Existenz einer Elementarfibrille von ca. 20 Å Durchmesser und kann sie im Metaphasechromosom in situ demonstrieren[16].

Summary. Tightly packed elementary fibrils with a diameter of 20 Å are demonstrated in human metaphase chromosomes in situ.

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Pycnotic Degeneration of Ventricular Cells in Embryonic Brain Following Transplacental Exposure to 5-Azacytidine

In the present work the transplacental effect of 5-azacytidine on ventricular cells[1] of 14-day embryonic mouse brain was studied. Of special interest was the timing of the appearance of pycnotic nuclei in the ventricular zone following 5-azacytidine[2], as well as the increase in their number and localization. In order to find out whether these nuclei migrate prior to their pycnotic degeneration and whether they are able to synthesize DNA at that time, thymidine-^{3}H has been administered to mothers and subsequently autoradiographic preparations have been made from embryonic mice killed at different time intervals following simultaneous administration of the label with 5-azacytidine.

Material and methods. For the experiments groups of 4 female mice (pregnancy day 14) belonging to the random-bred strain H, kept under standard conditions, were used. The mice with vaginal plugs or sperms in vagina were isolated from male, and that day was regarded as day 1 of gestation. Thymidine-^{3}H (5.0 Ci/mmol) was injected to mothers (100 µCi/animal) i.p. simultaneously with 5-azacytidine at the dose level of 3 mg/kg. The mice were killed 4, 8, 12 and 24 h after the administration, and the foetuses were embedded in paraffin. Sections were cut at 5 µ, coated with a stripping film Kodak AR. 10 and exposed for 2 weeks at −15°C. The slides were developed and stained with hematoxylin-eosin. Two embryos from each litter were processed for autoradiography. The average background values were less than 1 grain per 10 nuclei. In each slide 1250 ventricular nuclei[1], comprising 25 nuclear layers running parallel to the inner surface of the neural tube, were evaluated; 50 nuclei were counted in each layer.

Results. Table I indicates that following 5-azacytidine the labelling of ventricular nuclei is more considerable than in the control. At 12–24 h after 5-azacytidine the number of heavily labelled nuclei (more than 6 grains per nucleus) remains unchanged, whereas their amount diminishes progressively in controls. The number of pycnotic nuclei increases between 4 and 12 h following 5-azacytidine. At all times 60–66% of pycnotic nuclei are heavily labelled (Table II). Between 12 and 24 h, pycnotic nuclei migrate from inner to external layers of ventricular zone (Figure). The emergence of pycnotic nuclei is preceded by the accumulation of heavily labelled mitotic figures in the region of the inner surface of ventricular zone at 4 and 8 h, while at 12 and 24 h the mitotic index is significantly diminished (Table III).

Comparing the number of mitotic figures and pycnotic cells present in the ventricular zone (Table IV), it seems permissible to conclude that following 5-azacytidine mitoses in their majority undergo pycnotic degeneration. If the number of ventricular mitotic nuclei and that of pycnotic nuclei present at a given time interval is added together, the value thus obtained closely corresponds to the number of pycnotic nuclei actually observed at the following time interval (Table IV).

Discussion. The pycnotic degeneration of the ventricular cells occurs as a result of the disturbance of internal

[1] The Boulder Committee, Anat. Rec. *166*, 257 (1970).
[2] M. Seifertová, J. Veselý and F. Šorm, Experientia *24*, 487 (1968).

Table I. Labelling of nuclei in ventricular zone of mouse embryonic brain according to mean grain counts following simultaneous administration of thymidine-^{3}H and 5-azacytidine in vivo

Time after application (h)	Nuclei (%) Unlabelled		4–6 grains		7 and more grains	
8	68[a]	50[b]	24[a]	24[b]	8[a]	26[b]
12	80	60	16	16	4	24
24	88	64	12	12	0	24

[a] Controls. [b] Following 5-azacytidine. The nuclei have been arranged percentually into 3 classes according to their respective mean grain counts (unlabelled, 0–3; 4–6; and 7 and more grains per average nucleus).

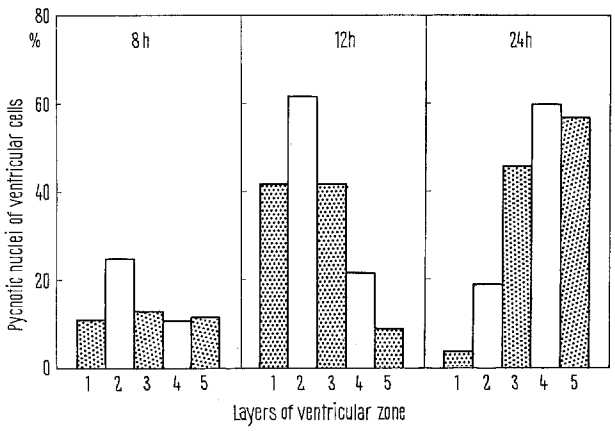
Table II. Percentage of pycnotic nuclei in ventricular zone of mouse embryonic brain and their labelling following simultaneous administration of thymidine-^{3}H and 5-azacytidine in vivo

Time after application (h)	Pycnotic nuclei (%)	Nuclei (%) Unlabelled	4–6 grains	7 and more grains
8	14	32	5	63
12	36	12	22	66
24	38	13	27	60

All pycnotic nuclei have been arranged percentually into 3 different classes according to their respective mean grain counts.

limiting membrane[3], and following transplacental effect of carcinogens[4]. The appearance of pycnotic ventricular cells labelled with thymidine-^{3}H (Table II) applied simultaneously with 5-azacytidine indicates that the analogue causes the degeneration of the ventricular nuclei without affecting their DNA replication at early phases following its application. Since the foetuses were exposed to a single dose of 5-azacytidine, only ventricular cells which were in the S-phase of the generation cycle were affected.

The labelled nuclei move toward the ventricular surface where they enter mitosis without completing it, and subsequently they undergo pycnotic degeneration. The pycnotic nuclei then migrate outward toward external ventricular layers, thus mimicking the movement of the normal unaffected daughter nuclei in the controls. The increased number of mitotic figures (Table III) at 4 and 8 h following 5-azacytidine is probably accounted for by their abnormal accumulation during the mitotic phase of the cycle due to the damage sustained by the drug[2].



Pycnotic degeneration of ventricular cell nuclei and their distribution in ventricular zone following 5-azacytidine. Abscissa: Ventricular zone of embryonic brain has been subdivided into 5 layers comprising the region from the ventricular surface (layer 1) to the external part of ventricular zone (layer 5). In each layer 250 nuclei have been evaluated. Ordinate: Pycnotic nuclei of ventricular cells 8, 12 and 24 h following 5-azacytidine have been counted (%).

Table III. Mitotic indices of ventricular cells of mouse embryonic brain following 5-azacytidine in vivo

Time after application (h)	Mitotic index ± S.E.	(%)
0	1.3 ± 0.2	(100)
4	9.0 ± 1.5	(692)
8	18.1 ± 0.2	(1392)
12	0.4 ± 0	(30)
24	0.05 ± 0	(4)

Table IV. Pycnotic degeneration of mitotic cells in ventricular zone following 5-azacytidine in vivo

Time after application (h)	Mitotic nuclei No.	Pycnotic nuclei No.	Expected number of pycnotic nuclei
4	170	0	0
8	240	175	170
12	5	450	415
24	1	475	455

The expected number of pycnotic nuclei has been calculated by adding the number of pycnotic and mitotic nuclei during the preceding time period; all of the mitotic nuclei have been assumed to undergo pycnotic degeneration..

Zusammenfassung. Das Cytostaticum 5-Azacytidin verursacht 8 bis 12 h nach Applikation Kernpyknosen in den ventrikelnahen Zellschichten des Gehirns bei Mäuseembryonen, ist aber ohne Wirkung auf die Replikation und die Auswanderung der Zellen.

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Bone Marrow Mesodermal Inducing Factor after Irradiation

In the investigations of Toivonen[1], Yamada[2], Tiedemann[3], it was proved that some substances have an inducing effect in the early embryogenesis. A method was found for the purification of a factor which causes mesodermal induction in cultures of gastrula cells[4]. It is known that the inducing factors are found not only in embryonic tissues but also in the adult organism. The mesodermal inducing factor is found in one of the most radiosensitive organs like bone marrow and its quantity sharply changes when malignant degeneration of the bone marrow takes place[5]. This fact urged us to investigate the changes of the mesodermal inducing factor isolated from guinea-pigs bone marrow (BMF) after irradiation. The animals were exposed to single dose of 450 r at approximately 48 r/min (with an X-ray apparatus 'Siemens Bomba' at 180 kV, 15 mA, h.v.l. 3 mmAl). The animals were killed by decapitation on the 3rd h after the irradiation. The marrow from the femurs, tibiae and humeri was suspended in 0.25 *M* sucrose. We used the method of Yamada and Takata[6] for extraction and purification of BMF (Table).