For the two membrane systems under investigation, the resolving power of crossed immunoelectrophoresis is similar to that of the widely-used SDS polyacrylamide gel electrophoresis, the former method giving 20 and 13 precipitates for the two systems, respectively, in comparison with 16 and 12 major bands when using the latter[14,15]. However, the separation obtained with the two methods differs, as it is based on different molecular properties. This has been clearly demonstrated for the erythrocyte membrane protein Spectrin, which appears as two bands in SDS-gel electrophoresis but reveals at least 7 precipitates by quantitative immunoelectrophoresis (Figure 1)[16]. Thus, these methods supplement each other; and immunochemical examination of membrane proteins separated by preparative SDS-gel electrophoresis is in progress.

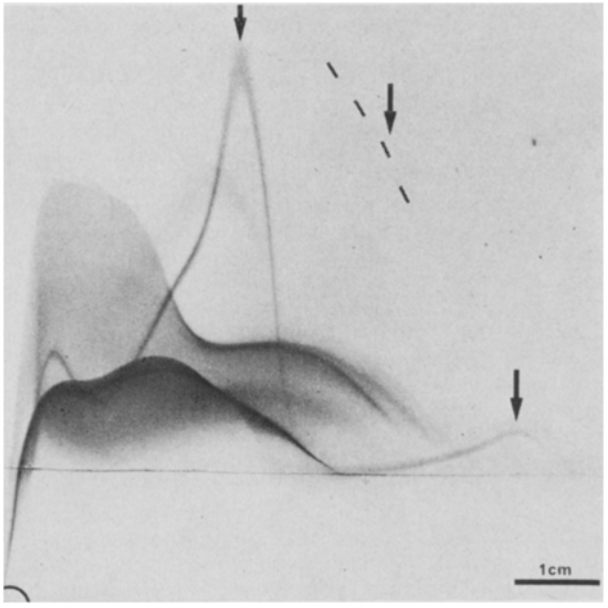

Fig. 2. Crossed immunoelectrophoresis of a 100 µl sample containing 145 µg Berol EMU-043 solubilized membrane proteins from bovine milk fat globules. The antibody content of the gel was 10 µg/cm². 11 different precipitates were seen on the original plate. The precipitates indicated with arrows were also present in bovine whey[1c] in very low concentrations. The conditions were as given in the legend to Figure 1, except for the agarose used (Batch 091 with lower electroendosmosis).

Zusammenfassung. Das Ergebnis der Antigen-Antikörper-Kreuzelektrophorese von Membranproteinen aus Erythrocyten und Fettkügelchenhüllen, solubilisiert mit nicht-ionischen Detergenten, wird beschrieben.

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[17] Acknowledgements. This work was supported in part by The Danish Medical Science Research Council. The author is grateful to P. LUNDAHL (Institute of Biochemistry, Uppsala), Å. H. PEDERSEN and J. ARNOLD (Government Research Institute for Dairy Industry), T. C. BØG-HANSEN for help and discussions and the skilled technical assistance of Miss A. MÖLLNITZ is greatly appreciated.

Organogenesis in Callus Culture of *Gazania splendens* MOORE Induced on New Medium

In vitro cultures of plants make it possible to obtain material of great breeding value (haploids, diploid isogenic lines, polyploids, gene mutations, parasexual hybrids, etc.)[1,2]. This type of culture has not yet been used in the tribe Arctotideae (Asteraceae)[3–5]. However, the induction of organogenesis is the basic requirement for the application of the in vitro culture in this tribe.

Mature leaves of the starting plant (Figure 1) *Gazania splendens* MOORE (Sempra, Prague) were used as primocultures. Leaves sterilized on the surface were cut vertically to the petiole to sections of 0.25 cm², and placed by the reverse side on a medium (Table) according to MURASHIGE and SKOOG[6]. The cultures were placed in a thermostat in the dark at 25 °C.

In about 20 days the cultivated pieces of the leaves created in the centre calli which grew, and 57–76 days after establishing the primocultures they could be passaged. Upon passage on the same medium the calli grew quickly and within 14 days attained an average fresh weight of 2 g. They were consistent and nodulous, their basic colour in the R.H.C. system[7] was 160 D. They showed no signs of organogenesis, even when 2,4-D was removed from the medium (Figure 2).

When the calli were passaged on a new medium designated NNS (Table) and placed in a thermostat in the light (25 °C, approx. 7000 lux), organogenesis took place rapidly. In 7 days white roots appeared on the calli growing heliotropically. After 14 days a branched root system growing geotropically was formed which after 10 days expanded into the whole medium. At the same time green buds appeared massively on the calli. After transferring the buds with a small piece of callus and a few small roots again on medium NNS, dark green leaves developed within 4 days to 2 weeks, corresponding morphologically to the young leaves of *Gazania splendens* MOORE. The calli from the 5th passage on the medium according to MURASHIGE and SKOOG also retained fully

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Fig. 1. Starting plant *Gazania splendens* MOORE[8].

Fig. 2. Callus on the medium according to MURASHIGE and SKOOG[6].

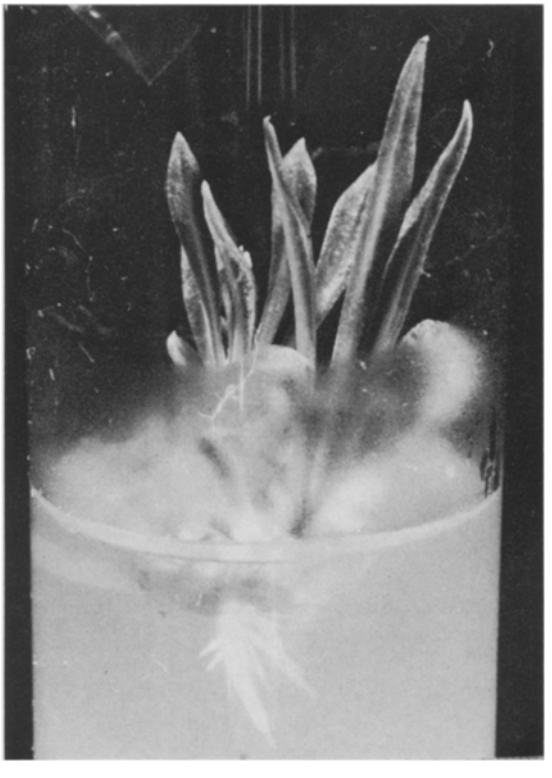

Fig. 3. Organogenesis on medium NNS (buds and roots).

Media formulations Constituents	MURASHIGE-SKOOG[6] (mg/l)	NNS (mg/l)
KNO_3	1900	500
NH_4NO_3	1650	500
$MgSO_4 \cdot 7H_2O$	370	150
$CaCl_2$	332	150
KH_2PO_4	170	100
$Ca(NO_3)_2 \cdot 4H_2O$	—	100
$MnSO_4 \cdot 4H_2O$	22.3	25
H_3BO_3	6.2	10
$ZnSO_4 \cdot 7H_2O$	8.6	10
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.25
$CuSO_4 \cdot 5H_2O$	0.025	0.025
$CoCl_2 \cdot 6H_2O$	0.025	0.025
Inositol	80.00	100
Glycine	—	5
Nicotinic acid	—	5
Pyridoxine · HCl	—	0.5
Thiamine · HCl	0.4	0.5
Folic acid	—	0.5
Biotine	—	0.05
Ca-pantothenate	5	—
Na_2EDTA	37.3	186.5
$FeSO_4 \cdot 7H_2O$	27.8	139
Kinetin	0.5	0.5
IAA	—	2
2,4-D	1	—
TCH	500	1000
Sucrose	30000	30000
Agar	7000	7000

Fig. 4. A regenerate in a high tube on medium NNS before transfer to the Perlit.

their organogenetic potency and differentiated roots and buds when transferred to medium NNS (Figure 3). When regenerates were transferred to tubes containing larger amounts of medium NNS, whole plants developed (Figure 4). After attaining leaf rosettes with 5 to 7 leaves, the regenerated plants were successively transferred to Perlit and then to the soil, where they grew normally (Figure 5). Experiments transferring the regenerates to the soil via liquid medium NNS with a bridge, made from Whatman paper No. 1, failed. The calli grew brown and regressed, and the plants died.

The karyological and genetical analysis of the regenerates obtained will be the subject of future work. The induction of regeneration of whole plants in callus cultures of *Gazania splendens* MOORE will make it possible to use the in vitro explant cultures in the explant breeding programmes for the tribe Arctotideae.

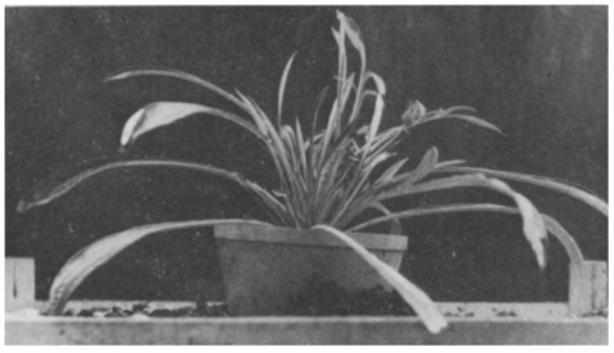

Fig. 5. A regenerate arisen in the 4th passage of the callus after developmental stages described in the text, fully growing; age 10 months from the starting day of the 4th passage[8].

Zusammenfassung. Es wird eine Methode zur Induktion der Organogenese und Entwicklung ganzer Pflanzen aus dem Kallus von *Gazania splendens* MOORE beschrieben.

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31 October 1973.

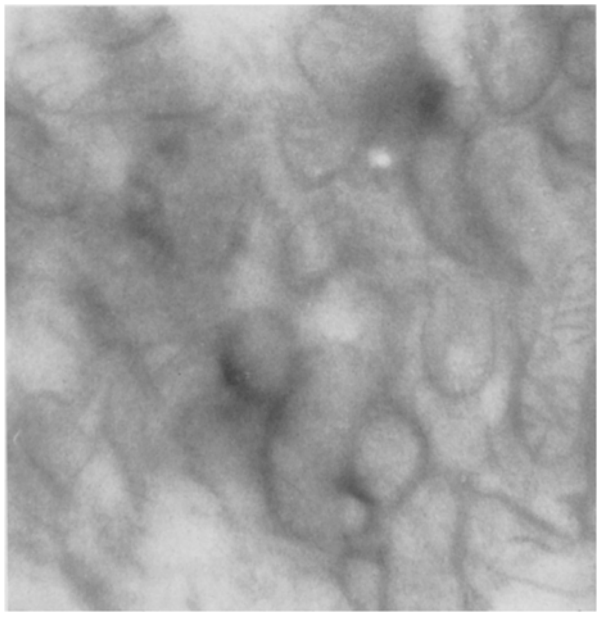
[8] Photo 1 to 4 by P. WANNER, 5 by Ing. F. KOCOUREK.

Ultracryotomy shows the crystae of mitochondria

The field of biological electron microscopy is founded on the assumption that the structure visible in the electron microscope is the 'true' structure of the living tissue. Normally, tissue is processed by fixation, dehydration and plastic embedding. Such extended chemical treatment might alter structure but this assumption, that it does not, has produced many successes covering the whole field of cell biology.

Recently, we have introduced[1] and refined[2] the process of ultracryotomy, where fresh tissue is frozen and cut into ultrathin sections at low temperatures (below −120 °C). Provided that the thin sections are freezedried and protected from rehydration by vacuum coating with a layer of carbon, membrane structure is preserved. In a recent paper[2] we were not able to report crystae in the visible mitochondria of hepatic cells. We have since improved our technique and, using a lower accelerating voltage in the electron microscope (40 keV), we are now able to confirm that mitochondria do indeed have crystae (Figure). The assumption that chemical processing preserves 'true' structure is supported.

This observation extends the resolution of ultracryotome sections down to 100 Å. It is hoped that shortly the resolution of ultracryotome sections will be comparable to sections cut through plastic embedded tissue.



Mitochondria in the cytoplasm of a parenchymatous hepatic cell. Although the section was prepared by ultracryotomy with no chemical treatment which could preserve structure or enhance contrast, the mitochondrial crystae are clearly visible. ×38,000.

Résumé. La technique des sections congelées de tissus biologiques (ultracryotomie) permet maintenant d'obtenir une résolution de 100 Å.

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