neurons[11]. Decarboxylation of exogenous L-dopa to dopamine seems to be necessary for resultant displacement of the endogenous serotonin from the vesicular stores[7]. On the other hand, experimental data give evidence that the decrease in serotonin content of rat brain after administration of L-dopa may be caused by reduction of the concentration of its precursor, tryptophan[6]. These possibilities of interactions may also be true in the case of 5-OH-dopa as the compound is metabilized to 5-OH-dopamine[1].

Further interactions between the catecholamines, 5-OH-dopamine and dopamine, and the indoleamine, serotonin are also demonstrated by the uptake studies described here. Both catecholamines dose-dependently decrease the amount of tritiated serotonin accumulated by striatal tissue slices in vitro. It is assumed that the decrease in the uptake of 5-HT is not due to the inhibition of the uptake mechanism for 5-HT, but that displacement of serotonin by the catecholamines takes place at the storage sites. This assumption is supported by kinetic studies with striatal tissue slices of rats in vitro. These investigations indicate the presence of at least 2 distinct uptake mechanisms for serotonin, thereby showing that serotonin may also enter catecholamine containing neurons, depending on the concentrations of 5-HT present[12]. This non-specific uptake of 5-HT into catecholaminergic terminals is also indicated by experimental studies with isolated P_2-fractions from hypothalamus and corpus striatum of rats[13]. To what extent the decrease in serotonin accumulation by 5-OH-dopamine and dopamine is due to displacement of non-specifically accumulated serotonin from the catecholaminergic terminals, and/or due to discplacement of serotonin from serotoninergic terminals, needs further elucidation. Nevertheless, this problem seems mainly to be a question of concentrations both of the catecholamines and of the indoleamine.

Zusammenfassung. 5-OH-Dopa und 5-OH-Dopamin senken in vivo den Serotoningehalt und vermindern in vitro dosisabhängig die Aufnahme von ^{3}H-Serotonin im Striatum von Ratten. Es kann daher angenommen werden, dass 5-OH-Dopamin in vivo überwiegend eine Verdrängung von Serotonin aus den serotoninergen Speichern verursacht, während in vitro zusätzlich eine Verdrängung von unspezifisch in katecholaminerge Speicher aufgenommenem Serotonin auftreten kann.

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Tissue Culture of *Nigella sativa* I. The Behaviour of Nucleus

The stability of chromosome number throughout a culture regime of $3^1/_2$ years in callus tissue of *Nigella sativa* is reported here.

Materials and methods. Callus tissue derived from the stem of *Nigella sativa* ($2n = 12$) was grown in modified tobacco medium[1] supplemented with NAA (0.05 mg/l) and coconut milk 15% (v/v). The medium was solidified with 0.7% bacto agar. Friable strain with faster growth was selected and transferred to the liquid medium. Tissues were grown in 250 ml. Erlenmeyer flask containing 25 ml medium on a gyro-rotatory shaker (60 rpm). Chromosomes were stained with 2% acetocarmine directly after pretreatment for 30 min in 0.001% colchicine solution at 14 °C during the maximum division.

Results and discussion. The most common observation was the divergency in the chromosomal complement in the

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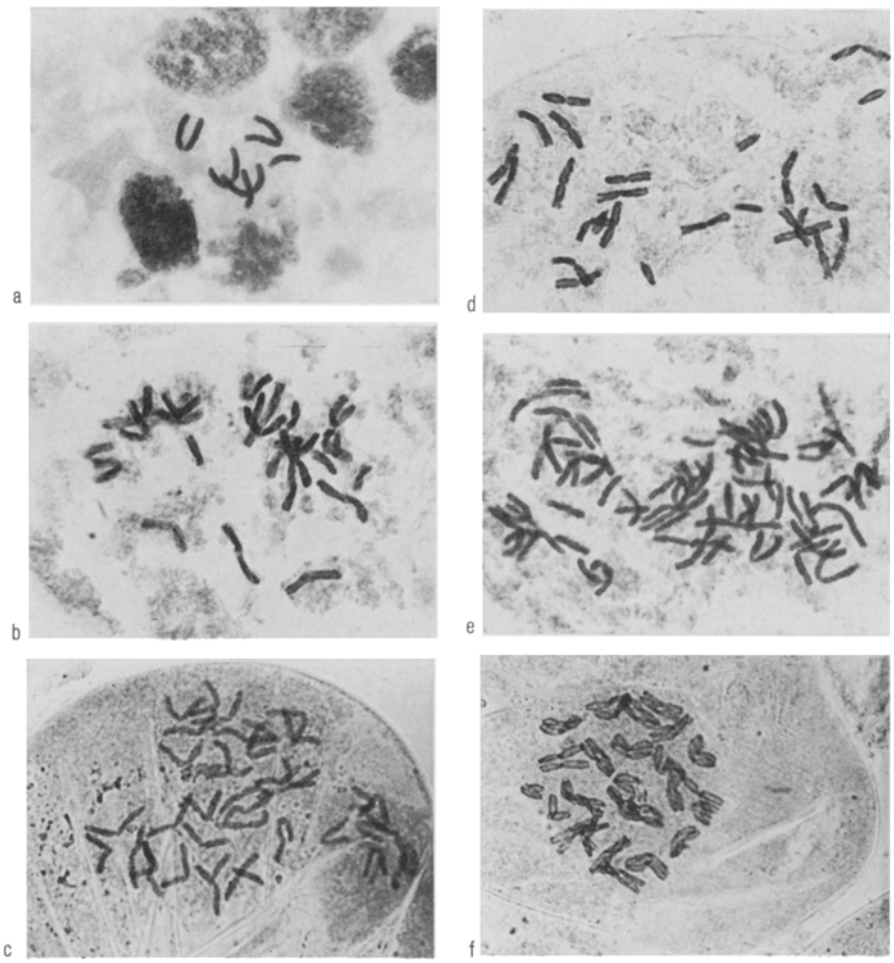
Percentage distribution of different ploidy cells at 6 month interval (total count 150–200 cells in each determination)

Tissue age in months	*n*	2*n*	3*n*	4*n*	5*n*	6*n*	7*n*	Aneuploid cells (including hypohaploid and higher than 7*n* cells	Cells with cytological irregularities[a]
1	6.10 ± 1.86	78.09 ± 3.23	—	12.20 ± 2.55	—	—	—	3.66 ± 1.46	5.22 ± 1.73
6	7.18 ± 2.08	64.41 ± 3.87	1.31 ± 0.91	13.07 ± 2.72	—	1.31 ± 0.91	1.31 ± 0.91	11.46 ± 2.57	9.48 ± 2.36
12	2.76 ± 1.56	65.81 ± 4.53	3.70 ± 1.89	7.41 ± 2.52	—	2.78 ± 1.58	—	16.51 ± 3.57	13.66 ± 3.30
18	4.90 ± 2.13	56.86 ± 4.90	7.84 ± 2.66	10.78 ± 3.07	—	—	1.20 ± 1.07	18.52 ± 3.84	15.13 ± 3.54
24	8.51 ± 2.34	53.19 ± 4.20	6.38 ± 2.05	11.35 ± 2.61	0.71 ± 0.70	2.13 ± 1.21	3.55 ± 1.55	14.18 ± 2.93	15.24 ± 3.02
30	10.05 ± 2.24	41.89 ± 3.68	4.47 ± 1.53	18.99 ± 2.93	0.57 ± 0.56	—	3.93 ± 1.45	20.10 ± 2.99	22.98 ± 3.14
36	8.40 ± 2.54	42.86 ± 4.53	7.56 ± 2.42	13.45 ± 3.12	—	—	1.68 ± 1.17	26.04 ± 3.45	17.13 ± 3.45
42	5.33 ± 1.69	46.66[b] ± 3.77	4.00 ± 1.48	14.67 ± 2.67	—	—	6.67 ± 1.88	22.67[b] ± 3.16	20.62 ± 3.05

[a] Total percentage of cells with unequal separation of chromosomes, lagging chromosomes and bridges at anaphase and micronuclei formation at telophase. [b] Significant at $p < 0.001$.



Photomicrographs of different ploidy cells of *Nigella sativa* at metaphase stage. a) Haploid ($n = 6$) × 600; b) Tetraploid ($4n = 24$) × 600; c) Heptaploid ($7n = 42$) × 600. d) Aneuploid cell with 26 chromosomes, × 600. e) Aneuploid cell with more than $7n$ chromosome number, × 700. f) Metaphase plate showing double the usual number of chromatids, × 415.

cell population as reported earlier in other materials[2–5]. In the present study, initially 78% of cells were diploid, 4% aneuploids with chromosome number ranging from 4 to about 96 per cell. while the rest were either haploids or tetraploids (Table and Figure). After about 42 months of culture the percentage of diploid cells decreased to 47 while that of aneuploids increased to 23. The differences in both the cases were highly significant ($p < 0.001$).

Chromosomal abnormalities such as lagging chromosomes, unequal separation of chromosomes, bridges occurred regularly and the frequency of such cells varied between 2.5 to 10%. Formation of micronuclei during telophase varied between 2.9 to 11.5%. In 1.5 to 2% of cells, double the usual number of chromatids were observed at metaphase stage (Figure f), suggesting the occurrence of endomitosis in *Nigella* tissue cells.

The heterogenity in chromosome number may be considered to be due to pre-extisting polyploid nuclei in the initial explant, which under favorable environmental conditions are capable of undergoing division. The use of auxins for initiating callus may also bring about division of such cells. The divergency thus induced is further enhanced by tissue culture method. In single cell cloned tissue where polysomaty should not normally occur, there was also a shift in the higher ploidy levels and aneuploids too[2,6,7]. This suggests, that pre-existing polyploid nuclei in the initial explant is not the only cause of chromosomal variability. The occurrence of polyploid nuclei may also be due to endomitosis, persistent chromosome bridges through telophase resulting in dumb-bell shaped nucleus, that ultimately form a single spherical nucleus with double the number of chromosomes and nuclear fusion in a multinucleate cell[8].

In tissue culture, occurrence of aneuploid cells was regularly observed, but in in vivo conditions rarely, possibly due to absence of suitable milieu and opportunity for cell division[9]. The aneuploid cells may possibly have arisen from mitotic aberrations described earlier.

Haploid and hyphaploid cells originated through gradual loss of chromosomes are generally considered nonviable. However, their occurrence in the present study, and also the report of WHITE[10], indicates that these cells remain viable at least for a few cell divisions.

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The change in chromosomal complement of tissue culture cells occurs slowly or rapidly with age[6,7,11]. In Citrus fruit tissue, culture change from essentially diploid cells to predominantly polyploid cells was brought about within 6 months[7]. But in the present investigation this shift in higher polyploids and aneuploids was brought about within 24 months.

Zusammenfassung. In Kallusgewebekulturen von *Nigella sativa* nimmt mit der Zeit der Anteil der diploiden Zellen ab, während sich die Anteile von aneupliden, von 2*n* abweichenden Zellen vermehren.

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Influence of Nutritional Factors on Chick Epidermal Differentiation

Differentiation of chick epidermis in vitro can be influenced by environmental factors such as the nutrients. Indeed, 5–6-day chick embryo skin keratinizes in a natural nutritional medium (chick embryo extract and chicken plasma)[1,2]; it does not if maintained with a protein-free chemically defined medium[3], which is able to support differentiation of 12-day embryo skin[3]. This different behavior suggests the possibility that embryo extract and/or adult serum contain differentiation promoting factor(s), which is (are) no longer necessary at 12 incubation days.

We have therefore examined the ability of adult serum, 6-day and 12-day chick embryo extracts to support development in vitro of 6-day incubation skin and the relationship between keratinization and cell division. To make comparison with these processes in vivo, we have also studied skin histodifferentiation in situ. As a criterion of proliferation we took the number of mitosis in the epidermis; as the criteria of differentiation, the epidermis structure, the presence of birefringent material and the presence and distribution of sulphidrils (SH)[3].

Methods. For studying normal differentiation in vivo, fragments from the thigh regions of 6-, 8-, 10-, 12-day White Leghorn embryos were used. Explants were derived from the same regions of 6-day chick embryos. Skin areas were removed under sterile conditions, rinsed in Tyrode's, carefully dissected and then placed in culture dishes on the vitelline membrane, according to WOLFF[4]. We carried out 5 sets of experiments using the following nutritional media: 12-day embryo extract and chicken serum (thereafter E_{12}+CS), 6-day embryo extract and chicken serum (E_6+CS), 12-day embryo extract (E_{12}), 6-day embryo extract (E_6) and chicken serum (CS) (see Table). Chick embryo extracts were prepared in our laboratories as follows: eyes removed and discarded, embryos pressed out through stainless steel grid in sterilized cylinder, equal volume of Tyrode's added, centrifuged and supernatant used.

In order to ascertain the importance of the presence of adult serum in nutritional medium, some cultures, after 24 and 48 h incubation in E_{12}+CS or E_6+CS, have been transferred to E_{12} or E_6.

Cultures were incubated at 37 °C for more than 6 days. Every day, tissues were fixed in Bouin's fluid and routine histological procedures were followed. Serial sections (8–10 μm) stained with hematoxylin-eosin were used for morphological examinations and thicker sections (25 μm mounted in 10% NaCl solution) for studying birefringence by means of a Leitz polarizing microscope. SH groups were localized with the ferric ferricyanide method according to CHÈVREMONT and FREDERIC[5]. Mitotic counts were performed in epidermal layers along the basement membrane, using an ocular micrometer.

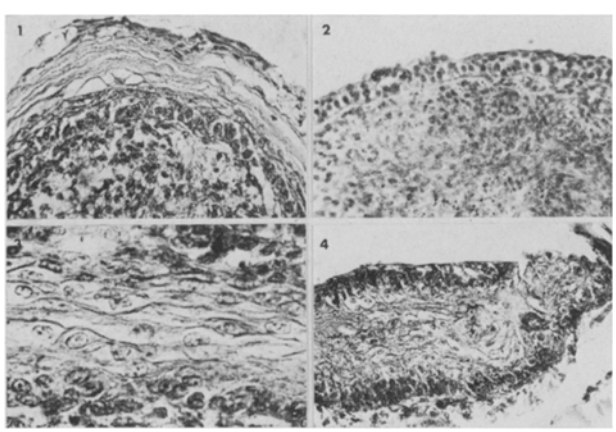

Fig. 1. 6-day skin explant after 6 days of incubation in E_{12} + AS. ×256.
Fig. 2. 6-day skin explant maintained in E_{12} for 6 day. ×125.
Fig. 3. 6-day skin culture after 4 days of incubation in CS; note the presence of a squamous layer. ×625.
Fig. 4. 6-day skin explant after 6 days in E_{12} nutritional medium to show the growth of the epidermis directly over the vitelline membrane (bottom) and the epidermal keratinization. ×125.

Composition of nutritional media

Media	Gelose 1% in Gey fluid	12-day embryo extract	6-day embryo extract	chicken serum (Difco)	Penicillin (10 Oxford U) in Tyrode's
E_{12} + CS	6[a]	3	–	3	1
E_6 + CS	6	–	3	3	1
E_{12}	9	3	–	–	1
E_6	9	–	3	–	1
CS	9	–	–	3	1

[a] Number of drops.

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