Bei den anschliessenden Vergessenstests sanken die Leistungen der Vt schneller unter die Signifikanzgrenze (Figur 2). (Unterschiede zwischen Vt und Kt nur für den 1., 3. und 6. Vergessenstest nach dem t-Test gesichert, nach dem X^2-Test alle Unterschiede mit einer Irrtumswahrscheinlichkeit von 1%). Das darf zunächst einmal auf das niedrigere Lernniveau der Vt zurückgeführt werden. Ob die schlechtere Ausgangsposition der Vt zu Beginn der Vergessenstests allerdings allein das schnellere Vergessen bewirkte, ist fraglich. Von Einfluss kann zusätzlich auch die stete Kampfaktivität der Vt während der Vergessensphase gewesen sein. Eine eindeutige Aussage liess sich mit diesem Versuch nicht gewinnen.

Versuch II. Um den Einfluss der Aggression auf das Vergessen genauer zu untersuchen, wählten wir einen etwas abgewandelten Versuchsmodus. Gearbeitet wurde wieder mit 20 Kampffischen (von denen im Verlauf der Versuche einige starben). Alle Tiere dressierten wir jetzt sofort auf die nicht bevorzugte Farbe. Während der Lernphase blieben nun alle Fische ungereizt. Nach Erlernen der Aufgabe (Lernkriterium s. Versuch I) teilten wir die Tiere nach ermittelten Spontantendenzen, Lerngeschwindigkeit und erreichtem Lernniveau in leistungsgleiche Paare (Vt und Kt) ein. Anschliessend wurden nur die Vt für die ganze Dauer der Vergessensphase in der zuvor beschriebenen Weise aggressionsspezifischen Reizen ausgesetzt. Die Kt blieben ungereizt. Die Vergessenstests führten wir in Abständen von 7 Tagen durch.

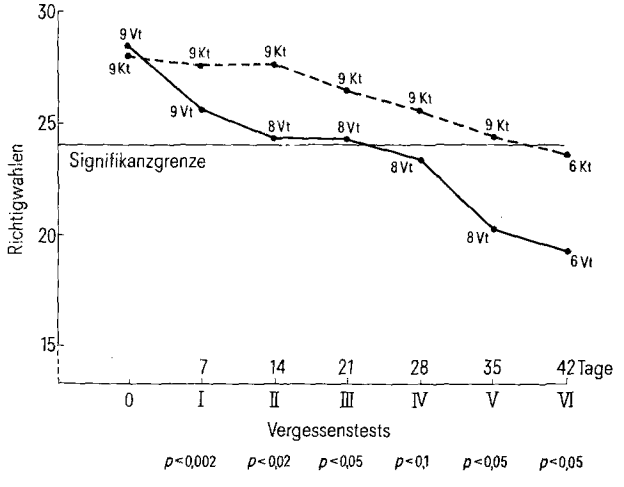

Fig. 3. Vergessenstests der Versuchsserie II. Bei 0: Ausgangswerte, gemittelt aus den drei letzten Werten der Dressur.

Wie Figur 3 zeigt, waren die in der Dressur erzielten Lernleistungen bei Vt und Kt nahezu gleich. Die Vt vergassen die erlernte Aufgabe aber schneller als die Kt. In allen Vergessenstests lagen die Durchschnittswerte der Vt unterhalb derjenigen der Kt. Alle Werte sind mit einer Ausnahme (4. Vergessenstest) nach dem t-Test gesichert (Figur 3). Nach dem X^2-Test waren die Unterschiede zwischen Vt und Kt mit einer Irrtumswahrscheinlichkeit von 1% gesichert. Das schnellere Vergessen der Vt kann hier nur auf den Einfluss der Aggression zurückgeführt werden.

Das Wirksamwerden der Aggression auf die Lern- und Gedächtnisleistungen kann durch mehrere Faktoren bedingt sein: a) durch retroaktive Hemmung infolge der Kampfaktivität, b) durch eine mit dem Aggressionsverhalten auftretende zentralnervöse Erregung, c) durch eine infolge der Erregung und der aggressionsspezifischen Aktivität auftretende Intensivierung des Stoffwechsels, die zu einem schnelleren Abbau von Engrammen führen kann.

Summary. Betta splendens-♂♂ were tested as to whether aggression-specific stimulation had any influence on learning and retention of a simple colour discrimination task. In comparison with control animals, the stimulated animals showed worse results in learning and retention.

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15. Oktober 1973.

Mitotic Activity in Lemon Fruit Explants (*Citrus limon* L.) Incubated on a Calcium-Potassium-Sucrose Medium

Changes in nucleolar morphology and starch production have been considered as being early cytological and physiological indications of growth in lemon fruit explants cultured on a mineral-sucrose nutrient medium[1]. Subsequent investigations have shown that these same cytological and physiological phenomena can be brought about by injury alone in complete isolation from any exogenous source of nutrient materials or growth-promoting substances[2-5]. Although lemon juice contains sugars, nitrogenous compounds, vitamins, and the six macroelements and most of the microelements known to be essential for plant growth[6,7], mitotic activity has not been described in lemon fruit explants incubated on anything less than basal mineral-sucrose nutrient media with or without growth-promoting substances[8-13]. Thus all materials required for the manifestation of starch production and changes in nucleolar morphology are available from endogenous sources within the explants themselves whereas this is not the case with respect to the material requirements needed for the manifestation of mitotic activity (Figure 1).

Citric acid is the most abundant organic acid in lemon juice[6] and is known to be a strong chelating agent for Ca[14]. The occurrence of Ca and K in lemons in combination

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with organic acids such as citric, malic, and oxalic and the relationship between high K and total acidity in the fruits[6] suggested the possibility that the Ca and K present in lemon juice may not be available endogenously for the manifestation of mitotic activity in explanted juice vesicle tissue by virtue of the close association of these ions with the organic acids present in the juice vesicle cells. In view of a) the relationship between Ca and K and the organic acids present in lemon juice[6], b) the importance of K in growing tips of plants[15], and c) the possible roles of Ca in mitotic activity[16] and in chromosome structure[17], it was considered to be of interest to examine nuclear behaviour in lemon fruit explants incubated on a Ca-K-sucrose nutrient medium.

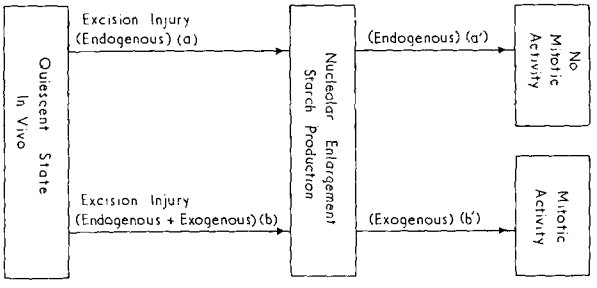

Fig. 1. Diagrammatic representation of cytological and physiological changes which occur in entire lemon fruit juice vesicles (sac plus stalk) after exicsion from the fruit and maintained under endogenous and exogenous nutrient conditions. a) First 48 h after excision from the fruit and maintained under endogenous nutrient conditions; a') continued exposure of the explants to completely endogenous nutrient conditions; b) first 48 h following excision from the fruit and maintained on an exogenous source of nutrients; b') continued exposure of the explants to the exogenous source of nutrients.

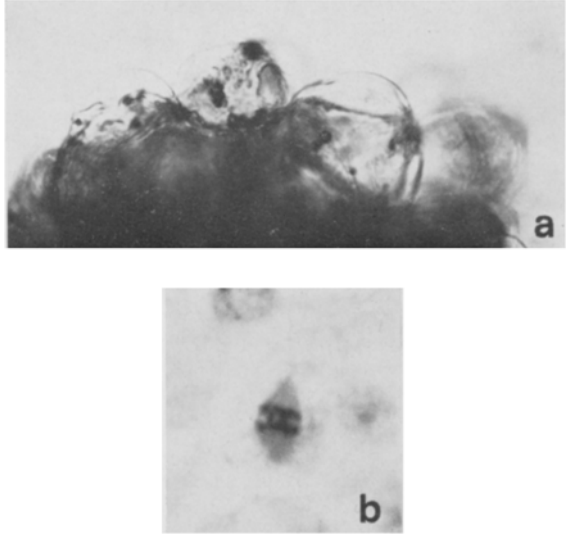

Fig. 2 a) Cells of a callus outgrowth from a 17-day-old stalk incubated on the Ca-K-sucrose medium. ×325. b) Mitotic figure from a 17-day-old stalk incubated on the Ca-K-sucrose medium. ×1800.

Materials and methods. Entire juice vesicles (sac plus stalk) were removed aseptically from firm mature yellow lemon fruits with green buttons (*Citrus limon* L.) as described previously[3] and placed on thin layers of the following liquids in glass Petri dishes immediately upon removal from the fruit: a) glass-distilled water; b) 4% sucrose; and c) 2 mM/l $CaCl_2$-2 m*M*/l KCl-4% sucrose. The stalks were severed from the sacs, the sacs were discarded, and the stalks were transferred to glass Petri dishes containing: 1. 1% Oxoid agar No. 3; 2. 1% Oxoid agar No. 3 plus 4% sucrose; and 3. 1% Oxoid agar No. 3 plus 2 m*M*/l $CaCl_2$-2 m*M*/l KCl-4% sucrose. The stalks in each of the treatments were overlayed with the following liquids (see reference [10] for liquid overlay technique): 1. glass-distilled water; 2. 4% sucrose; and 3. 2 m*M*/l $CaCl_2$-2 m*M*/l KCl-4% sucrose. All Petri dishes of all 3 treatments were sealed with 'Parafilm' and placed in the dark at 25–27 °C.

After 14–21 days of incubation, the explants were harvested for examination and placed in Lillie's AAF solution[18], embedded in paraffin wax[3], and sectioned at 10–12 µm. The sections were dewaxed, brought down to water, stained with aceto orcein-fast green[19], dehydrated in cellosolve[9], cleared in xylene, and mounted in 'Sira' mountant or Canada balsam.

Results and discussion. Definitive callus outgrowths as well as mitotic figures were evident in the stalks of the 3rd treatment after 14–21 days of incubation on the Ca-K-sucrose medium (Figures 2a and b) whereas there were no callus outgrowths or mitotic figures in the 14–21-day-old explants of the 1st and the 2nd treatment. Thus the results presented here show that excised vesicle stalks from mature lemon fruits are capable of manifesting mitotic activity when incubated on an exogenous nutrient medium far simpler in composition than any hitherto employed.

An average batch analysis of Oxoid agar No. 3 shows that this material may contain significant quantities of inorganic elements which are known to be essential for plant growth[20]. It is possible, therefore, that the tissue explants were being supplied with one or more of these minerals from the agar itself. However, the apparent absence of callus outgrowths as well as the absence of mitotic figures from the 14–21-day-old explants in the 1st and 2nd treatment indicate that whatever nutrient material might have been supplied by the agar itself, it evidently was not sufficient for bringing about mitotic activity in the cells under the experimental conditions employed here. Consequently, the manifestation of mitotic activity in lemon fruit vesicle stalks incubated on the Ca-K-sucrose medium in the 3rd treatment lends support for the suggestion that the Ca and K present in lemon juice may not be available endogenously for the manifestation of mitotic activity in explanted entire juice vesicles isolated from all exogenous nutrients by virtue of the close association of these ions with the organic acids present in the juice vesicle cells.

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The purpose of the present investigation was not that of stimulating or inducing mitotic activity in lemon fruit explants as an end in itself but rather to investigate the relationship between the endogenous and exogenous nutrient factors associated with the transformation of quiescent cells in vivo into mitotically active cells in vitro. Thus the principle of thought underlying the present investigation is well expressed by the following quotation from FILNER[21]: 'Wouldn't it be nice to have systems in which a developmental event is initiated by a metabolite, particularly one whose biochemistry is well known? Then, with a little bit of luck, perhaps the site of initiation and the chain of biochemical steps leading to the developmental event would be found in the known biochemistry'[22].

Zusammenfassung. Nachweis einer Mitose-Aktivität bei Zitronenfrucht-Gewebeschnitten, die auf einem Calcium-Kalium-Saccharose-Medium ohne andere bekannte Nährsubstanzen bebrütet wurden.

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[22] I thank the Dr. HADWEN, Trust For Humane Research and the Air Chief Marshall The Lord Dowding Fund For Humane Research for funds received in support of this research.

Observations on Nucleolar Staining with Osmium Tetroxide

A simple technique for nucleolar staining in plant cells, based on the osmium tetroxide (OsO_4) fixation, has been reported by BATTAGLIA and MAGGINI[1]. The application of this technique has allowed us to study the topographic distribution of the osmiophilic component in normal and segregated nucleoli of *Allium cepa* meristematic cells[2,3]. The mechanism of nucleolar staining with OsO_4 remains unknown, although a reaction between OsO_4 and unsaturated lipids has been suggested[1,4]. We here present some observations in relation to a mechanism which could account for nucleolar staining with OsO_4.

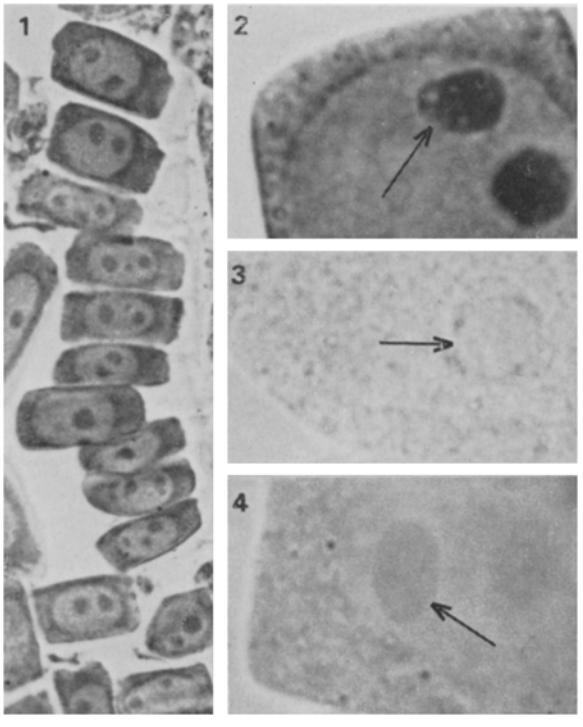

Fig. 1. *Allium cepa* root-tip cells after osmium staining.
Fig. 2. Greater magnification of an osmium-stained cell, showing the high contrast of the nuleolus (arrow).
Fig. 3. RNase digestion before OsO_4 fixation. Notice the unstained nucleolus (arrow).
Fig. 4. Ethanol fixation and tryipsin digestion before osmium staining. The nucleolus appears slightly stained (arrow).

Materials and methods. Roots of *Allium cepa* bulbs growing in tap water at room temperature were fixed with OsO_4 for 3–10 min in one of the following solutions: a) 1% in distilled water; b) 5% in distilled water; c) 2.5% in 0.1 *M* phosphate buffer at pH 7.0, and d) 2.5% in 0.4 *N* NH_3. Other fixatives were also used: e) 5% formaldehyde in 0.1 *M* phosphate buffer at pH 7.0 for 30 min, followed by OsO_4 as in b); f) 1 h in 96% ethanol, followed by 50% and 25% ethanol, distilled water and then OsO_4 as in b). After fixation and washing in distilled water the samples were heated: g) at 60 °C during 15 min; h) at 80 °C during 5 min; i) at 100 °C during 2 min, or j) treated 10 min with 1% *p*-phenylendiamine in 70% ethanol[5].

Before the OsO_4 treatment, extractive procedures and enzymatic digestions were carried out as follows: k) RNase, 1 mg/ml in 0.1 *M* phosphate buffer at pH 7.0, 1–2 h at room temperature or at 37 °C, followed by washing in 5% cold perchloric acid for 10 min; l) trypsin, 1 mg/ml in distilled water at pH adjusted to 8 with 0.01 *N* NaOH, 1 h at 37 °C; m) 5% perchloric acid, 16 h at 4 °C; n) absolute ethanol 1 h, followed by chloroform for 3–48 h, and then absolute, 96%, 70% and 50% ethanol; o) 5 *N* HCl, 1 h at room temperature; p) DNase, 1 mg/ml in 0.1 *M* phosphate buffer at pH 7.0, 2–4 h at 37 °C.

After all these procedures the root-tips were flattened in 1–2 drops of distilled water. The Feulgen reaction was carried out in roots previously fixed for 10 min as in b). After a 10 min hydrolysis in 1 *N* HCl at 60 °C, the roots were treated with the Schiff's reagent for 30 min. Some samples fixed as in c) and treated as in g) or j), were dehydrated in alcohols and embedded in Maraglas. Thin sections were obtained with a Porter Blum microtome and observed in a Zeiss 9A electron microscope without staining. Other sections were stained with uranyl acetate and lead citrate as usually.

Results and discussion. The observations are summarized in the Table. Any of the OsO_4 fixations, followed by any of the heating procedures or by the treatment with

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