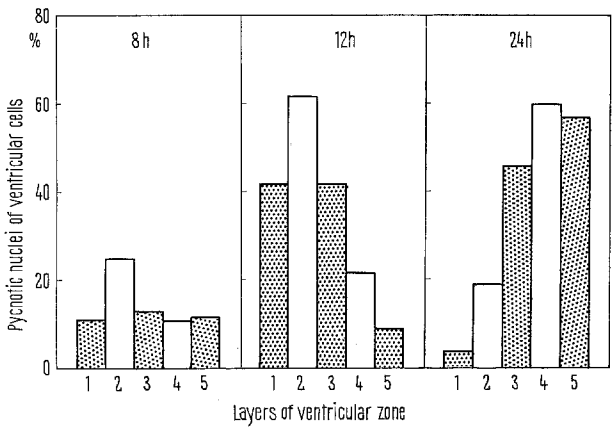
limiting membrane[3], and following transplacental effect of carcinogens[4]. The appearance of pycnotic ventricular cells labelled with thymidine-^{3}H (Table II) applied simultaneously with 5-azacytidine indicates that the analogue causes the degeneration of the ventricular nuclei without affecting 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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[3] E. Klika and R. Jelínek, Revue roum. Embryol. Cytol. *3*, 141 (1966).

[4] S. L. Kauffman, Devl. Biol. *20*, 146 (1969).

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).

The inducing effect of BMF after irradiation of marrow donors

Preparation steps after YAMADA[2]	Donors	Frequencies (%) of Induction: No induction + Desinteeration							
		g/μg/ml → 100		50		25		10	
		Days of observation: 3	7	3	7	3	7	3	7
1. Supernatant	Normal	20	40	0	30	0	0	0	0
		80 + 0	60 + 0	100 + 0	70 + 0	100 + 0	100 + 0	100 + 0	100 + 0
100,000 ×g	Irradiation	0	0	0	0	0	0	0	0
		100 + 0	60 + 40	100 + 0	100 + 0	100 + 0	100 + 0	100 + 0	100 + 0
2. Fraction	Normal	40	70	10	40	0	10	0	0
		60 + 0	30 + 0	90 + 0	60 + 0	100 + 0	90 + 0	100 + 0	100 + 0
AP	Irradiation	20	0	0	20	0	0	0	0
		80 + 0	40 + 60	100 + 0	80 + 0	100 + 0	100 + 0	100 + 0	100 + 0
3. Fraction	Normal	90	100	60	80	40	60	0	30
		10 + 0	0 + 0	40 + 0	20 + 0	60 + 0	40 + 0	100 + 0	70 + 0
ASP	Irradiation	40	40	0	30	0	20	0	0
		60 + 0	40 + 20	100 + 0	60 + 10	100 + 0	80 + 0	100 + 0	100 + 0
4. Fraction	Normal	100	100	90	100	70	90	20	50
		0 + 0	0 + 0	10 + 0	0 + 0	30 + 0	10 + 0	80 + 0	50 + 0
E15	Irradiation	70	90	40	70	20	40	0	0
		30 + 0	10 + 0	60 + 0	30 + 0	80 + 0	60 + 0	100 + 0	100 + 0
5. New fraction E25	Irradiation	0	0	0	0	10	0	0	0
		20 + 80	0 + 100	50 + 50	20 + 80	80 + 10	60 + 40	100 + 0	100 + 0

1. Supernatant after centrifugation at 100,000 ×g for 90 min of bone marrow homogenate in 0.25 *M* sucrose (centrifugated at 6000 ×g for 5 min). 2. Precipitate (at 10,000 ×g for 20 min) of the 1st supernatant brought to pH 4.8 with dilute acetic acid. 3. Precipitate with 50% saturation of ammonium sulfate (in 0.1 *M Tris*-HCl buffer pH 7.4) of the 2nd precipitate dissolved in the same buffer after dialysis against the same buffer. 4. Fraction eluted at 0.15 *M* NaCl by chromatography of the 3rd precipitate over DEAE-cellulose column with increasing concentrations from 0.1 to 1.0 *M* NaCl (in 0.1 *M Tris*-HCl pH 7.4) in stepwise. 5. Fraction eluted at 0.25 *M* NaCl in the same conditions.

All fractions were tested for inducing activity, applying YAMADA's explantation method. We treated the prospective ectoderm of late blastula (in the beginning of gastrulation) with each fraction in 4 concentrations (100, 50, 25 and 10 μg protein content estimated by a Folin test). The duration of the treatment was 3 h at 18°C; subsequently the explants were washed and cultured in Holtfreter solution. Differentiation of the explants thus treated was observable in gross morphology of the explants after 3 days and 7 days of culture (then we observed the histological features: in the case of induction-over-all, notochord; in the absence of induction-cell-division without differentiation and some cells with cilia; in some cases the explants disintegrated).

The Table shows the inducing effect of BMF in the different preparation steps. Our data indicate that in the course of purification of the product isolated from normal guinea-pigs the specific inducing activity increases. The last step of purification, the chromatography by DEAE-cellulose, gives only one maximum at 0.15 *M* NaCl (absorbance at 230 nm). This fraction has an inducing effect in all concentrations observed, even on the 3rd day after treatment.

The product isolated from the bone marrow of irradiated guinea-pigs shows a weaker inducing activity. The products obtained in 1st and 2nd preparation steps applied in concentration of 100 μg/ml have no inducing effect, but, on the 7th day of observation, disintegration of the explants takes place. The chromatography by DEAE-cellulose indicates 2 peaks at 230 nm: at 0.15 *M* NaCl and at 0.25 *M* NaCl. The first fraction has an weaker inducing effect in comparison with the controls. The second fraction causes a disintegration on the explants. We think that the late insignificant inducing effect observed when using 25 μg/ml of the second fraction is due to admixtures of the first fraction. Many problems remain open; primarily what is the role of the new toxic product obtained after irradiation?

Zusammenfassung. Aus Meerschweinchen-Knochenmark, das nach der Methode von YAMADA[6] isoliert wurde, wird der Einfluss des mesodermalen induzierenden Faktors auf dem äusseren Keimblatt von Triton bei beginnender Gastrulation geprüft. Das Produkt wirkt nach der Bestrahlung nicht indizierend, sondern destruktiv auf das Explanat.

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1 L. SAXEN and S. TOIVONEN, *Primary Embryonic Induction* (Academic Press, London 1962).
2 T. YAMADA, J. cell. comp. Physiol. Suppl. 1, *60*, 49 (1962).
3 H. TIEDEMANN, J. Cell Physiol. *72*, 2 (1968).
4 H. TIEDEMANN, H. TIEDEMANN and J. BORN, Science *164*, 3884 (1968).
5 S. TOIVONEN and L. SAXEN, Expl. Cell Res., Suppl. *3*, 346 (1955).
6 T. YAMADA and K. TAKATA, Devel. Biol. *3*, 411 (1961).