The dysmorphogenetic effect of tryptophane on the developing chick embryo

Type	Total No.	No. dead	Normal embryos	Abnormal embryos			
				Limb deformities	Rumplessness	Visceral abnormalities	Total
Control I	10	1	9	—	—	—	—
Control II	10	—	10	—	—	—	—
Experimental	30	4	5	10 (33.5%)	4 (13.33%)	7 (23.33%)	21 (70%)

embryonic development. Imbalance in the amino acid pool may be caused by the excess addition of one amino acid which results in relative deficiency of the remaining amino acids. Since tryptophane is an essential amino acid, there is every reason to infer that excess addition of tryptophane will cause a relative deficiency of the other essential amino acids. It may tilt the nitrogen equilibrium of the essential amino acids and may cause a total imbalance in the amino acid pool, ultimately bringing about dysmorphogenetic effects.

Hence the incidence of abnormalities may be due to the imbalance of the essential amino acids in the amino acid pool that is available for the synthesis of proteins of the developing embryo, or may be due to the toxicity of high tryptophane content or its accumulated metabolic byproducts.

Zusammenfassung. Nachweis einer teratogenen Wirkung von Tryptophan beim Hühnerembryo als mögliche Folge einer Störung der Proteinbiosynthese.

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Localization of the Labelled 5-Azacytidine in Cultured Mouse Embryonic Cells

5-Azacytidine is a pyrimidine analogue which affects primarily the synthesis of RNA thereby interfering also with the formation of DNA and proteins[1,2]. It has been observed that the drug causes chromosomal aberrations[3], affecting the cells predominantly in the S phase[4–7]. In the present work, we have studied the uptake of 5-azacytidine-4-^{14}C into cultured mouse embryonic fibroblasts and we observed its localization over heterochromatin.

Materials and methods. 5-Azacytidine-4-^{14}C (33.7 mCi/mmole) was prepared in this Institute. The cells were maintained in Eagle's minimal essential medium containing antibiotics supplement with vitamins and 10% calf serum. The primary cultures were prepared from 13-day-old mouse foetuses. The cells were grown in Roux bottles (1×10^6 cells/ml) and 10^{-5} *M* 5-azacytidine-4-^{14}C (0.2 µCi/ml) was added after 18 h of cultivation at 37 °C. 1 h later the radioactive medium was removed and a fresh medium was added. The cells were harvested at 2-h intervals following 1-h exposure to colchicine (0.05 µg/ml). Cultures were fixed in acetic methanol after hypotonic treatment (0.075 *M* KCl) for 12 min at 37 °C. The monolayer was than dispersed in 60% glacial acetic acid and drops of cell suspension were placed on clean glass slides prewarmed to 56 °C. After drying the slides were washed (5 min) 3 times in 5% trichloracetic acid, rinsed with distilled water and coated with a stripping film Kodak AR. 10. The exposition at −20 °C lasted for2 months. Following the development and fixation the cells were stained by Giemsa stain in a phosphate buffer (pH 6.8). The preparations for the evaluations were photographed using Orwo-film NP 15. In each instance 25–30 individual karyotypes were counted. The localization of grains over individual chromosomes was registered separately over proximal (centromeric), medial and distal parts. The labelling over the proximal part was considered to correspond to constitutive heterochromatin whereas that over the distal part of chromosomes to euchromatin. The number of grains over the medial segment was constantly low in all instances where the labelling of this region was disregarded.

Results and discussion. The number of nucleoli in mouse fibroblasts and their size considerably varied during the experiment. The largest nucleoli were found 8 h after the removal of medium containing 5-azacytidine-4-^{14}C. The nucleolar enlargement following this analog has been also observed by Voigt et al.[8]. Furthermore it has been shown recently[9] that 5-azacytidine inhibits the maturation of 45 S precursor ribosomal RNA to 28 S and 18 S rRNA.

[1] A. Čihák and H. M. Rabes, Neoplasma, in press.
[2] M. Reichman and S. Penman, Biochim. biophys. Acta *324*, 282 (1973).
[3] V. Fučík, A. Michaelis and R. Rieger, Mutation Res. *9*, 599 (1970).
[4] L. H. Li, E. J. Olin, T. J. Fraser and B. K. Bhuyan, Cancer Res. *30*, 2770 (1970).
[5] B. K. Bhuyan, L. G. Scheidt and T. J. Fraser, Cancer Res. *32*, 398 (1972).
[6] B. S. Zain, R. L. P. Adams and R. C. Imrie, Cancer Res. *33*, 40 (1973).
[7] R. A. Tobey, Cancer Res. *32*, 2720 (1972).
[8] W. H. Voigt, E. A. Löbbecke and E. Grudmann, Acta histochem., Suppl. *13*, 165 (1973).
[9] J. W. Weiss and H. C. Pitot, Arch. Biochem. Biophys. *160*, 119 (1974).

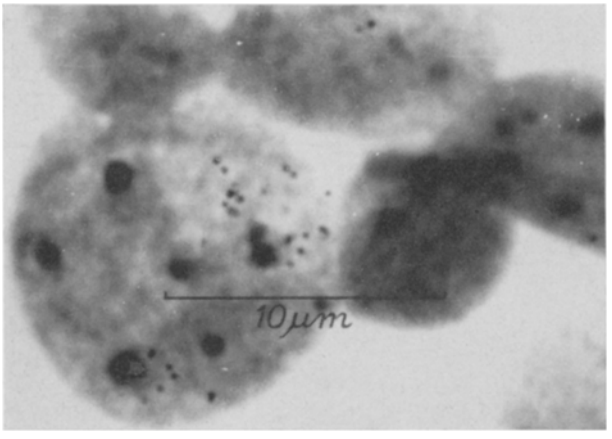

Fig. 1. The localization of the labelled 5-azacytidine in the nuclei of mouse embryonal fibroblasts. The label was concentrated close to the nucleoli or to the nuclear membrane.

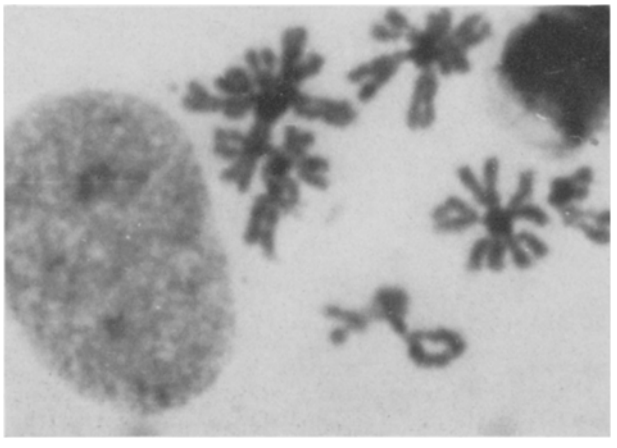

Fig. 2. The arrangement of chromosomes in 'star-shaped chromosomal associations' 6 h after the removal of the medium containing 5-azacytidine.

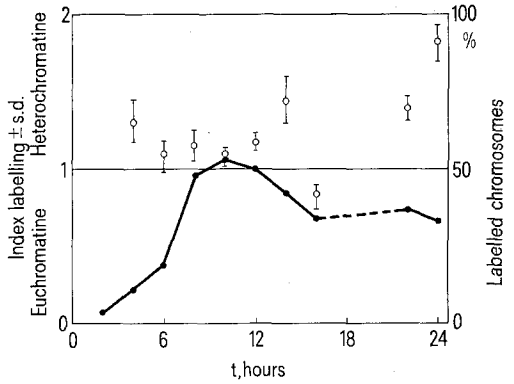

Fig. 3. The percentage of labelled chromosomes and the localization of the radioactivity in the chromosomes following the exposure to 5-azacytidine-4-^{14}C in vitro. The fraction of labelled chromosomes (continuous line) and the chromosomal localization of the label (individual points) are given as percent and as numerical indices (left and right ordinate, respectively). The numerical indices for the localization were obtained by dividing the number of chromosomes labelled over centromeres (heterochromatin) by the number of chromosomes labelled over telomeres (euchromatin). Abscissa: Recovery time.

At all the recovery time intervals the label was concentrated close to the nucleoli or to the nuclear membrane; this pattern was most marked 6 h after the removal of the radioactive medium (Figure 1). The localization of label over fibroblast nuclei after 5-azacytidine-4-^{14}C shows same pattern as that following the cytological hybridization of mouse liver cells with RNA complementary to mouse satellite DNA[10]. The presence of heterochromatin close to the nucleolar region has also been described by VAGNER-CAPODANO and STAHL[11] in fibroblasts from 8-day-old quail embryos.

Arrangement of chromosomes in metaphase plates follow 5-azacytidine changes depending on the recovery time. In controls there was a certain tendency for the centromeric heterochromatin to aggregate into clusters giving the appearance of several 'star-shaped chromosomal association'[12]. This phenomenon observed in controls is much more pronounced following 5-azacytidine, especially at 4–6 h after the removal of the drug (Figure 2). Thread-like extensions or 'fusions' of centromeres associated in some cases with decondensed heterochromatin are clearly noticeable. At 6 to 24 h achromatic gaps, elongated chromosomes or chromatids and fragmented chromosomes are seen. The chromosomal aberrations preferentially localized in the constitutive heterochromatin have also been described following the administration of mitomycin C[13] and benzimidazole[14].

The labelling and the localization of 5-azacytidine-4-^{14}C over the chromosomes is summarized in Figure 3. The highest percentage of labelled chromosomes has been observed at 10 h after the removal of the radioactive medium. Between 16–22 h the number of mitoses was extremely small and these values were not included in the Figure 3. Except 16–22 h following the exposure to the analogue the grains are more frequently observed over heterochromatin. On the contrary, at 16 h the label prevails over the euchromatin region and as already mentioned, mitoses diminish in number at 16 to 22 h. Apparently the cells which have taken up 5-azacytidine at drug-sensitive period of their generation cell cycle have undergone pycnotic degeneration. This contention is further strengthened by the presence of a large number of pycnotic cells in the cultures at respective time intervals. An analogous phenomenon was observed previously in mouse embryos in vivo[15,16]. Mitotic figures with chromosomes labelled prevalently over heterochromatin begin to reappear at 22 to 24 h. The percentage of labelled chromosomes remains relatively high. The cell division is evidently delayed or even completely blocked[17]. DEV et al.[18] identified individual mouse chromosomes on the basis of their fluorescence patterns and found that mouse satellite DNA was concentrated in centromeric heterochromatin and had been found to be replicating sooner. GONTCHAROFF[19] suggests that RNA which depends on the early synthesized DNA is essential for normal structure and function of nucleoli. Probably

[10] P. M. M. RAE and W. W. FRANKE, Chromosoma *39*, 443 (1972).
[11] A. M. VAGNER-CAPODANO and A. STAHL, Experientia *30*, 3 (1974).
[12] J. C. STOCKERT and J. A. LISANTI, Chromosoma *37*, 117 (1972).
[13] A. T. NATARAJAN, G. AHNSTRÖM and R. P. SHARMA, Mutation Res. *22*, 73 (1974).
[14] I. HILWIG and A. GROPP, Expl Cell Res. *81*, 474 (1973).
[15] M. SEIFERTOVÁ, J. VESELÝ, A. ČIHÁK and F. ŠORM, Experientia *28*, 841 (1972).
[16] J. LANGMAN and M. SHIMADA, Am. J. Anat. *132*, 353 (1971).
[17] M. SEIFERTOVÁ, A. ČIHÁK and J. VESELÝ, Neoplasma *20*, 243 (1973).
[18] V. G. DEV, M. S. GRAVAL, D. A. MILLER, R. E. KOURI, J. J. HUTTON and O. J. MILLER, Cytogenetics *10*, 436 (1971).
[19] M. GONTCHAROFF and B. RAO, Chromosoma *38*, 441 (1972).

the early synthesized DNA, or at least a part of it, is associated with nucleolus and acts as template for ribosomal RNA. Predominantly lethal action of 5-azacytidine in the S phase[5–7], inhibition[9] of maturation of 45 S rRNA, the effect of the drug on nucleolus[8], and the localization of 5-azacytidine in the nuclear and in the chromosomal heterochromatin indicate that this analogue may be of value for the investigation of the function of nucleolus and its relation to heterochromatin.

Zusammenfassung. Die Inkorporation des ^{14}C-markierten 5-Azacytidins in die embryonalen Mäusefibroblasten, die in Zellkulturen gehalten worden waren, wurde autoradiographisch untersucht. Die Radioaktivität in Kern und Chromosomen ist häufig mit dem Heterochromatin assoziiert. Die Nukleolen werden vergrössert, und die Chromosomen, die sich in der Metaphase befinden, weisen sternförmige Gebilde auf.

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Tubuloreticular Structures in a Case of Bronchialadenom (Carcinoid Type)

Tubuloreticular structures, reported often in collagen diseases[1], have also been seen in several other diseases, including neoplastic diseases[2,3]. To our knowledge they have not been previously reported in carcinoid tumours.

One bronchialadenoma (carcinoid type), resected from the left main bronchi of a 39-years-old woman admitted to the Department of Pathological Surgery of Sta. Maria's Hospital in Lisbon, was studied by electron microscopy. Fragments of less than 1 mm^3 wide were fixed sequentially in 3% glutaraldehyde in cacodilate buffer, pH 7.3, 2% osmium tetroxide in veronal acetate buffer, pH 7.3 and in 0.5% uranyl acetate in bi-distilled water. Following dehydration in ethanol, they were embedded in Epon 812 (Luft). The patient showed no evidence of other diseases, namely collagen diseases.

[1] F. Györkey, J. G. Sinkovics, K. W. Min and P. Györkey, New Engl. J. Med. *280*, 333 (1969).
[2] Z. Scaff, U. Heine and A. J. Dalton, Cancer Res. *32*, 2696 (1972).
[3] B. G. Uzman, H. Saito and M. Kasac, Lab. Invest. *24*, 492 (1971).

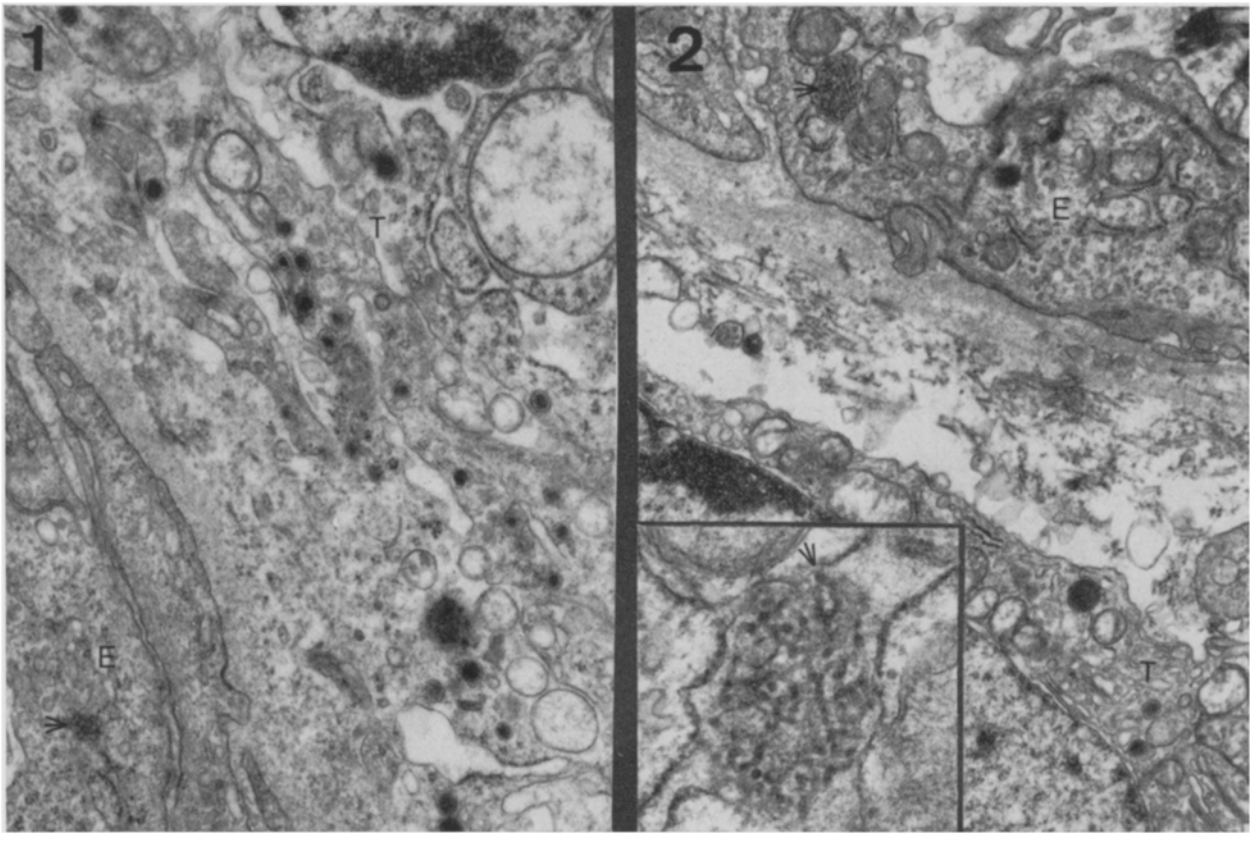

Fig. 1. T, tumor cell; observe the presence of neurosecretory-type granules. E, endothelial cell. Arrow points to tubuloreticular structure. Fig. 2. T, tumor cell. 2 neurosecretory-type granules can be seen. E, endothelial cell with tubuloreticular structure inside endoplasmic recticulum (arrow). Inset-enlargement of tubuloreticular structure. The tubules are associated with intracisternal dense material. Arrow points to apparent continuity of 1 tubule with endoplasmic recticulum membrane.