Transplacentar Induction of Neurogenic Malignomas by 1,2-Diethyl-Hydrazine, Azo-, and Azoxy-Ethane in Rats[1]

Ethyl-nitroso-urea, when given to pregnant BD-rats after the twelfth day of gestation, produced malignant tumours of the brain, spinal cord or peripheral nerves in the whole progeny lateron, mainly occurring at the age between 150 and 300 days[2,3]. In the meantime we observed more than 700 cases. Even a single dose as low as 5 mg/kg, corresponding to 2% of the LD_{50} was effective[4]. This indicated a surprisingly high susceptibility of the nervous system to carcinogenic changes in prenatal life. With respect to the importance of this problem both for experimental and etiological cancer research, we extended our studies to other groups of carcinogens.

1,2-Diethyl-hydrazine (I), azo- (II), and azoxy-ethane (III), after repeated s.c. injections in adult rats, produced neuroaesthesio-epitheliomas of the olfactorius bulbus, brain tumours, hemangioendotheliomas of the liver, mammary carcinomas, and leukemias respectively, but not local sarcomas at the site of injection[5,6]. Accordingly, their carcinogenic action presumes a metabolic activation. Incubation with liver microsomes, TPNH and oxygen yielded acetaldehyde. Hence, α-C-hydroxylation and subsequent desalkylation occurs. Since ethyl-acetaldehyde-hydrazone (IV) showed no carcinogenic activity, the intermediate formation of the highly unstable diazohydroxide and ethyldiazonium-ions is assumed, eventually leading to carbonium-ions as the ultimate alkylating carcinogen.

1,2-Diethylhydrazine-dihydrochloride (m.p. = 168°) has been synthesized according to RIED and WESSELBORG[7]. The freshly prepared aqueous solution was neutralized under addition of 10^{-4} (g/ml) EDTA to prevent dehydrogenation to azo-ethane, otherwise rapidly occurring in presence of traces of metal. Azoethane, prepared after the method of RENAULD and LEITCH[8], is a volatile yellowish fluid, characterized by the b.p. (58°) and absorption spectrum (water) with maximum at $\lambda = 194$ nm, $\log \varepsilon = 2.97$. Azoxyethane was obtained by oxydation of azoethane with perbenzoic acid.

Pregnant rats of our inbred strains BD VI (CPaH, black) and BD IX (CPAH, agouti) at the fifteenth day of gestation received a single dose of the respective compound. The litter rats were reared and carefully observed for clinical symptoms. When moribund, the animals were killed by gas, and the autopsies performed with preparation of the nervous system. For the histological diagnoses in many cases we are indebted to Professor K. J. ZÜLCH (Köln) and Dr. C. THOMAS (Bonn).

1,2-Diethyl-hydrazine was given by i.v. injection to 2 groups of pregnant rats as a single dose of 50 and 150 mg/kg body weight respectively, corresponding to 12 and 36% of the LD_{50}. 19 and 12 descendants could be reared, the latter mostly showing malformations of the paws. With the exception of only 1 in each group, all 29 remaining rats died between the 126th and 498th day of life with malignant tumours of the brain, the spinal cord or the peripheral nervous system (Figure and Table).

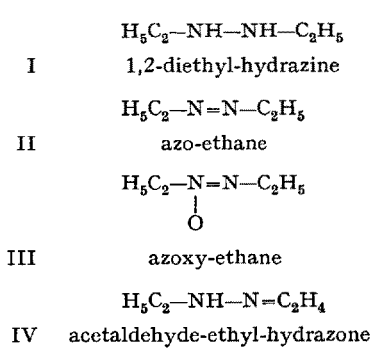
Azoethane, which is highly volatile at room temperature, has been used in inhalation experiments. Pregnant rats in 2 dosage groups were exposed in a gas chamber for 1 h to 4800 and 9600 ppm respectively, corresponding to an uptake of about 300 and 600 mg/kg (14 and 28% of the LD_{50}). In the first group 41 from 42 rats of the progeny developed neurogenic malignomas, many of them in 2 or more localisations. The individual induction times, plotted in a log probit network, yielded a practically linear regression corresponding to normal distribution with t 50 equals 220 ± 45 days. The higher dose again was teratogenic and produced ectrodactylia and other malformations of the paws. In this group the whole offspring with 27 rats died with cancer of the nervous system at the age between 126 and 405 days.

Further experiments with azoxyethane by i.v. injection of 50 mg/kg b.w. are still in progress. Up to now 11 rats died, 9 of them with neurogenic malignomas.

The results are summarized in the Table. On the whole, 107 from 114 rats of the progeny died from neurogenic malignomas. The histological types of the tumours were identical in all experimental groups and independent of the type of the carcinogen administered to the mother rat. The brain tumours mostly were isomorphic (oligodendrogliomas, ependymomas, astrocytomas) or polymorphic gliomas and glio-sarcomatous tumours, often of considerable size (Figure a). Similar types were observed in the spinal cord (Figure b). As to the brain nerves, almost all of them were malignant neurinomas of the trigeminus (Figure c), but in 3 cases tumours of the vagus nerve have been observed. The tumours of the peripheral nerves, likewise malignant neurinomas, showed every localization, as e.g. plexus brachialis or lumbosacralis (Figure d).

From these results it follows that hydrazo-, azo-, and azoxy-ethane are transmitted through the placenta, and activated metabolically by the fetus to the ultimate carcinogen. In contrast with the high yield of tumours in the progeny, we observed only 1 neurogenic malignoma, 4 carcinomas of the ovaries and 2 nephroblastomas in the 32 treated mother rats. This again indicates the high susceptibility of the nervous system to carcinogenic transformation in prenatal life, as already observed with ethyl-nitroso-urea[4].

Comparative experiments with hydrazo- and azoxy-methane, although in adult rats more potent carcinogens[9]



[1] This paper is dedicated to Prof. Dr. h.c. A. BUTENANDT on the occasion of his sixty-fifth birthday.

[2] H. DRUCKREY, S. IVANKOVIC and R. PREUSSMANN, Nature *210*, 1378 (1966).

[3] S. IVANKOVIC, H. DRUCKREY and R. PREUSSMANN, Naturwissenschaften *16*, 410 (1966).

[4] H. DRUCKREY, R. PREUSSMANN, S. IVANKOVIC and D. SCHMÄHL, Z. Krebsforsch. *69*, 103 (1967).

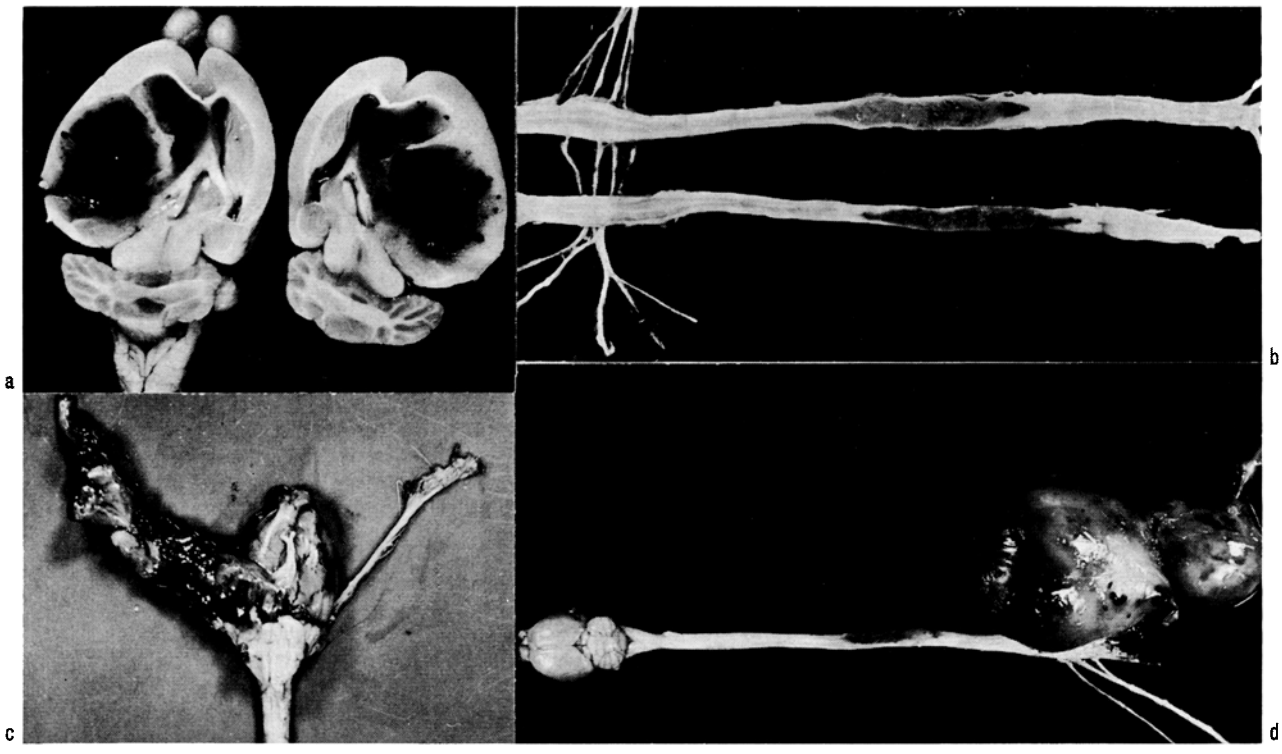
[5] H. DRUCKREY, R. PREUSSMANN, S. IVANKOVIC, B. T. SO, C. H. SCHMIDT and J. BÜCHELER, Z. Krebsforsch. *67*, 31 (1965).

[6] H. DRUCKREY, R. PREUSSMANN, F. MATZKIES and S. IVANKOVIC, Naturwissenschaften *21*, 557 (1966).

[7] W. RIED and K. WESSELBORG, Justus Liebigs Annln Chem. *611*, 71 (1958).

[8] R. RENAULD and L. C. LEITCH, Can. J. Chem. *32*, 545 (1954).

[9] H. DRUCKREY, R. PREUSSMANN, F. MATZKIES and S. IVANKOVIC, Naturwissenschaften *54*, 285 (1967).



Transplacentar induction of neurogenic tumours in rats. (a) Brain, polymorphic glioma, azoxyethane i.v., 50 mg/kg, 232 days. (b) Spinal cord oligodendroglioma, 1,2-diethylhydrazine i.v., 50 mg/kg, 126 days. (c) Trigeminus nerve, malignant neurinoma, azoethane inhalation, 9600 ppm for 1 h, 195 days. (d) Plexus lumbosacralis and spinal cord, malignant neurinomas azoxyethane i.v., 50 mg/kg, 175 days.

Neurogenic malignomas in the progeny of rats, treated on the fifteenth day of pregnancy with 1 single dose of hydrazo-, azo-, and azoxyethane respectively

Substance	Dose mg/kg	Progeny	Neurogenic malignomas			
		No.	Positive	Brain	Spinal cord	Peripheral nerves	Total
1,2-Diethyl-hydrazine (i.v. injection)	50	19	18	11	2	5	18
	150	12	11	4	2	12	18
Azo-ethane (inhalation, 1 h)	300	42	41	25	20	29	74
	600	30	28	16	6	24	46
Azoxy-ethane (i.v. injection)	50	11	9	3	4	6	13
Total		114	107	59	34	76	169

than the corresponding ethane compounds, remained completely negative. No tumours occurred in the progeny up to 525 days. With cycad-meal, the active principle of which is methyl-azoxy-methanol, however, SPATZ and LAQUEUR[10] obtained gliomas of the brain in 5 from 75 rats of the progeny. If correspondingly α-C-hydroxylation is considered as the essential step in the metabolic activation of hydrazo-, azo-, and azoxy-alkanes, then different biochemical mechanisms for the methane and ethane compounds are to be assumed, as indicated already by the striking difference in the organotrophy of these 2 groups[6,9].

Zusammenfassung. Schwangere Ratten erhielten am 15. Tage post coitum eine einmalige i.v. Injektion von 1,2-Diäthyl-hydrazin bzw. von Azoxyäthan oder wurden einmal 1 h lang Azoäthan-Dämpfen exponiert. 107 von 114 aufgezogenen Nachkommen starben mit malignen Tumoren im Gehirn, Rückenmark und peripheren Nervensystem.

H. DRUCKREY, S. IVANKOVIC,
R. PREUSSMANN, C. LANDSCHÜTZ,
J. STEKAR, U. BRUNNER
and B. SCHAGEN[11]

Forschergruppe Präventivmedizin am Max-Planck-Institut für Immunbiologie,
78 Freiburg i.B. (Germany),
4 March 1968.

[10] M. SPATZ and G. L. LAQUEUR, J. natn Cancer Inst. *38*, 233 (1967).

[11] The authors are indebted to the Deutsche Forschungsgemeinschaft for generous support.