Some of the water released could have arisen from acid- or base-catalyzed thermal degradation of carbohydrates present in gluten or browning reactions between them. Plain bleached wheat flour (10% protein content approximately), impure starch (commercial corn flour) and a sample of soluble starch were examined by the same technique. No detectable conductivity change occurred in the air-dried starch. The wheat flour showed little change in conductivity until about 170 °C. When starch and wheat flour were moistened peaks in the 30–80 °C range were obtained. Only small peaks were obtained in 140–170 °C range (Figures 3 and 4). These findings suggest that water is not produced by carbohydrates in these circumstances at least below 140 °C since trypsin has negligible carbohydrate content and the higher levels of starch in wheat flour, in comparison with gluten, did not yield large peaks of conductivity. If heating rates are increased from 5–7 °C/min to 10 °C/min a similar though more compact 'spectrum' is produced.

Further evidence that the electrothermal method described was detecting water held by gluten has been found in the detection, in the gluten samples used here, of 'hygroscopic' water by classical differential thermal analysis techniques[6]. Other support has been the presence in similar gluten samples of protons, as revealed by NMR studies, with a certain amount of restricted motion[7] corresponding approximately to the loss in weight at 105 °C (24 h).

Thermogravimetric results[6] over the range 20–140 °C and Wallace-Shawbury curometer measurements (carried out at the Rubber and Plastics Research Association, Shawbury) indicate changes which are in keeping with the electrothermal studies. Gas-liquid chromatography (kindly carried out at the Applications Laboratory, F and M Scientific Corporation), using a g.l.c. linear programming system over 60–215 °C at 11 °C/min identifies the sole low temperature volatile as water. The electrothermal changes found would thus appear not to be due to a.c. conductivity artefacts.

The evidence presented would thus seem to indicate that the electrothermal method is measuring water release and that the 'spectra' presented provide the patterns of release[10].

Zusammenfassung. Mit Hilfe einer elektrothermischen Methode wird das Freiwerden von Wasser aus Proteinen gemessen und die Dehydratation der Eiweisse im elektrothermischen Spektrum genauer als bisher erfasst.

C. B. COULSON[8]

Arthur D. Little Research Institute[9],
Inveresk (Midlothian, England), 14 December 1970.

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[7] E. R. ANDREW, private communication.
[8] Present address: National College of Food Technology, University of Reading, St. George's Avenue, Weybridge (Surrey, England).
[9] Now: Inveresk Research International.
[10] Thanks are due to Hercules Powder Co Inc., Delaware for supporting this work.

Overcoming Self-Incompatibility in Petunia. Differential Treatment in vitro of Whole Placentae

In the first paper we reported the overcoming of self-incompatibility in *Petunia axillaris* (Lam.) B.S.P. in aseptic cultures[1] of whole placentae, and indicated that the new technique of 'placental pollination' can help to bring about selfing in part of the placentae while the remainder of the placentae can be subjected to any desired treatment. Exploratory work showed that during growth the pollen tubes can cross from one placenta to the other. Thus, to treat differentially the 2 placentae (of the same ovary), a mechanical barrier between the placentae became necessary. In this report the results of the differential treatments of the placentae in aseptic cultures are described.

Material and method. The experimental plant and the methodology of placental pollination have already been described[1,2]. For differential treatment, the 2 placentae were slit with a sterilized scalpel almost to the base of the septa, and a piece of cellophane (6 mm long and 4 mm broad and previously dipped in absolute ethyl alcohol and dried) was inserted in the slit made between the placentae (Figure A). And then the placentae were given one of the following 3 treatments: a) 1 of the 2 placentae was left unpollinated as control and the other was self-pollinated (i.e. control vs selfed), b) 1 of the placentae was left as control and the other was cross-pollinated (control vs crossed), and c) 1 of the placentae was self-pollinated and the other was cross-pollinated (selfed vs crossed).

For each treatment 48 cultures were raised in 4 replicates of 12 each. All explants were grown on agar-sucrose culture medium described earlier[1] and the cultures were maintained at diffuse light conditions (100–200 Lux) at 22 ± 2 °C. For statistical analysis both correlation coefficient (r)[3], and t value[4] were determined.

Results and discussion. In the first two treatments, the control placentae invariably shrivelled whereas the pollinated placentae showed regular pollen germination and pollen tube growth amidst the ovules. In 5 days of culture 10–50 ovules enlarged and eventually developed into mature seeds in most of the cultures (Figure B). Of the 48 control vs selfed placental cultures, 11 became infected, 17 shrivelled, and the remaining cultures produced a total of 458 seeds on the pollinated placentae; the corresponding figures for the 48 control vs crossed placental cultures were 12, 12, and 523[5].

In the third differential treatment (selfed vs crossed), pollen germination as well as pollen tube growth occurred equally well on both placentae. Within 5 days of pollina-

Performance of selfed vs crossed placentae (Differential treatment No. 3)

Cultures raised	48
Infected cultures	10
Cultures harvested	38
Shrivelled cultures	17
Cultures which set seed	21
Cultures in which seeds developed on selfed placenta only	4
Cultures in which seeds developed on crossed placenta only	6
Cultures in which seeds developed on both placentae	11

	Selfed placenta A	Crossed placenta B
Total seed number	339	376
Average seed number	8.92	9.89

	r value	t value	Degrees of freedom
B and A	+0.868	0.314	150

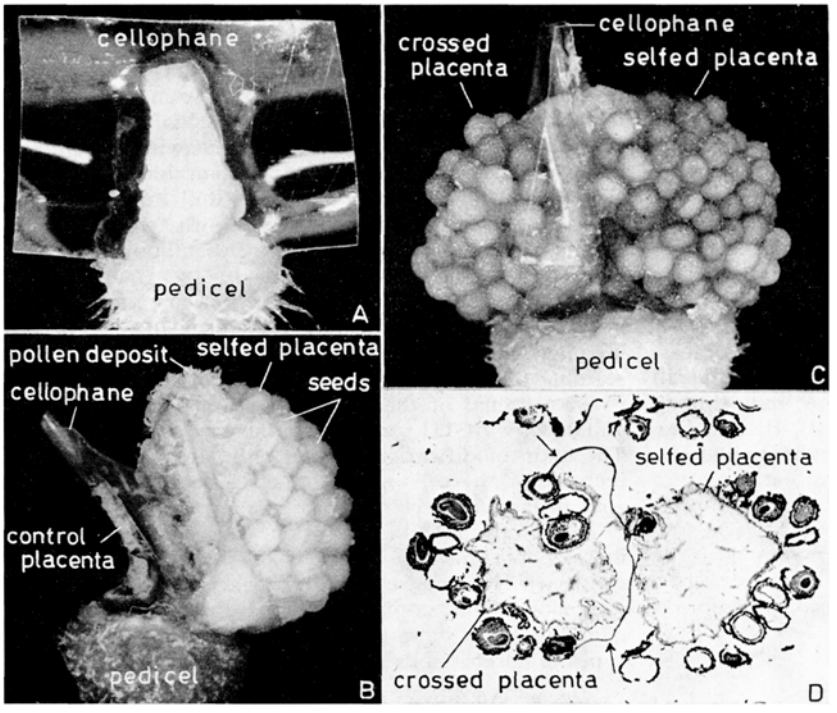

Figure A–D. Differential treatments of placentae in vitro in *Petunia axillaris*. A) Explant after insertion of cellophane as seen in face view of one of the placentae. ×7,8. B) Culture as seen 14 days after differential treatment (control vs selfed). Control placenta (left) has shrivelled and on the selfed placenta (right) several seeds are obvious. ×12,8. C) Culture 21 days after differential treatment (selfed vs crossed). Both placentae bear numerous seeds. ×13,3. D) Transection through middle region of the culture shown in C. Cellophane between the placentae is seen cut as a black coil (arrow-marked); seeds cut in favourable plane show the embryo. ×10,7.

tion, many cultures showed beginnings of seed development on both placentae, and mature seeds were formed in 21 days after pollination (Figure C, Table). Irrespective of their affiliation either to the selfed or to the crossed placenta, the majority of seeds contained normal embryo and endosperm (Figure D). There was a high degree of positive correlation between the seed set obtained on selfed placenta and that on the crossed placenta; the t value also showed no significant difference. The seeds (crossed as well as selfed) readily germinated and gave rise to normal seedlings. Squash preparations of root tips of seedlings obtained from selfed placentae showed the diploid chromosome number 14.

Thus, the technique of introducing a mechanical barrier such as cellophane between the placentae has made differential treatments of the 2 placentae of the *same ovary* possible. Further, this technique reduces the sample variation between the treatments to a minimum and facilitates a direct deposition of pollen grains on *definitive loci* of a placenta; also it offers a wide scope for studies of pollination with mixed pollen, irradiated pollen, and labelled pollen.

Zusammenfassung. Es wird eine Methode zur Vornahme verschiedener Bestäubungsversuche an ein und derselben Plazenta beschrieben.

K. R. Shivanna[6]

Department of Botany, University of Delhi, Delhi 7 (India), 10 August 1970.

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[5] The r value, the t value and f values for the 2 treatments were +0.557, 0.636 and 144 respectively.
[6] Acknowledgments. I am grateful to Dr. N. S. Rangaswamy for guidance, to Prof. B. M. Johri for facilities and to Dr. K. M. M. Dakshini for his help in statistical analyses.

Demonstration by a New Staining Method of Different Types of Cells in Adrenal Glands

In recent years much has been learned about hormone biosynthesis of adrenal glands but no substantial progress was made about their light microscopical appearence. However, some evident changes in physiological conditions in the classical zones of the adrenal cortex suggest that a better knowledge of the adrenal morphology is essential. In spite of this, the information required for adrenal morphology up to the present is obtained from sections stained with haematoxylin and eosin or with a few other histological and histochemical routine methods.

A new staining method recently proposed by Novelli[1] for collagen and reticulum gives interesting and sometimes surprising results if it is applied to the study of the histophysiology of adrenal glands, provided that a suitable fixation with dichromate has been used; several different types of cells in the various cortical zones and medulla can be demonstrated.

The following procedure is suggested: Fixation for 48 h at room temperature (22 °C) in Mueller fluid with 10% formalin; pH between 5–6, eventually adjusted with a 5% aqueous solution of potassium chromate. Paraffin sections of 5 µm. 1. Bring sections to water and then into 0.04% Evans blue solution in picric acid (aqueous saturated) for 20 min. 2. Rinse in water and place in 1% aqueous fucsin acid solution for 10 min. 3. Rinse in water, dehydrate, clear and mount.

Results. Adrenal cells assume different color combinations of the 3 acid stains employed. These combinations