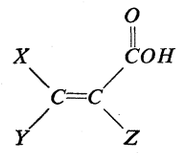
Table 1. Cotton defoliating properties of various derivatives and analogs of *cis*-3-chloroacrylic acid



applied at the rate of 2 kg per hectare (2 lb/acre).

| *X* | *Y* | *Z* | Defoliation 10 days after application |
|---|---|---|---|
| Cl | H | H | Complete |
| H | Cl | H | None |
| Cl | Cl | H | None |
| Br | H | H | Complete |
| Cl | H | $CH_3$ | Complete |
| Cl | H | $C_2H_5$ | None |
| Cl | H | $C_6H_5$ | None |
| Cl | H | Cl | None |
| Cl | $CH_3$ | H | None |
| Cl | COOH | H | None |
| COOH | Cl | H | None |
| $CH_3$ | H | H | None |
| $CH_3$ | Cl | H | None |
| H | $CH_3$ | H | None |
| H | Cl | $CH_3$ | None |

desiccant (*4*). It also has a relatively low mammalian toxicity ($LD_{50}$ rat: oral, 320 mg/kg; $LD_{50}$ rat: skin penetration, 400 mg/kg) (*5*).

*cis*-3-Chloroacrylic acid can be synthesized by reacting sodium acetylide with $CO_2$ with the formation of sodium propiolate, which, when treated with aqueous HCl in the presence of $Cu_2Cl_2$, forms *cis*-3-chloroacrylic acid almost exclusively. Recrystallization from hexane gives a good yield of product, melting point 60° to 62°C, which may be compared to 63° to 64°C (*6*). Calculated for $C_3H_3ClO_2$ are C, 33.83 percent; H, 2.84 percent; Cl, 33.29 percent; found: C, 33.68 percent; H, 2.95 percent; Cl, 33.23 percent.

The defoliating activity of *cis*-3-chloroacrylic acid and several closely related compounds was determined by applying approximately 2.0 kg per hectare (2.0 lb/acre) to the foliage of greenhouse-grown cotton (*Gossypium hirsutum*, L. var. Coker 100A) at the stage when four to eight leaves had appeared. The comparative defoliating properties of these compounds are presented in Table 1.

Activity is associated with the *cis*-isomer and its salts. The corresponding *trans*isomer and its sodium salt are virtually without activity. Substitution of bromine for chlorine does not alter activity. Replacement of the $\alpha$-hydrogen with an $\alpha$-methyl does not reduce activity. However, when the $\alpha$-substituent is ethyl, phenyl, or chloro, activity is eliminated. Replacement of the $\beta$-hydrogen with a methyl, chloro, or carboxyl group also results in inactivation. Chloromaleic acid, *cis*- and *trans*-crotonic acid, and halogenated saturated aliphatic acids, such as 3-chloropropionic acid, 2,2-dichloropropionic acid, and 3,3-dichloropropionic acid are all without activity.

Amides, substituted amides, and esters of *cis*-3-chloroacrylic acid, were ineffective. Subsequent studies indicated that hydrolysis by the plant to the acid was negligible; however, soil organisms were capable of hydrolyzing both the ester and the amide to the active acid.

From these data, it is possible to construct structural criteria for defoliation activity in this series of related compounds. (i) The beta carbon of the acrylic acid derivative must be substituted with a halogen oriented *cis* to the carboxyl group and a hydrogen oriented *trans* to the carboxyl group. (ii) Unsaturation must be present for activity. (iii) The carboxyl group must be readily available. Esters and amides will not be effectively hydrolyzed by the plant but may be by soil organisms. (iv) Substitution in the $\alpha$-position is limited to size since substitution of groups larger than methyl results in inactive molecules.

The coincidence of defoliation activity with a high degree of structural and stereospecificity leads us to propose that *cis*-3-chloroacrylic acid and the few other active analogs act as inhibitors of a specific biochemical mechanism in the regulation of leaf abscission.

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26 June 1963

## Thymectomy: Prolongation of Immunological Tolerance in the Adult Mouse

Abstract. *The loss of acquired immunological tolerance of mice to bovine gamma globulin depended on the presence of the thymus. Mice were repeatedly injected with bovine γ-globulin from birth until the age of 5 to 10 weeks and then thymectomized or sham operated. After 130 to 160 days without antigen, an accelerated (immune) disappearance of $I^{125}$ bovine γ-globulin could be uniformly induced in controls while thymectomized mice remained tolerant. Adult thymectomized mice made tolerant by a single injection of bovine γ-globulin lost tolerance more slowly than sham-operated controls.*

The thymus is thought to be essential for the development of immunologically competent cells capable of producing specific antibody. Mammals thymectomized at birth have a reduced capacity to produce antibodies when later stimulated with antigen. They fail to reject skin homografts and have fewer small lymphocytes in their blood and tissues (*1*). The thymus is also active in adult life. Adult animals treated with whole-body radiation will not recover immunological reactivity if they are thymectomized before x-ray is given (*2*). It is not known whether immunologically competent cells are produced in the thymus and transported to the lymphoid tissues or whether the development of these lymphoid cells is under the indirect, perhaps humoral, influence of the thymus.

A state of immunological unresponsiveness to soluble complex antigens may be induced in normal animals by injecting these antigens at birth. Such unresponsiveness may also be produced in adult animals if the antigen is injected in large enough doses, or if the animal is treated with x-ray or radiomimetic drugs (*3*). Although the mechanism is unknown, the duration of this form of immunological tolerance is proportional to the dose and persistence of antigen. When tolerance disappears, the capacity to respond to antigen reappears and in some instances antibody may be formed without further antigenic stimulation (*4*). The waning of tolerance may be explained on a cellular basis either by the loss of unresponsiveness by cells capable of producing specific antibody or by the development of new uninhibited cells.

Table 1. Effect of thymectomy on the waning of tolerance induced in adults, challenged with BGG adsorbed on bentonite, followed in 7 days by tracer $I^{125}$-labeled BGG.

| Group* | No. | Day of challenge | Mean ADR (%) per day | |
|---|---|---|---|---|
| | | | Thymectomy | Control |
| A | 10 | 20 | 25 (21–30)† | 24 (23–25) |
| B | 10 | 69 | 32 (31–33) | 49 (33–92) |
| C | 10 | 118 | 38 (20–93) | 72 (36–97) |
| D | 12 | 131 | 45 (27–64) | 67 (53–92) |

* Each group had an equal number of thymectomized and control mice. † Figures in parentheses represent the range in the elimination of the labeled BGG.

On the basis of a theory of cellular selection, we predicted that thymectomy in an adult animal tolerant to a specific antigen would delay or prevent the reappearance of reactivity to that antigen (*5*).

Newborn $C_{57}$BL/6J mice were injected intraperitoneally twice weekly with 1.2 mg of supernatant from bovine γ-globulin (BGG) centrifuged for 30 minutes at 59,000*g* in the center of the tube. Injections began during the first 3 days of life and continued for 5 to 10 weeks. One day after the last injection, the mice of each litter were separated at random; half were thymectomized by aspiration under pentobarbital anesthesia and half had sham operations.

One week after thymectomy, two litters were tested for tolerance by the intraperitoneal injection of 1.9 mg of BGG adsorbed onto bentonite followed 1 week later by a tracer dose of $I^{125}$-labeled BGG antigen. Tolerant mice tested by this technique have an "antigen disappearance rate" (ADR) of 15 to 25 percent per day, similar to the disappearance of tracer in untreated animals. The ADR for immune mice is 70 to 98 percent per day (*6*). In the two litters tested, the ADR fell within the tolerant range in all six mice (two sham-operated and four thymectomized).

Except for the two test litters, mice received no treatment until 130 to 160 days after operation. At that time each mouse was given 2.0 mg of BGG adsorbed onto bentonite intraperitoneally followed again in 1 week by $I^{125}$-labeled BGG. Although different litters were operated at different ages and had different periods of "antigen lapse," all the mice in a given litter were operated and later tested for tolerance on the same day. A total of 24 mice from eight litters was used.

In 11 sham-operated mice the $I^{125}$-labeled BGG was eliminated in rapid immune fashion with a mean ADR of 84 percent per day (range 68 to 99 percent). The 13 thymectomized mice remained tolerant, eliminating the radioactive BGG with a mean ADR of 39 percent per day (range 27 to 66 percent).

The role of the thymus was assessed in a different system. Forty-two adult male CBA/J mice were thymectomized or sham-operated at the age of 9 weeks (day 0). One week later, tolerance to BGG was induced by a single intraperitoneal injection of 1.9 mg supernatant BGG (*7*). At varying intervals after operation, groups of five or six each of the thymectomized and control animals were given 2 mg BGG-bentonite intraperitoneally followed a week later by a test tracer dose of $I^{125}$-labeled BGG. The results shown in Table 1 indicate that although tolerance in each group waned with time, it disappeared more slowly in the thymectomized animals.

These data give further evidence that the adult thymus is active in the development of immunologically competent cells. They support the hypothesis that long-standing acquired immunological tolerance occurs through irreversible inhibition or death of competent cells and that the waning of tolerance occurs through the development of newly arisen uninhibited cells. These new cells may arise in the thymus (*8*). The experiments do not eliminate the possibility that the thymus directs the development of these cells in the peripheral lymphoid tissue.

The waning of tolerance which eventually occurred in the thymectomized mice might be caused by incomplete thymectomy (to be analyzed at autopsy) or by very slow development of immunological cells by somatic mutation in peripheral tissues. The more rapid waning of tolerance in thymectomized adults given a single dose of BGG indicates that tolerance was less complete than in those mice repeatedly injected from birth.

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## Heterogeneity of DNA in Density and Base Composition

Abstract. *Chromatography, on methylated-albumin columns, of DNA from calf thymus, mouse testis, and* Bacillus subtilis, *yielded, on elution by a sodium chloride gradient, fractions differing in density. The fractions eluted by higher sodium chloride concentrations had lower densities in a CsCl density gradient. Since DNA with higher guanine-cytosine content is eluted from the column with lower concentration of sodium chloride and has higher density, the density heterogeneity of DNA is best interpreted as a result of heterogeneity of base composition. An extra band observed in calf-thymus DNA had a higher density than that of the main DNA; it was eluted at a lower concentration of NaCl, indicating a higher content of guanine and cytosine. On the other hand, an additional DNA component in the mouse-testis DNA had a lower density and also it was eluted at a lower salt concentration, possibly an indication of an unusual base component in its structure.*

Heterogeneity of density of DNA molecules from an organism detected by density-gradient centrifugation has been interpreted as the reflection of heterogeneity of base composition (*1–4*), although other possibilities are not excluded (*5*). A methylated-albumin column can separate DNA into fractions differing not only in molecular size (*6*) but also in base composition (*7*). This technique provides an opportunity to investigate the relationship between heterogeneity in density and base composition of DNA. It also makes possible an examination of some properties of extra components of DNA in various organisms (*2, 8*).

The DNA was fractionated on the methylated-albumin column by applying a linear gradient of sodium chloride concentration. Elution with a gradient