Our main research concerning the development of the central nervous system is performed on the cerebral hemispheres from chick embryos. For this purpose 7-day-old embryos have so far been used[3], but sometimes it may be of interest to vary the age of the embryo at the start of the culture to study certain aspects of differentiation. It has been found that when using 7-day-old embryos, optimum culture development is obtained by using 1 hemisphere per 8 ml medium with collagen as substrate, and 1 hemisphere per 4 ml when cultivating directly on a plastic surface (SENSENBRENNER, personal communication). From our results, these are equivalent to 3.5 and 7 million cells/ml medium respectively. To obtain the same density for all embryonic ages when cultivating on collagen, the amount of medium to be used was calculated for each case, and the results are shown graphically in Figure 2. These data can be used as a guideline when setting up cultures from embryos of different ages. When cultivating on plastic alone, the above quantities of medium should be halved[4].

Résumé. Le nombre total de cellules obtenues après dissociation du cerveau d'embryons de Poulet de divers âges a été déterminé au «Coulter Counter». La quantité de milieu nutritif nécessaire pour avoir un nombre constant de cellules dans la suspension a été calculée pour chaque âge embryonnaire, afin de pouvoir réaliser des cultures comparables.

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Suspension Culture of *Nigella sativa*

Cell suspension culture is an excellent system to study different cellular mechanisms. The behaviour of the suspended cells of *Nigella sativa* is reported here.

Materials and methods. Cell suspensions of leaf callus tissue of *N. sativa* (Fam. Ranunculaceae; $2n = 12$) were grown in 50 ml MURASHIGE and SKOOG's (MS) medium[1] supplemented with NAA (0.5 mg/l) and coconut milk (15% v/v from fresh green coconut) in projecting flasks (1 l) on revolving shaker (4 rev/min)[2] in dark at 25° ± 1 °C. The medium was sterilized at 121 °C for 20 min. Cells were counted in a counting chamber with dimensions of 50 × 20 × 1 mm and a capacity of 1 ml. The suspended cells in 10 random fields were counted at a magnification of ×100. Suspension culture was initiated by transferring 0.8–1 g 2-week-old callus tissue to the 50 ml liquid medium. During subculture, 5 ml of the suspension from the previous passage was inoculated to a count of 800–900 cells/ml at the onset of the experiments. Cells were subcultured at 30 day intervals. Chromosomes were stained with 1% acetocarmine directly. Coconut milk alone, or in combinations with NAA/2,4-D, was tested to see if there was any increase in the number of free cells.

Results. A good number of free cells were obtained in Ms + NAA (0.5 mg/l) + CM medium, whereas 2,4-D supported clone formation (Table I). In presence of casein hydrolysate or coconut milk, there was considerable increase in total cell number (Table II). A unique suspen-

Fig. 1. Cell suspension of *Nigella sativa* with mostly elongated cells.

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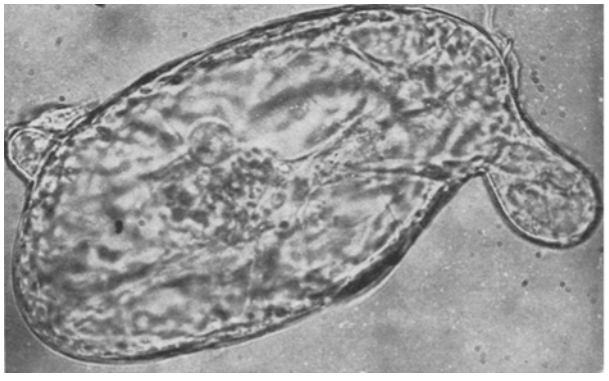

Fig. 2. A free cell of *Nigella* undergoing division by budding.

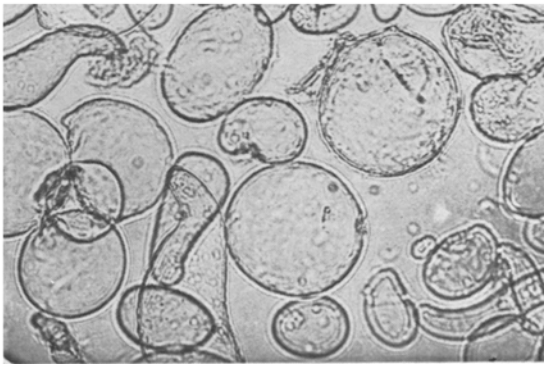

Fig. 3. Cell suspension of *Nigella* with a majority of spherical cells.

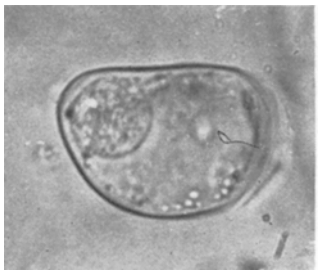

Fig. 4. A small *Nigella* cell with a large nucleus and starch grains.

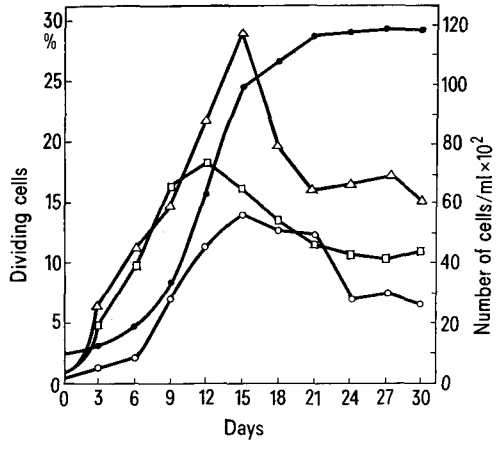

Fig. 5. The number of cells and the occurrence of mitosis in free cells, small clumps and tissue pieces in suspension culture of *Nigella sativa* grown in MSNCHCO medium for 30 days. Mitotic count represents the percent of cells in mitosis of 1000 cells for each sample. △—△, small clumps; □—□, tissue piece; ○—○, free cells; ●—●, growth curve.

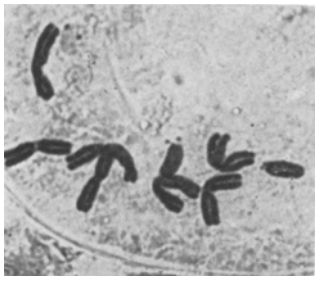

Fig. 6. Metaphase plate of *Nigella* cell with 9 chromosomes.

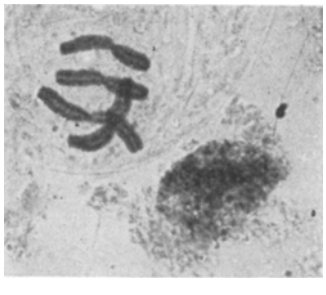

Fig. 7. Metaphase plate of *Nigella* cell with 4 chromosomes.

sion with 90% free cells (Figure 1) was obtained in WHITE's medium[3] containing casein hydrolysate (100 mg/l) inositol (50 mg/l) and adenine sulphate (2 mg/l).

In suspension culture cells were divided by cell plate formation, budding (Figure 2) or by internal septation. In MS and WHITE's medium generally spherical cells (Figure 3) were obtained but when WHITE's medium was supplemented with CH (100 mg/l) + inositol (50 mg/l) + adenine sulphate (2 mg/l), 98% cells were elongated (Figure 1). Besides the parenchyma cells, small cells (8–15 μm) with dense cytoplasm and heavy starch grain appeared (Figure 4).

Frequency of cell-division reached a maximum between 10–15, 12–16 and 18–22 days by tissue piece, small clumps and free cells respectively (Figure 5). Percentage of division was maximum (29%) in small clumps (5–10 celled). The ploidy distribution patterns in free cells and cell clumps of different sizes were similar (Table III). Percentage of aneuploids (Figure 6) was significantly more (p 0.01) in free cells. Minimum chromosome number observed was 4 (Figure 7). The small starch-filled cells were mostly diploid.

Embryogenesis in *Nigella* cell suspension culture was observed only in W + CH + Ino + Ad medium at the end of third passage after subsequent omission of NAA and coconut milk from the MSNCo medium and with subsequent addition of casein hydrolysate (Table IV). 8–12 embryoids/ml (Figure 8) of suspension were obtained. Further development of the embryoids occurred in the agar medium of the same composition.

Discussion. Nigella tissues were fast growing and friable in nature. Amongst the different media used, W + CH + Inositol + Adenin sulphate was suitable for accelerating cell separation and preventing clump formation. Presence of casein hydrolysate in the medium usually favoured cell separation. Requirement of coconut milk in suspension culture was reported by BAJAJ and DIONNE[4]. But we observed with *Nigella* tissue that omission of coconut milk favoured cell separation. In suspension culture, as the cells were usually free from the restraint of surrounding cells and even from the formative influence of tissue mass, variations in cell size and shape were very common. The degree of variation and proportion of a particular type of cell in suspension culture has been attributed to species difference[2,5,6]. In *Nigella* suspension cells were mostly spherical, but when the same culture was grown in WHITE's medium containing casein hydrolysate, inositol and adenine, most of the cells were elongated. This indicated that different nutrients in the medium might have some influence on cell nature. In suspension culture where 108–116/ml small cells (8–15 μm) were present, embryogenesis was observed. The possible role of the small cells has also been considered by STEWARD et al.[7] and REINERT et al.[8]; 3–5% division in the free cells clearly indicated that single cells in culture were capable of undergoing mitosis, and their percentage could be enhanced by adequate supplements of proper nutrients. Variation in chromosomal complement may be due to lagging chromosome, unequal separation and bridges etc. In free cells, more irregularities were observed as they

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Table I. Average No. of free cells, cell aggregates and total No. of cells during 21st and 24th day of culture period

Media	Free cells/ml × 10^2	Cell aggregates/ml × 10^2					Total No. of
		2-celled	3–5 celled	5–10 celled	10–50 celled	Above 50 celled	cells/ml × 10^2
MS + CM	10.60	1.50	2.10	2.52	1.44	0.48	117.50
MS + CM + NAA (5 mg/l)	13.72	2.00	2.40	2.80	1.20	0.31	112.60
MS + CM + NAA (0.5 mg/l) + CH (100 mg/l)	18.80	4.20	3.00	2.91	1.22	0.38	127.30
MS + CM + 2,4-D (0.05 mg/l)	0.63	0.21	0.25	0.66	1.76	0.50	87.70
W + CH (100 mg/l) + Ino (50 mg/l) + Ad (2 mg/l)	79.30	5.32	1.80	0.09			96.60

MS, MURASHIGE and SKOOG's medium; W, WHITE's medium; CM, 15% v/v coconut milk; CH, casein hydrolysate; Ino, inositol; Ad, Adenine sulphate.

Table II. Total cell count of *Nigella sativa* in different media during 30-day suspension culture period

Media	Approximate total No. of cells/ml × 10^2 after each 3 days intervals										
	0	3rd	6th	9th	12th	15th	18th	21st	24th	27th	30th
MS + CM	10.2	12.0	18.8	34.2	64.5	100.1	108.2	117.5	117.6	118.0	118.3
MS + CM + NAA (0.5 mg/l)	10.0	11.7	15.1	31.4	59.9	86.3	101.5	112.4	112.6	113.1	113.6
MS + CM + NAA + CH (100 mg/l)	9.8	11.2	14.4	38.0	75.0	112.4	120.5	126.4	128.2	130.8	133.1
MS + CM + 2,4-D (0.05 mg/l)	10.3	11.0	13.2	25.9	57.6	83.5	85.3	86.2	89.2	89.2	89.3
W + CH (100 mg/l) + Ino (50 mg/l) + Ad (2 mg/l)	9.5	11.0	14.3	28.7	61.1	87.4	93.1	95.5	97.8	98.2	98.4

For abbreviations see Table I.

Table III. Percentage of different ploidy cells in free cells and cell clumps

Material	Ploidy level									
	n	*2n*	*3n*	*4n*	*5n*	*6n*	*7n*	*8n* + ab.	Aneuploid	Total
Free cells	5.36	30.36	10.71	98.57	1.79	—	1.79	5.36	16.07	224
5–10 celled clumps	8.70	60.69	6.52	15.22	—	—	—	2.17	6.70	230
10–50 celled clumps	4.50	51.20	9.55	18.00	2.25	4.50	—	4.50	5.25	250
Tissue piece	9.50	53.20	7.25	11.73	—	—	3.53	8.15	5.00	411
Small starch filled cells (8–15 μm)	4.67	93.33	—	2.00	—	—	—	—	—	75

Table IV. Schematic representation of sequential treatment and its time in *Nigella* cell suspension culture

Treatment	Time in passages	No. of small cell (8–15 μm/ml)	No. of globular embryo/ml
MSNCO	2	10–30	Nil
↓			
MSCO	2	40–60	Nil
↓			
MSCHCO	2	80–101	16 compact celled stage
↓			
MSCH	1	108–116	16 compact celled stage
↓			
WCHInoAd	3	108–116	4–6

MSNCO, MURASHIGE and SKOOG's medium + 0.5 mg/l NAA + 15% v/v coconut milk; MSCO, MS + 15% v/v coconut milk: MSCHCO, MS + Casein hydrolysate (100 mg/l) + 15% v/v coconut milk; MSCH, MS + casein hydrolysate; WCHInoAd, WHITE's medium + casein hydrolysate (100 mg/l) + inositol (50 mg/l) + Adenine sulphate (2 mg/l). Passage + 30 days growth period.

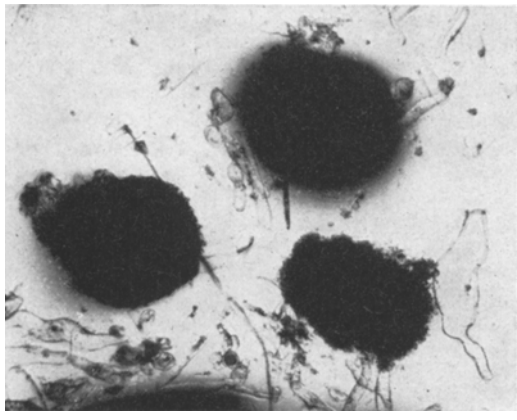

Fig. 8. Globular embryo in the suspended cells.

were in direct contact with the environment and free from physiological restraint of others. Limiting factors controlling differentiation in callus tissue were assumed to be due to auxin/kinin ratio[9,10] or sequential omission of auxin and kinetin from the basic medium[11]. In the suspension culture of *Nigella*, embryogenesis was obtained by omission of auxin and kinetin from the Ms + NAA + CM medium and sequentially growing the cells in different media as described.

Summary. In suspension culture 91% free cells of *Nigella sativa* was obtained in WHITE's medium supplemented with casein hydrolysate, inositol and adenine. Ploidy distribution pattern was similar in cell clumps of different sizes and free cells. Chromosomal irregularities were more in free cells. A number of globular embryoid were formed when casein hydrolysate, inositol and adenine were added in the medium after subsequent omission of auxin and coconut milk.

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Mitoseaktivität und Zellzyklus unter Begasung mit subletalen SO_2-Konzentrationen bei der Wasserlinse (*Lemna minor* L.)

Effects of Low SO_2 Concentrations on the Mitotic Activity and on the Cell Cycle of Duckweed (*Lemna minor* L.)

Niedere SO_2-Konzentrationen in der Luft vermögen in Pflanzen nachweisbare stoffwechselphysiologische Umstimmungen hervorzurufen[1]. Wirkungen auf die Meristemtätigkeit und auf den damit verbundenen Metabolismus sind selten untersucht worden, obschon diese Bereiche von zentraler Bedeutung für das Überleben einer Pflanzenart in immissionsgefährdeten Gebieten sind. Die wenigen bekannten Befunde bekräftigen diese Feststellung. In den vergangenen Jahren wurde berichtet von einer mutagenen Wirkung von Na-bisulfit[2], von einer Störung der DNS-Replikation[3], von einer Blockierung der Phenylalanin-tRNS[4], von einer Erhöhung der Chromatidenbruchrate[5], von einer Limitierung des Thymidineinbaus und Reduktion der Mitosekerne[6] und von einer Hemmung einer Nukleosidphosphotransferase[1].

Die Wasserlinse (*Lemna minor* L.) hat sich in verschiedener Hinsicht als geeignete Indikatorpflanze für die Auswirkungen niedriger SO_2-Immissionen erwiesen[7,8]. Sie wurde daher, obschon ihre Morphologie noch viele Rätsel aufgibt und sie zytologisch schwer zu bearbeiten ist, herangezogen um die Mitoseaktivität und den Zellzyklus unter Begasung mit subletalen SO_2-Konzentrationen zu überprüfen.

Die Anzucht und die Begasung der Versuchspflanze ist andernorts beschrieben[7,9]. Die zytologischen Untersuchungen wurden an 5 µm Dünnschnitten und an Quetschpräparaten durchgeführt. Als geeignete Farbstoffe haben sich Chrysoidin, Hämatoxylin «Delafield» und basisches Fuchsin erwiesen[10]. Die stark gefärbten Partien setzen sich aus verschiedenen Meristemen zusammen. Sie umfassen die basalen Pole der jungen Glieder, die Seitenknospen in den lateralen Taschen und die rückwärts gelegenen Adventivwurzeln. Beim Lemnenglied handelt es sich um das ehemalige Apikalmeristem, das infolge frühzeitiger Abflachung zu einem Phyllokladium umgewandelt wird[11]. Die Präparation der Mikroautoradiographien erfolgte nach dem Strippingfilmverfahren[12]. Für die 1 h Pulsfütterung wurde ^{3}H-markiertes Thymidin verwendet (1 µCi/ml Nährlösung, spez. Aktivität 6,7 Ci/m*M*).

Veränderungen des Kerndurchmessers und des Kernvolumens können bei gleichem Ploidiegrad als generelles Indiz gesteigerter oder reduzierter Kernfunktionen betrachtet werden[13]. Die Bestimmung des Kerndurchmessers in verschiedenen Meristembereichen begaster und unbegaster Wasserlinsen ergibt, dass die untergetauchte Adventivwurzel im Gegensatz zum «Apikalmeristem» kaum auf die Immission anspricht (Tabelle I).

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