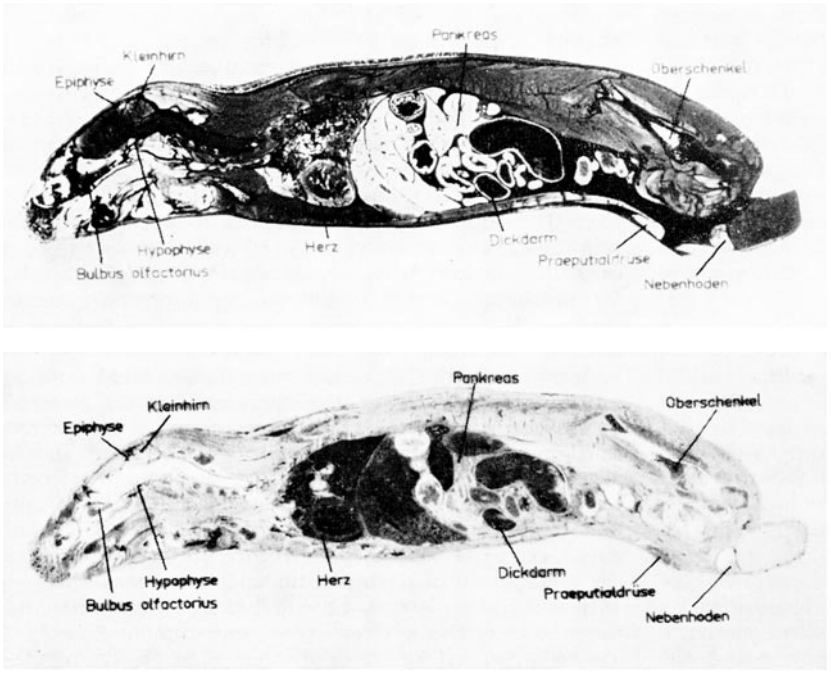

a Sagittalschnitt (Medianebene) durch eine 220 g schwere, mit ^{14}C-markierter Substanz behandelte Ratte. a) Autoradiogramm, Negativ-Darstellung. b) Fixierter Kryostatschnitt.

führen. Dadurch wird die bei autoradiographischen Untersuchungen notwendige Zuordnung von Schwärzungen der Autoradiogramme zu den entsprechenden Gewebsstrukturen, die oftmals nur durch einen visuellen Vergleich mit den Nativschnitten exakt möglich ist, wesentlich erleichtert (Figur).

Methode. Die auf einer selbstklebenden Trägerfolie haftenden Nativschnitte (50–80 µm) werden nach Anfertigung der Autoradiogramme gefriergetrocknet (Gefriertrocknungsanlage Delta II, Fa. M. Christ, Osterode; $p < 10^{-3}$T orr, Eiskondensatortemperatur -90 °C), unter Vakuum auf Raumtemperatur gebracht und im Vakuumexsikkator über Paraformaldehyd (5–10 g) 4–5 h auf 60 °C erwärmt. Zur Entfernung des bei der Fixation gebildeten Wassers werden die Schnitte anschliessend 1–2 h bei Raumtemperatur vakuumgetrocknet. Die freie Schnittfläche wird mit Tesafilm® verklebt; das Gesamtpräparat kann zusätzlich in eine Folie eingeschweisst werden.

Auf diese Weise ergeben sich fixierte Dauerpräparate, die zur Information und Dokumentation geeignet sind. Die natürlichen Farben sind durch eine geringe Ausbleichung nur wenig verändert. Weiterhin kann in den Grossflächenschnitten die Vorbestimmung optimaler Exponierzeiten und die Messung der relativen Radioaktivitätsverteilung mit dem Dünnschicht-Scanner schnell durchgeführt werden. Das Präparat darf hierbei nur mit einem möglichst dünnen Film überklebt sein. Nach Ausschneiden einzelner Organe bzw. umschriebener Gewebsabschnitte lässt sich ihre relative Aktivität auch mit einem 2π-Methandurchflusszähler messen. Bei diesem Verfahren entfällt die für die Densitometrie problematische Mittelwertsbildung bei ungleicher Verteilung der Radioaktivität innerhalb eines Organs. Die quantitative Bestimmung der Aktivität eines Organs bzw. eines bestimmten Gewebsabschnitts kann nach automatischer Verbrennung der Proben (z. B. im Probenverbrennungssystem nach Wegner/Winkelmann, Typ BF 5010 der Fa. Berthold/Frieseke GmbH) im Flüssigkeitsszintillationszähler durchgeführt werden.

Summary. By exposure to formaldehyde vapor after freeze-drying large cryostat sections are brought into a stable form. These sections can be used for documentation and may be compared easily with autoradiograms made of the same slices. Furthermore, a method is shown of evaluating these sections with a thin-layer scanner and a 2π-flow counter, or by measuring quantitatively the radioactivity of single, excised organs or parts of tissues after automatic combustion by liquid scintillation counting.

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Electrothermal Studies on Protein Powders. Electrical Conductivity of Gluten in Thermal Gradients

'Bound' water present in proteins is considered to be intimately connected with the character of their native state[1]. Information on the energy levels of binding and differentiation between these levels might therefore aid studies on variation in the native state of protein powders. Classical differential thermal analysis of gluten and other protein powders indicates that water is released over a relatively small temperature interval[2]. ELEY et al.[3,4] in their studies on *dry* protein powders have shown that a linear relationship exists between log ϱ_{10} and T^{-1} over the range 30–160 °C for d.c. conductivities thus excluding the possibility of conductivity effects arising from second order transitions or abrupt changes in the surface contact of powder particles. A study of the changes

in electrical conductivity of protein powders in a thermal gradient thus seemed a way to assess these different energy levels of water binding in *moist* or *air-dry* powders provided the method could be sufficiently refined to reveal them.

The behaviour of air-dry protein powders in thermal gradients was studied in terms of a.c. (50 c/sec) electrical conductivity. A pressure conductivity cell, similar to those used in commercial moisture meters (model, Marconi Ltd.) was designed to include a simple heating unit (a spiral of resistance wire fitted between cylinders of compact asbestos, Sindanyo). The heat was conveyed by a solid cylinder of aluminium to the face of the powder disc furthest away from the annular electrodes. The temperature was measured by inserting the thermocouple into a space immediately behind the electrodes. This heater unit, with suitable voltage input, provided an acceptable temperature gradient. The conductivity cell was connected to a valve voltmeter bridge circuit. The output (0–10 mV) of this bridge circuit and that of an iron-Constantin thermocouple system used to measure the cell temperature was fed into a 2 channel Honeywell-Brown Electronik recorder. The complete unit represented a convenient recording conductivity meter.

Freeze-dried gluten (7.6% moisture content) was examined in the conductivity meter at several stages of hydration (Figure 1). Two peaks of conductivity are shown for the partially hydrated sample. This experiment would appear to indicate that water release with increasing temperature is being measured in the form of conductivity changes. If this is the case then water would appear to be bound at 2 different main energy levels.

Trypsin powder (BDH) also shows major peaks of electrical conductivity (Figure 2) but in this case they occur at rather lower temperatures than in gluten. This may be due to a lower thermal denaturation temperature for trypsin. Gluten is reported to show appreciable resistance to thermal denaturation[5].

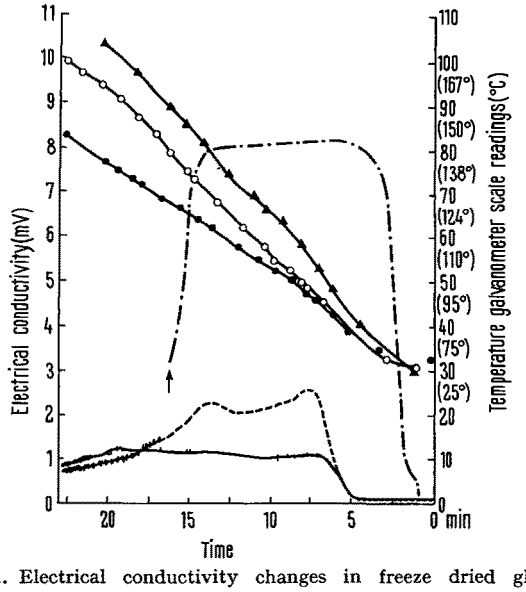

Fig. 1. Electrical conductivity changes in freeze dried gluten powder in thermal gradients. 1. Original sample (Conductivity — Temperature ●—●). 2. 'Partially' hydrated sample (Conductivity --- Temperature ▲—▲). 3. 'Fully' hydrated sample (Conductivity -·-·- Temperature, given in parenthesis, ○—○). (Arrow indicates thermal degradation point (also probable melting point) of 'fully' hydrated sample.)

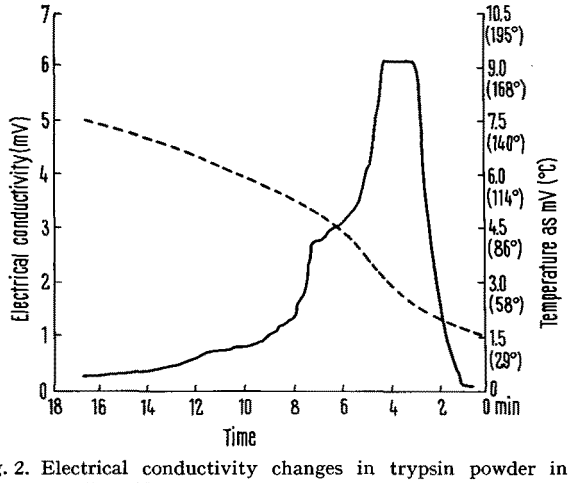

Fig. 2. Electrical conductivity changes in trypsin powder in a thermal gradient (Conductivity — Temperature ---).

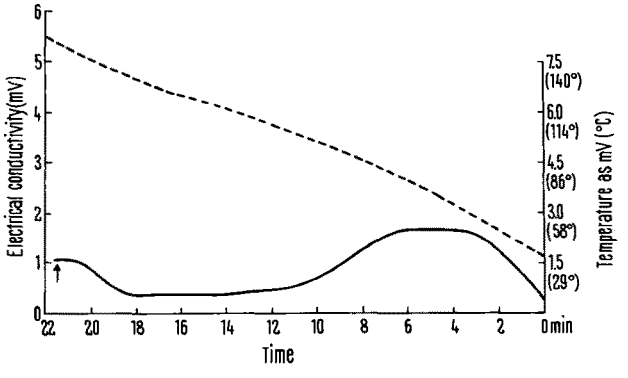

Fig. 3. Electrical conductivity changes in plain wheat flour (damp) in a thermal gradient (Conductivity — Temperature ---).

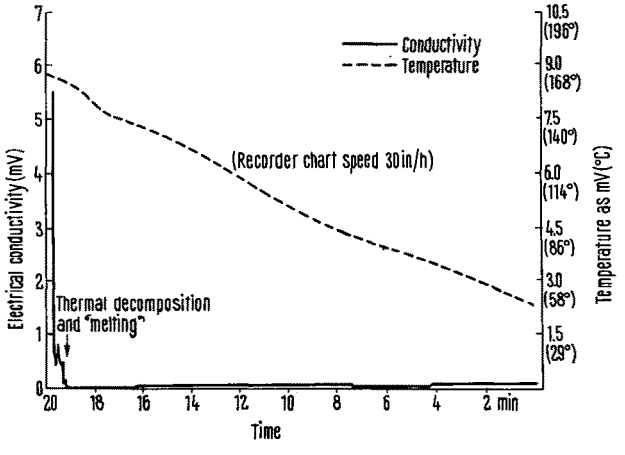

Fig. 4. Electrical conductivity changes in purified air dried starch (Connaught, starch hydrolysed) in a thermal gradient (Conductivity — Temperature ---).

[1] W. Kauzmann, Adv. Protein Chem. *14*, 1 (1959).
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[5] C. B. Coulson and A. K. Sim, Biochem. J. *80*, 46P (1961).

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.

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[6] B. D. MITCHELL, private communication.
[7] E. R. ANDREW, private communication.
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[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