with the value of 0.753 obtained by Michel for males (*6*) and the values obtained by other investigators for both males and females, but is lower than the value of 0.861 obtained by Wolfsie and Winter (*7*). The value obtained for plasma activity of males (0.91) was higher than the value found by Michel (0.703) but agrees with the value obtained by Wolfsie and Winter (0.912).

The symptoms caused by overexposure to anti-ChE agents usually do not appear until the ChE level of the plasma is near zero and that of the red cells is below 30% (*2, 3*). Since the color indicator method can detect 75% or less of normal activity, it should be quite adequate for relating symptoms to ChE activity. If the symptoms are found to be due to a low ChE activity, atropine should be administered immediately in recommended doses (*8, 9*).

In addition to relating symptoms to ChE activity, the color indicator test could be used as a screening test to detect individuals having a low ChE activity either as the result of overexposure to anti-ChE compounds or as the result of some pathological condition. A ChE activity below 75% should dictate the removal of such an individual from further exposure to anti-ChE compounds until his ChE activity returns to normal.

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Mutation of Mating Type in *Saccharomyces cerevisiae*[1]

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Two mating type genes (*a* and α) were first described in *Saccharomyces cerevisiae* by Lindegren and Lindegren (*1*). These authors reported that haploid cultures maintained vegetatively may ultimately lose their mating ability, but noted no interconversion between *a* and α. Leupold (*2*) has found that mating type in the yeast *Schizosaccharomyces pombe* is mutable, apparently in all directions. Such mutability has not been proved in *S. cerevisiae*, although it has been inferred by Winge (*3*), based on the observation that "a diploid area . . . is frequently encountered at the margin of an otherwise haploid colony. . . ."

TABLE 1

SEGREGATION OF ASCOSPORES FROM A CROSS BETWEEN A176.1 (*a* Tr⁻ Me⁻ Ad⁺ Ur⁺) × A191.1 (*a* Tr⁺ Me⁺ Ad⁻ Ur⁻)*

Ascospore	M.T.	Tr	Me	Ad	Ur
A667.1	α	+	+	+	−
A667.2	α	+	−	+	+
A667.3	a	−	−	−	+
A667.4	a	−	+	+	−
A668.1	a	+	−	+	−
A668.2	a	−	−	−	+
A668.3	α	−	+	+	−
A668.4	Died				
A670.1	α	+	−	+	+
A670.2	a	−	+	+	−
A670.3	a	−	−	−	−
A670.4	Lost during dissection				
A671.1	a	−	+	+	+
A671.2	α	+	−	−	−
A671.3	a	−	−	+	+
A671.4	Died				

* Tr = tryptophan-synthesizing gene; Tr⁺ = independent of tryptophan requirement; Tr⁻ = requires tryptophan for growth; Me = methionine; Ad = adenine; Ur = uracil; M.T. = mating type.

To examine this question directly the following experiment was carried out. A haploid clone of *a* mating type, which required tryptophan and methionine for growth, was mixed with another *a* haploid which required adenine and uracil. The cells were handled as described elsewhere (*4*) for prototroph recovery. Controls were run with each haploid alone. One ml of each washed cell suspension was plated on minimal agar (lacking tryptophan, methionine, adenine, and uracil). Neither control plate had any colonies; i.e., the frequency of double mutation was too low to be revealed by the plating method. The plate in which the mixture had been plated had about 10 colonies. Several of these were isolated and induced to sporulate.

The segregation data for one of these isolates are shown in Table 1. Mutation of mating type from *a* to α has probably occurred to give rise to the observed results. Ascus A667 segregates 2*a* : 2α, and the three incomplete asci show heterozygosity for mating type. It may be noted that A667 yields a 3+ : 1− ratio for the adenine gene. Population analyses run on the independent cultures revealed no heterogeneity, suggesting that mutation occurred very early in ascospore development or germination. It is unlikely that there is a significant correlation between the mutation for mating type (which must have occurred prior to conjugation) and that for adenine independence (which must have occurred after fusion).

These data, demonstrating mutation of mating type prior to a cross between two *a* haploids, do not rule out the possible occurrence of an *a* × *a* cross, giving rise to an "illegitimate" diploid (*5*). Such diploids would be homozygous for mating type, and have been reported to sporulate only poorly if at all. It has been

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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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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*.