# Technical Papers

## Ovular Tumors and Inhibition of Embryo Growth in Incompatible Crosses of *Datura*[1]

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For some time the Smith College Genetics Experiment Station has been making a somewhat detailed study of the various barriers to crossability among the 10 herbaceous species of the genus *Datura.* Of the 90 interspecific combinations, with each species used both as a male and as a female parent, only 19 have resulted in viable hybrid seed. In some cases the pollen tubes burst in the style of the foreign species or grow too slowly to reach the ovary. It seems to be the rule, however, that if the sperm can be gotten into contact with the egg cell, fertilization results. The postfertilization barriers to crossability which result in abortion of the hybrid embryos can be overcome in many crosses by cultivating the excised embryos on artificial media before they abort.

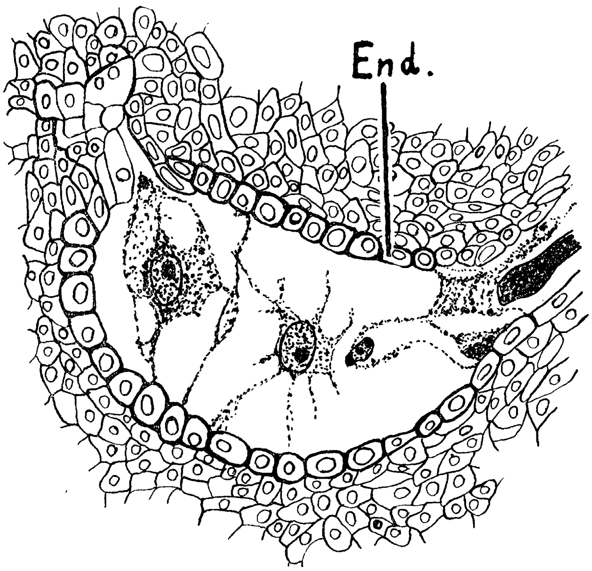


FIG. 1

There are great differences in the behavior of tissues within the ovules of incompatible and compatible crosses or selfs. In compatible crosses and selfs, a single layer of cells, the endothelium (marked "End." in Fig. 1), which is epidermal in origin, surrounds the embryo sac and these cells act as nurse cells in passing on nourishment to the developing embryo. The cells of the integument outside the endothelium are at first filled with starch grains. With the growth of the embryo this starch is depleted. As the endosperm develops inside the embryo sac it becomes filled with fat and aleurone grains. The endothelium remains a single layer of cells which lose their contents and become much flattened. Ultimately the layers outside the embryo sac, including the endothelium itself, become digested.

In incompatible crosses the condition in the young ovule is similar to that just described for compatible crosses. Soon, however, the cells of the endothelium stain more deeply and enlarge, as shown in Fig. 2. The outlying cells of the integument do not lose their starch. Chemical tests seem to show that the ovular contents of selfs and compatible crosses convert starch (presumably

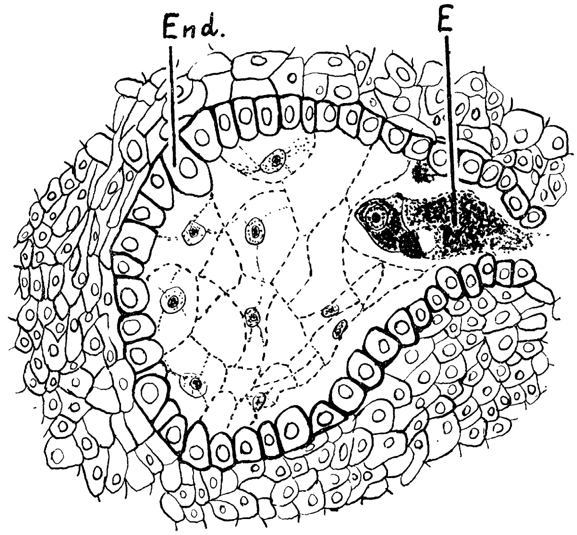


FIG. 2

due to an amylolytic enzyme), whereas the contents of ovules from an incompatible cross are apparently unable to convert starch. It would seem, therefore, that in incompatible crosses either an amylolytic enzyme is absent or its activity is inhibited. Accompanying the growth in size and division of the endothelium in all planes, there is abortion of the contents of the embryo sac. Sometimes the endosperm aborts first and sometimes the embryo, as is the case in Fig. 2 (the embryo is marked "E."). In more advanced stages, such as that shown in Fig. 3, the endothelial tissue may proliferate at several points and penetrate into the embryo sac to form groups of tumoral tissue. Sometimes such ovular tumors form definite masses visible to the naked eye, which from their appearance have been called "pseudo embryos."

The contents of ovules from incompatible crosses may be watery, jellylike, milky, or cheeselike, dependent somewhat upon which parents had taken part in the cross and upon the age of the ovule. It should be

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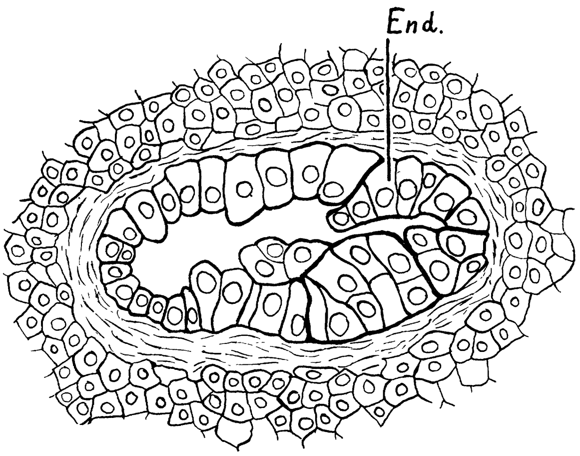


FIG. 3

pointed out that the stimulus to incompatibility and ovular-tumor formation is not confined to species crosses in which qualitative differences in the genes of the two parents might be supposed to be in some way responsible for the failure of embryo growth. Ovular tumors and incompatibility occur also in $4n \times 2n$ crosses within inbred lines of the same species.

Ovular tumors associated with embryo abortion in incompatible crosses of *Datura* have been found to contain a water-soluble thermostable substance unrelated to plant hormones, which is capable of inhibiting growth of embryos from selfed *D. stramonium* both *in vitro* and *in vivo*. The contents of such inhibited ovules contain a substance capable of inhibiting another set of capsules. This substance has been found effective in three successive passages, suggesting a self-duplication such as occurs in viruses or a new formation of inhibitor stimulated by the originally injected ovular-tumor extracts. Fig. 4 diagrams the effects of these injections.

In the upper part of the diagram is shown that one-fourth of the extract from a single inhibited ovule (a) will not inhibit ovules in another capsule, whereas the extract from a whole incompatible ovule (A) inhibits the

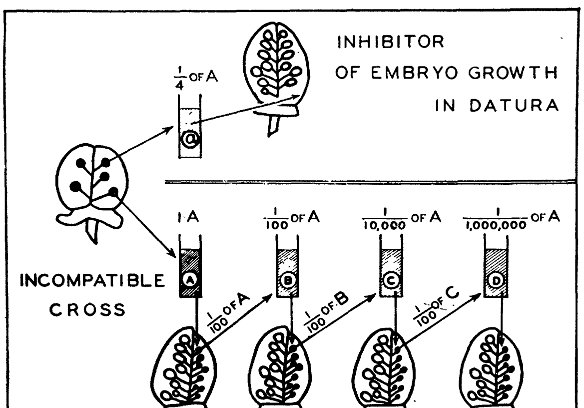


FIG. 4

$\pm 100$ ovules of the locule of the capsule into which it has been injected. The extract (B) from one of these $\pm 100$ inhibited ovules will contain one-hundredth of the amount of inhibitor present in A. Since, however, it inhibits a second series of $\pm 100$ ovules it is believed that the strength of the inhibitor had in some way been increased. The strength of the extract (D) which inhibits ovules in capsule IV must be only one-millionth of that in the original (A) if no such increase had taken place.

The basis of this increase in potency of the inhibiting substance, as well as the nature of the inhibition, is receiving further study.

Solution of this problem might well lead to methods whereby the postfertilization barriers to crossability could be removed, with a great increase resulting in the number of wide species hybrids possible. It might also throw new light on the broader problems of both normal and abnormal growth and differentiation in other forms.

Details of our studies on ovular tumors in *Datura*, together with literature citations, will be given in two papers now in press in the *American Journal of Botany.*

## A Mechanical Heart with Coagulable Blood

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As far as we know, no artificial heart with coagulable blood has yet been devised. Our method, which will be described briefly, aims to take blood (arterial, venous, or mixed) without extravasation, from any part of the body, raise or lower its pressure to any wanted level, and perfuse any organ, organs, or part of the body with this blood, which is sent back to the right heart through a jugular vein.

This artificial heart is made from an aorta in which the blood is propelled by a roller-pulley. The peripheral resistance is provided, on the one hand, by the perfused organ or organs, and on the other, by a shunt such as we described previously (*1*), which is coated inside by a carotid. The air pressure exerted on this vessel regulates the arterial pressure.

The aorta of a dog of 15–30 kg is dissected from the heart up to and including the iliac division, and all branches carefully ligatured. It is cut 5 cm below the subclavian artery. The central end of the peripheral part is turned inside out over a Payr's cannula of 10–12 mm diam. The cannula is tied at its other end inside the end of a piece of rubber tubing of 12-mm diam and 80-mm length. The distal part of the vessel emerges from the rubber tube. The superior mesenteric branch is connected with the shunt, which is the pressure regulator. One of the renal arteries is connected with a manometer. One of the iliac arteries leads to the perfused organ or organs (i.e., kidneys); the other iliac artery, clamped, is used for rinsing the air out of the preparation.

The crook of the aorta, which has been separated, is tied at its peripheral end over the central end of the other part of the aorta, which is fixed on the Payr's cannula.