Influence of N-methylformamide on the development, the NAD synthesis, and the activity of the ADPR transferase of rat embryos

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Summary. When N-methylformamide is administered to rats on the 11th day of pregnancy approximately 50% of the fetuses are resorbed and a reduced weight of the developed animals is found in comparison to the controls on the 21th day (delivery by Caesarian section). The toxic effect is increased by using nicotinamide and methionine. If a combination of these substances is employed practically all fetuses are resorbed. Tryptophan, however, has a considerably protective influence.

N-Methylformamide has no influence on the NAD-synthesis induced by nicotinamide or tryptophan. It does, however, inhibits the activity of the ADPR transferase.

Various workers, especially Lorke have performed embryotoxic studies with N-methylformamide which has a strong embryotoxic effect[1-6]. In this paper, we report on the toxic effects of N-methylformamide on rat embryos, and on their NAD synthesis, (nicotinamide adenine dinucleotide) its effects activity on the ADPR transferase activity (this enzyme is also designated as poly(ADPR)polymerase or as poly(ADPR)synthetase. ADPR = adenosine diphosphateribose).

Methods. Animal experiments. Wistar rats (on the 11th day of pregnancy) derived from the 'Zentrale Versuchstier-Anlage of the Bundesgesundheitsamt Berlin' were used for the experiments. N-Methylformamide (1 ml/kg) and the other substances (500 mg/kg) were injected i.p. These substances were used because they interfere with NAD metabolism[7]. The embryos were delivered by Caesarian section on the 21st day of gestation. (Day one of pregnancy was the day on which spermatozoa were found in the vaginal smear.)

Determination of $NAD + NADH_2$. This was performed according to Nisselbaum and Green[8].

Determination of the ADPR transferase. The nuclei were isolated according to Blobel and Potter[9]; the activity of enzyme within the nuclei was determined as described by Kidwell and Burdette[10].

Material. N-Methylformamide (Ega-Chemie, Berlin); L-methionine (Schwarz-Mann, Orangebourg, N.Y.); L-tryptophan, nicotinamide (Merck, Darmstadt); 1-methylnicotinamide (Sigma, Munich); nicotinamide adenine dinucleotide U-^{14}C, 265 mCi/mmole Batsch 21, CFA 497 (Amersham/Buchler, Brunswick).

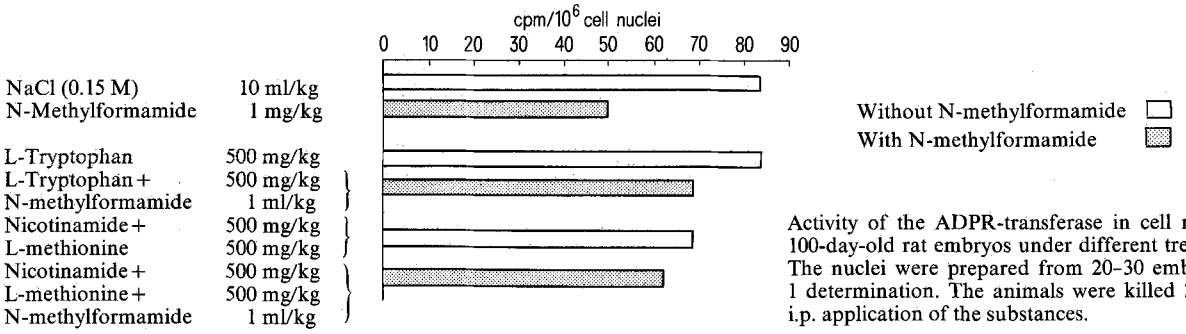
Results. When N-methylformamide is administered i.p. to rats on the 11th day of pregnancy, 44% of the fetuses are resorbed (table 1). The remaining fetuses show a growth retardation, as can be seen from a comparison of weight with that of the controls. The rate of resorption is increased by methionine and especially by a combination of nicotinamide and methionine. Tryptophan, on the other hand, has a protective effect. A simultaneous administration of methionine and tryptophan reduces the protective effect of the tryptophan. Testing the substances alone, it can be seen that the rate of resorption is only a little higher with the combination of nicotinamide and methionine and with the administration of 1-methylnicotinamide (table 2).

From these results we suggested that N-methylformamide could be involved in NAD metabolism. We therefore

Table 1. Influence of different substances upon the embryonal development of rats

Experiment	Total No. mothers	Total No. embryos	Embryos alive (%)	Embryos resorbed (%)	% weight MFA + Substance / NaCl-control	% weight MFA + Substance / MFA	% weight MFA + Substance / Substance	% weight Substance / NaCl-control	% weight Substance / MFA
NaCl (0.15 M)	12	141	96.7	3.3	100	-	-	100	119
N-Methylformamide	13	138	55.8	44.2	80.6	100	-	-	-
N-Methylformamide + nicotinamide	10	107	57.9	42.1	64.4	76.6	66.8	-	-
N-Methylformamide + L-methionine	7	95	15.9	84.1	81.0	100.1	76.7	-	-
N-Methylformamide + L-tryptophan	11	120	79.9	20.1	89.3	106.2	82.5	-	-
N-Methylformamide + nicotinamide + L-methionine	6	60	1.0	99.0	54.5	64.2	56.4	-	-
N-Methylformamide + L-tryptophan + L-methionine	9	95	49.3	50.7	78.8	98.0	78.4	-	-
Methylformamide + N-methylnicotinamide	7	81	32.1	67.9	80.5	95.8	79.9	-	-
Nicotinamide	7	71	96.3	3.7	-	-	-	96.3	114.6
L-Methionine	6	65	98.4	1.6	-	-	-	107.0	121.2
L-Tryptophan	10	103	96.3	3.7	-	-	-	108.2	128.8
Nicotinamide + L-methionine	8	82	93.9	6.1	-	-	-	96.4	114.7
L-Tryptophan + L-methionine	9	90	98.3	1.7	-	-	-	101.8	121.1
N-Methylnicotinamide	7	88	88.4	11.6	-	-	-	101.3	120.5

The substances (500 mg/kg) were given to the animals twice at intervals of 2 h. In the case of a combination with N-methylformamide (MFA) (1 ml/kg), the substances were administered 1 h before MFA and 1 h after MFA.



Activity of the ADPR-transferase in cell nuclei of 100-day-old rat embryos under different treatments. The nuclei were prepared from 20–30 embryos for 1 determination. The animals were killed 2 h after i.p. application of the substances.

investigated the NAD synthesis it is known that tryptophan as well as nicotinamide causes an increase of the NAD content in animal cells. We tested the NAD synthesis in 11-day-old rat embryos. It could be seen that nicotinamide led to a higher increase of NAD than L-tryptophan (table 2). In neither case could a change in the NAD synthesis due to the influence of N-methylformamide be observed. We were also interested in the activity of the ADPR transferase. For this purpose, we isolated nuclei of 11-day-old embryos and determined the activity of the enzyme in vitro. The results of the experiments have to be estimated with caution; however, it can be said that in contrast to L-tryprophan, a combination of nicotinamide + methionine decreases the activity of the ADPR transferase (fig.). N-Methylformamide considerably inhibits the activity of the enzyme. In the presence of L-tryptophan, the decrease is lower.

Discussion. Since the publications of Warburg[11], it has been known that NAD as coenzyme is involved in oxidation-reduction processes. Experiences within the last few years have shown that NAD also serves as a substrate. ADP-ribosylation is a postsynthetic modification of proteins[12]. Caplan and Rosenberg[13] concluded from their experiments in 1975 that this metabolism is involved in differentiation. Several indications of the last few years have confirmed this postulate[14,15]. Many results also give us reason to assume that the metabolism of NAD is involved in repair processes of the DNA[16,17].

We were able to demonstrate that the embryo-toxigenicity of N-methylformamide is decreased by L-tryptophan, but increased by a combination of nicotinamide and methionine. These reactions indicate an involvement of NAD metabolism. Even in teratological studies with cyclophosphamide, we found an increase due to nicotinamide and methionine (manuscript under preparation). In former experiments using cyclophosphamide – which has carcinostatic as well as teratogenic properties – we observed a decrease of the NAD concentration in tumors[18–20]. Moreover, our studies on ascites cells showed an accumulation of nicotinamide parallel to the NAD decrease[21,22]. Since it is known that alkylating substances activate the ADPR transferase[23,24], the accumulation of nicotinamide found by us in earlier experiments has also to be ascribed to this reaction. Although we also observed an influence of N-methylformamide upon the ADPR transferase of the embryos, further studies will have to show in detail by which processes the potentiating effect of nicotinamide and methionine and the protective effect of L-tryptophan are induced.

Table 2. NAD content of rat embryos under influence of different substances

	Control	+ MFA
NaCl	0.199	0.222*
Nicotinamide	0.365	0.329
L-Methionine	0.245	0.252
L-Tryptophan	0.248	0.234
Nicotinamide + L-methionine	0.329	0.353
L-Tryptophan + L-methionine	0.245	0.257
N-Methylnicotinamide	0.235	0.268

The substances were i.p. administered to the animals on the 11th day of pregnancy and the embryos delivered by Caesarian section for the determination of NAD 8 h later. N-Methylformamide (MFA): 1 ml g/kg. All the other substances: 500 mg/kg 1 h before and 1 h after administration of N-methylformamide. For each determination 5–8 animals were used. * Given as µmoles NAD + $NADH_2$/g tissue.

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