The fluorescence histochemical method developed by FALCK and HILLARP[4] was used to demonstrate the presence of the monoamines. The cultures were rapidly frozen in liquid nitrogen and freeze-dried for 1–2 days. After the freeze-drying procedure, the tissue was exposed to paraformaldehyde vapour at 80 °C for 1 h, afterwards rinsed in xylol for 5 min and mounted on slides. To differentiate between non-specific and specific monoamine-fluorescence, the following tests were performed. Reserpine (Fluka; 10^{-7} and $10^{-6}M$) was added to the nutrient medium of some cultures 24 h before the freeze-drying. Other cultures were treated with sodium borohydride (0.03–0.1% in 90% isopropanol for 2 min at 20 °C)[5] and a few cultures were incubated without paraformaldehyde vapour.

Fluorescence microscopy was performed with a Zeiss microscope WL. The light from an Osram HBO 200 Hg-lamp was passed through a Schott BG 38 filter (red absorption) and through a Schott BG 3 filter. The barrier filter in the microscope tube had a spectral transmission of > 500 nm.

Neurones developing specific fluorescence were observed in almost all the cultures treated with paraformaldehyde vapour. Although there was usually a high proportion of fluorescent cells, their number varied considerably between the different cultures. Monoamine-containing cells were found in the dense zone of the explant as well as in the zones of migration. It was often observed that cells in the marginal zones showed a weak or medium fluorescence, whereas cells in the dense zones of the explant developed a remarkably high fluorescence intensity. The monoamines were diffusely distributed in the cytoplasm of the cell body and in some processes the nuclei being non-fluorescent. No attempt was made to distinguish between catecholamine and 5-hydroxytryptamine fluorescence.

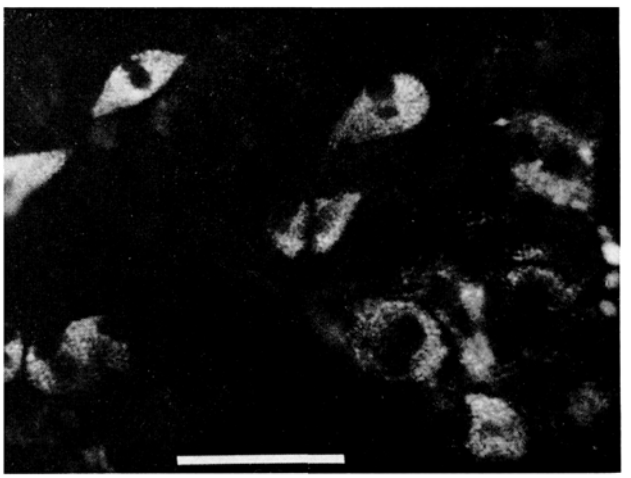
Figure 1 illustrates monoamine-containing neurones of brain stem tissue cultivated in vitro. The cells showed a strong greenish yellow to yellow fluorescence, whereas the plasma clot appeared white fluorescent. The fluorescence of the plasma clot but not of the cells was also present in cultures treated without paraformaldehyde (autofluorescence).

Addition of reserpine (10^{-7} and $10^{-6}M$ for 24 h) to the nutrient medium caused a marked decrease of the fluorescence of the neurones, whereas the fluorescence of the plasma clot was unaffected. Treatment of the cultures with sodium borohydride also markedly reduced the specific fluorescence which could be regenerated by reincubation in paraformaldehyde vapour.

In a series of cultures in which the cells were degenerated (due to toxic calf serum), no fluorescent neurones could be detected.

From these results, it appears that the observed fluorescence is due to the presence of monoamines, suggesting that neurones grown in vitro for several days are able to store and/or to synthesize monoamines[6].

Zusammenfassung. Monoaminhaltige Neurone konnten mit Hilfe der Fluoreszenz-Mikroskopie in Hirnstammgewebe, welches während mehrerer Tage in vitro gezüchtet wurde, nachgewiesen werden. Reserpin und Natriumborhydrid bewirkten eine deutliche Abnahme dieser Fluoreszenz. Diese Untersuchungen weisen darauf hin, dass Neurone, welche während mehrerer Tage in vitro gezüchtet wurden, die Fähigkeit haben, Monoamine zu speichern und/oder zu synthetisieren.



Brain stem neurones showing medium to strong yellow and greenish yellow fluorescence; culture of rat brain stem 14 days in vitro. Bar represents 50 µm.

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Cell Fusion as a Mechanism for the Origin of Polyploid Cells in vitro

In any mammalian cell culture a certain proportion of polyploid cells can usually be found. Since polyploid cells occur in vivo, their presence in culture may be regarded as the legacy of the original tissues. However, the frequent occurrence of endoreduplication and the discovery of cell fusion suggest that some polyploids arise de novo in cell cultures. The present communication suggests that tritium autoradiography may be used to detect the occurrence of cell fusion in homologous cell populations.

In studies on the sequence of DNA replication in cell cultures of *Peromyscus maniculatus* ($2N = 48$), we routinely expose the cells to tritiated thymidine at 1 µc/ml of growth medium for 2.5 to 3 h. Colcemid is introduced

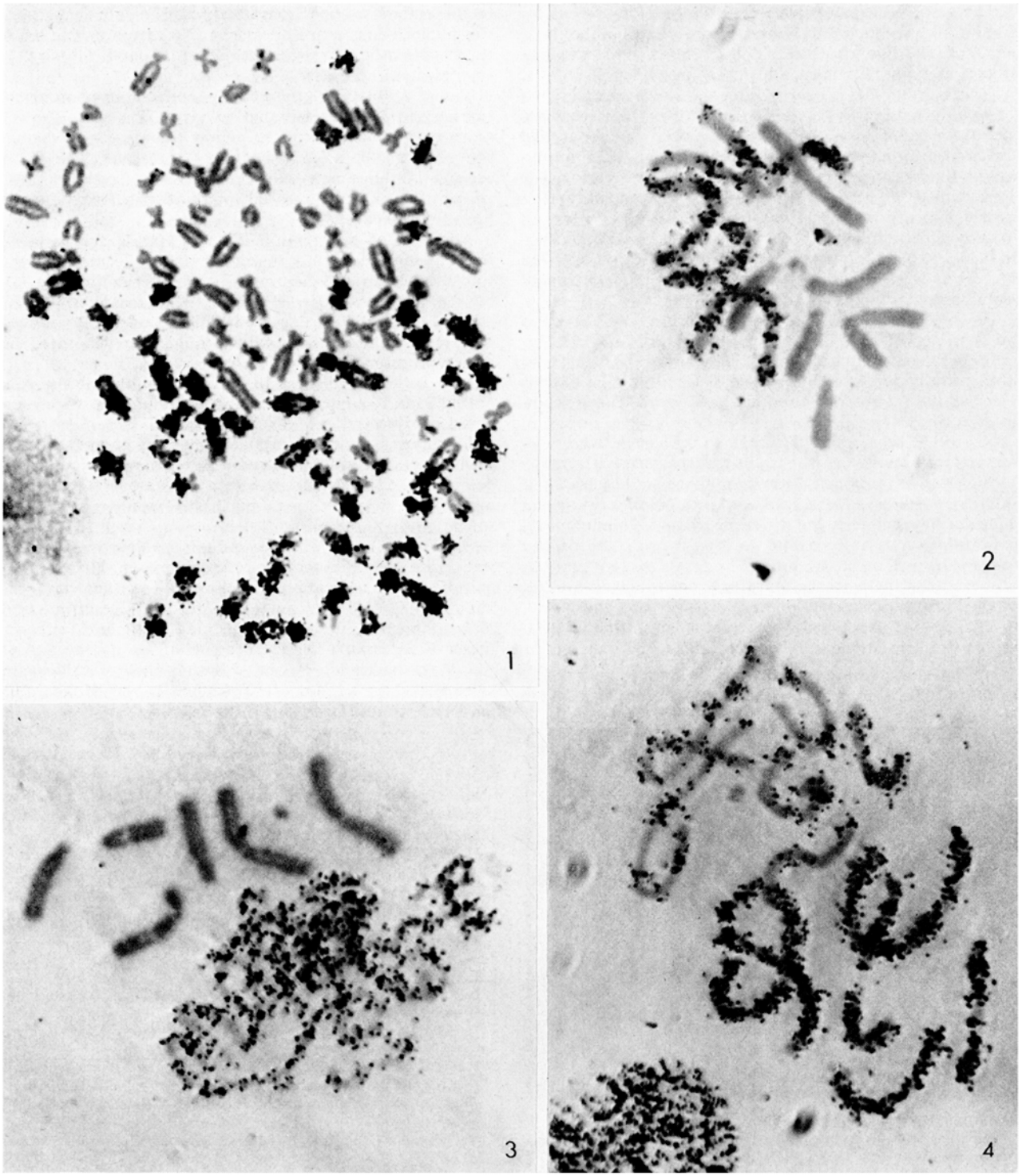

Fig. 1. Autoradiograph of a tetraploid metaphase from a cell culture of *Peromyscus maniculatus*. One diploid set of chromosomes heavily labeled and one set lightly labeled or unlabeled.

Fig. 2–4. Autoradiographs illustrating the types of abnormally labeled tetraploid metaphases from a cell culture of *Muntiacus muntjak*. Culture treated with H^3TdR for 30 min and collected 5 h later.

Fig. 2. Tetraploid cell with one diploid set of chromosomes labeled and one set unlabeled.

Fig. 3. Tetraploid cell with one unlabeled set of chromosomes in metaphase and one labeled set in prophase. The cytoplasm surrounding the cell is not reproduced in the photograph but can be observed microscopically. The possibility that this figure is two separate cells is also eliminated by the labeling pattern. The muntjac cells have a 2.5 h G_2 period, and at 5 h after H^3TdR addition all diploid metaphase cells are heavily labeled.

Fig. 4. Tetraploid prometaphase with one diploid set showing a terminal S labeling pattern, the other an earlier S pattern.

into the cultures 1.5 h prior to harvesting the cells, and autoradiography is used to reveal the late S labeling pattern. Occasionally in these autoradiographs, polyploid cells are found which show 2 different types of labeling within the cell, 1 for each diploid set as demonstrated in Figure 1.

This unusual labeling pattern is seen rarely in *Peromyscus maniculatus* cultures. However, in our studies on a male cell line of the Indian Muntjac, *Muntiacus muntjak* ($2n = 7$) established by WURSTER and BENIRSCHKE[1], this labeling pattern was observed frequently in polyploid cells. The culture had been pulse labeled with H^3TdR (2 μc/ml of growth medium) for 30 min. After labeling, the cells were rinsed with warm Hanks' solution and grown in fresh medium containing non-radioactive TdR (2 μg/ml). Samples were collected at 2 h intervals after labeling, and squash preparations were made for autoradiography.

Figures 2, 3, and 4 show polyploid cells containing 14 chromosomes which were collected at 5 h after H^3TdR addition. In Figures 2 and 3 one diploid set of chromosomes is labeled, while the other is not. In Figure 4 both diploid sets are labeled, but one shows an earlier DNA replication pattern than the other. The chromosomes in some of the diploid sets can also be differentiated by the degree of condensation. In Figure 2 the chromosomes with incorporated H^3TdR are 1.5–2 times as long as the unlabeled set, suggesting that one set lags behind the other in prophase coiling as well as in DNA synthesis. Figure 3 is an extreme example in this regard, with one diploid set in prophase and the other in metaphase. The frequency of occurrence of these heterophasic polyploid cells is presented in the Table.

These heterophasic cells are interpreted as the products of fusion between 2 diploid cells that are at different stages of the cell cycle. The 2 diploid elements may synchronize and enter S phase following the first mitosis after fusion. However, it is probable that some of these mitoses will be 'terminal' mitoses due to the inability of the 2 individual genomes to synchronize prior to division.

SANDBERG et al.[2] have described tetraploid mitoses in which one diploid set of chromosomes is labeled and one is unlabeled in a cell line derived from the blood of a patient with acute myeloblastic leukemia. In contrast to the Indian muntjac cells, the chromosomes within a given metaphase in the human cells did not vary in degree of condensation. The authors suggested that the polyploid cells observed originated from multinucleated megakaryocytes, plasma cells, or normoblasts.

Recently, JOHNSON and RAO[3] used synchronized HeLa cultures to show that fusion can be accomplished between 2 cells in different stages of the cell cycle. According to these authors, when G_2 or metaphase cells are fused with G_1 or S cells, an induction of chromosome condensation may occur resulting in aberrant chromosome morphology in the lagging nucleus. They called this phenomenon, which is very similar to the fragmentation induced by myxoviruses[4] or prolonged Colcemid treatment[5], premature chromosome condensation. Our observations confirm their conclusions that cells may fuse while in different stages of the cell cycle, but we found no examples of premature chromosome condensation. Perhaps this is because the fused cells we observed were not widely separated in the cell cycle. However, it is also possible that the technology employed by JOHNSON and RAO[3] (Colcemid treatment, double thymidine block, etc.) in synchronizing the cell populations resulted in metabolic imbalances which enhanced the occurrence of premature chromosome condensation. Under more normal growth conditions the induction of chromosome condensation in fused heterophasic cells may be reduced or absent[6,7].

Zusammenfassung. In Kulturen von *Peromyscus maniculatus* und in *Muntiacus muntjak* wurden Tetraploidzellen gefunden, die als Produkte von Zellfusionen aufgefasst werden. Es wird angenommen, dass die ursprünglichen diploiden Elemente sich im Zeitpunkt der Fusion in verschiedenen Phasen des Zellzyklus befunden haben.

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Frequency of heterophasic polyploid cells expressed as percent of mitotic cells[a]

Hour after H^3TdR pulse	Diploid mitotic cells (%)	Homophasic polyploids (%)	Heterophasic polyploids (%)
1	87.0	9.0	4.0
3	91.3	5.0	3.7
5	85.8	8.7	3.3

[a] Mitotic cells counted at each collection point = 250.

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Short Adrenergic Neurons Innervating the Female Urethra of the Cat

Fluorescence histochemistry has demonstrated that the uro-genital tract of different mammals receives an adrenergic nerve supply originating from ganglion formations located within or in the vicinity of the effector organ. This special type of short adrenergic neurons innervates the internal male accessory genital organs[1–3], to a certain extent the female reproductive tract[4–6] and the trigonum of the urinary bladder[7,8]. Besides, the