocado, barley, cocklebur, and Sudan grass have given callus-inhibiting results similar to that of the crystalline material from oats used in the tests reported here.

A second test of the antiauxin property of the crystalline material was made by measuring its effect upon inhibiting epinasty induced by indolacetic acid. A test is made as follows: 0.8–0.9 mg of the crystals (originally extracted from oats in the boot stage) were placed in logitudinal slits in the sixth internode from the base of 12 weeks' old cocklebur plants. Control plants without crystals were also slit. Three to 10 days after the time of applying crystals various lots of plants were sprayed with several concentrations of indolacetic acid. Plants that had been previously implanted with crystals developed less epinasty (Fig. 1). The amount of epinasty was determined by measuring the angle between the stem axis and a line extending from along the petiole where it attaches to the leaf blade and subtracting from this value the averaged angles of leaves of the same ages on untreated plants. The mature leaves at the fifth to eighth nodes from the tip of the stem were used in determining the degrees of epinasty. The averaged results of four experiments are shown in Fig. 2. Two to 4 plants for each concentration of indolacetic acid were used in each experiment.

The reason for using dry crystals in the slit plants instead of a solution that would give a more quantitative measurement is that the crystalline substance is not soluble in solvents that are practicable for use with plants. It was determined that cocklebur plants become "saturated" at a low level of active extract: triple dosages of crystals applied by using 3 slits/plant gave no more inhibition of auxin activity than a single dose.

Additional effects from the placing of crystals in the plants were a reduction in the stimulation of adventitious roots characteristic of auxin and also a normal development of shoots on topped plants, instead of the reduced growth typical of plants treated with indolacetic acid applied in lanolin.

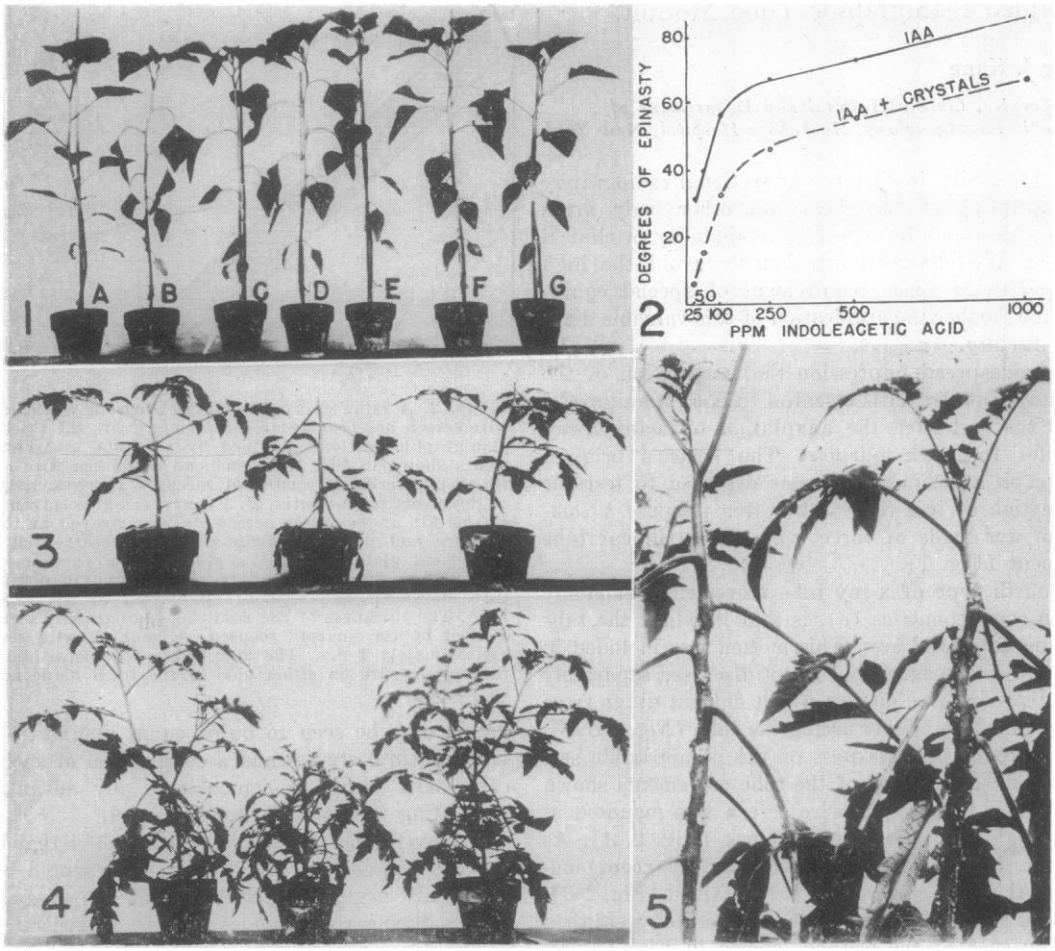
Similar demonstrations of the antiauxin property of the crystalline material were obtained when tomato, *Lycopersicon esculentum* var. Bonny Best, was used as a test plant. Three plants were used in each treatment in triplicated experiments. Crystals were placed in slits in the fourth internode from the base of plants prior to treatment with indolacetic acid either as a water spray of 100 ppm or 1% in lanolin paste. The presence of crystals reduced epinasty by an average of 61% (Fig. 3): reduced adventitious rooting from an average of 86 to 53 roots/plant, and gave near normal suckering of topped plants treated with indolacetic acid (Fig. 4). The presence of crystals in the plants also inhibited the increase in diameter resulting from an application of indolacetic acid. Untreated plants averaged 6.5 mm in diameter, treated plants averaged 9.1 mm, and treated plants with implanted crystals averaged 7.0 mm.

To provide another antiauxin test, tomato plants were inoculated with the crown gall organism *Agro-*

[1] Published by permission of the director of the Agricultural Experiment Station.

[2] Karl Weinke in the laboratory of Mark Stahmann, Department of Biochemistry.

[3] After this paper had been accepted for publication, a paper by M. Ahmed (*Nature*, **170**, 546 [1952]) appeared describing an independent demonstration of mutation of mating type in *S. cerevisiae*.



FIGS. 1–5. Fig. 1, *Xanthium* epinasty. *A*, untreated; *B*, 1000 ppm indolacetic acid; *C*, same as *B* with crystals; *D*, 250 ppm; *E*, same as *D* with crystals; *F*, 100 ppm; *G*, same as *F* with crystals. Fig. 2, *Xanthium*. Graph showing inhibiting effect of crystal injections on epinasty caused by indolacetic acid. Fig. 3, topped tomato var. Bonny Best. Left, untreated; center, 1% IAA in lanolin on cut end (note epinasty); right, same but with crystal injection (epinasty inhibited). Fig. 4, plants similar to Fig. 3, but 18 days later; note shoot inhibition (center) and near normal shoot length where crystals were used (right). Fig. 5, tomato inoculated with the crown gall organism. Right, no crystals used. Note larger galls, marked epinasty, numerous adventitious root initials, and inhibition of stem elongation. Left, inoculated plant but with crystals; auxin effects much inhibited.

bacterium tumefaciens (Smith and Town) Conn, strain A6.[3] Plants infected with this bacterium developed large galls, strong leaf epinasty, numerous adventitious roots, and marked cambial activity and made less terminal growth. All the responses, which are typical auxin effects, were related to the high auxin level that arises as the galls develop (*2*). On plants treated with crystals prior to inoculation with the bacteria, early growth of the galls was slower, and leaf epinasty, adventitious rooting, and cambial activity were much inhibited. Nearly normal terminal growth was also made by treated plants (Fig. 5).

The formative effects typical of 2,4-dichlorophenoxyacetic acid, 25–50 ppm, on cocklebur are also inhibited by previous implantation with antiauxin crystals. This reduction in effect of 2,4-D is comparable to the reduced distortion produced by this chemical on plants when they are in flower (*3*).

A record of the effects that active crystals have upon the maturation of the excess tissue produced by stems treated with auxins will be reported elsewhere.

Obviously the warm chloroform-soluble fraction of the extract from flowering plants is highly active physiologically and would appear to exert a marked antiauxin effect.

[3] Inoculations by H. W. Klemmer in association with A. J. Riker, Department of Plant Pathology, and O. N. Allen, Department of Agricultural Bacteriology.

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Manuscript received April 14, 1952.