Qualitative fluorescent studies are highly misleading in this respect.

Sprague Dawley male rats weighing 150–250 g and bearing the Walker 256 carcinosarcoma were injected i.p. with 50 mg tetracycline/kg of body weight 2 and 3 days prior to sacrifice. Groups of rats were sacrificed 24, 48 and 72 h later.

The amount of necrosis in the Walker 256 carcinosarcoma increased with the age and the size of the tumor. Grossly visible tetracycline fluorescence was noted in viable and necrotic tumor, but appeared to be of greatest intensity in areas of early necrosis. Intense fluorescence was never observed in the actively growing margins of the tumor. Other tissues and visceral organs displayed essentially no visible tetracycline fluorescence. Ultraviolet microscopy revealed scattered focal areas of intense tetracycline fluorescence localized solely in necrotic portions of the tumor. No fluorescence was observed on microscopic examination of the other tissues.

Quantitative analysis of carefully separated portions of viable and necrotic areas of tumor uniformly revealed greater tetracycline concentrations in the necrotic regions (Table). Tetracycline concentration was determined in viable and necrotic portions of the tumor and several visceral organs including the liver, spleen and kidney. The quantitative spectrophotofluorometric determination of tetracycline was performed by a recently described technic[7]. The concentrations of tetracycline in the liver, spleen and kidney prove to be high compared to those found in the viable portions of tumor in the same animal. These values are only slightly lower than those found in the necrotic tumor tissue.

No gross or microscopic fluorescence was observed in areas of the tumor where hemorrhage had occurred. However, quantitative determination of tetracycline revealed higher concentrations of tetracycline in areas of hemorrhage than was present in several other tissues including viable tumor, where fluorescence was actually observed. This apparent discrepancy is partially attributable to the 'internal filter effect' of the blood pigments, which absorb the narrow band of the spectrum employed for exciting tetracycline fluorescence (excitation maximum 378 nm).

Abundant evidence already exists that complexing with calcium ion is necessary for tetracycline fluorescence in vitro[8] and it has also been suggested as contributory to fluorescence in vivo[9,10]. We, therefore, examined the role of ionic calcium in determining the apparent fluorescence of tetracycline in the tumor and other tissues.

A homogenate was prepared from portions of necrotic tumor which exhibited gross fluorescence in a pH 9.0 *Tris* buffer without calcium or barbital. The fluorescence was then measured quantitatively and compared with that of a homogenate of the same tissue at an identical concentration but prepared in a pH 9.0 *Tris* buffer solution to which disodium ethylenediamine tetracetate (EDTA) had been added in a final concentration of 0.063 molar. This concentration of EDTA effectively removes unbound calcium ion from solution. The amount of fluorescence observed in the necrotic tissue homogenate in buffer solution alone was significantly higher than that of the same homogenate prepared with buffer containing EDTA. This effect was reversable by the addition of excess calcium ion to the EDTA-containing homogenate, with restoration of fluorescence. Finally, the calcium concentration in necrotic and viable tumor tissue, which had been dried to constant weight and digested in concentrated nitric acid, was determined by atomic absorption spectrophotometry. Necrotic tumor had 8 times the calcium concentration of viable tumor; 6.65 vs 0.76 mg/g dry wt.[11].

Tetracycline concentration in various organs and experimental tumor in rats 24 h after parenteral administration of tetracycline

| Rat No. | Tetracycline concentration (γ/g) | | | | | |
|---|---|---|---|---|---|---|
| | TC Dose mg | Liver | Kidney | Spleen | Tumor | |
| | | | | | Viable | Necrotic |
| 1 | 7.5 | 8.00 | 10.60 | 4.40 | 5.40 | 18.10 |
| 2 | 3.0 | 10.75 | 12.80 | – | 3.60 | 13.20 |
| 3 | 5.0 | 9.80 | 8.90 | – | 3.50 | 12.79 |
| 4 | 12.5 | 6.95 | 9.52 | 4.48 | 2.16 | |
| 5 | 12.5 | 15.75 | 12.30 | 6.96 | 2.20 | |

*Zusammenfassung.* Quantitative Studien an Karzinomsarkomen von Ratten haben gezeigt, dass lebendes Krebsgewebe Tetracyclin nicht stärker anreichert als normales Gewebe.

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## Monoamine-Containing Neurones in Cultures of Rat Brain Stem

Fluorescence microscopic studies have shown that monoamine-containing neurones and nerve terminals are widely distributed throughout the brain stem of the rat[1,2]. We were therefore interested to investigate whether monoamines are also present in brain stem neurones cultivated in vitro.

The explants were prepared from tissue of the medulla oblongata and pons of 2–4-day-old rats, and were grown on a plasma clot on cover slips. The cultures were kept at 37 °C in Falcon plastic tubes filled with nutrient medium consisting of Parker's TC 199, 5% fetal calf serum, 10% bovine serum, glucose and antibiotics[3].

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.

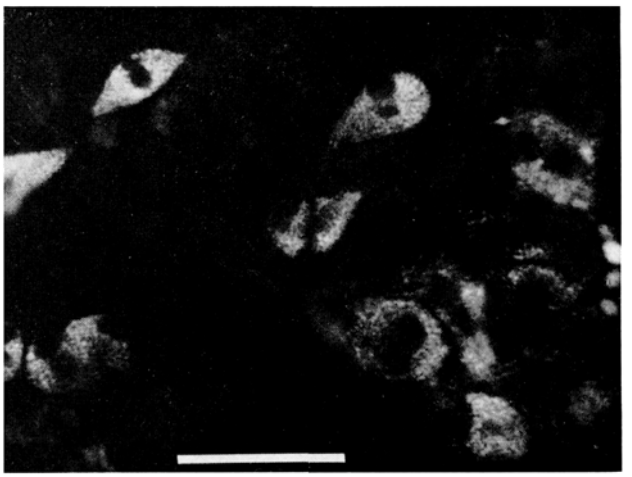
Figure 1 illustrates monoamine-containing neurones of brain stem tissue cultivated in vitro. The cells showed a strong greenish yellow to yellow fluorescence, whereas the plasma clot appeared white fluorescent. The fluorescence of the plasma clot but not of the cells was also present in cultures treated 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