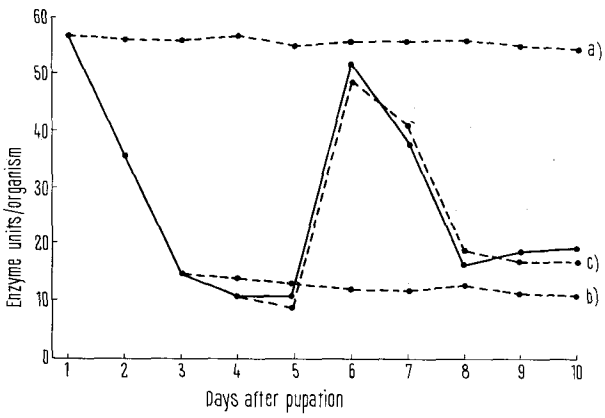
necessitated by implantation of 5FU crystals, but without any material implanted. The assay for ADH activity has been described earlier[5].

Treatment with 5FU during the first 3 days following pupation inhibits the pattern of change in ADH activity, the level of activity remaining constant to at least the 10th day of pupal life. As seen in the Figure, a high level is maintained with treatment on the 1st day after pupation, while a constant low level results from treatment on the 3rd day after pupation. When 5FU is administered on the 4th day after pupation or later, the ensuing pattern of ADH activity is not appreciably different from that of normal development. For the sake of clarity only 3 experimental series and 1 control series are included on the Figure.

Apparently the entire program of developmental change in ADH activity is dependent on a 5FU-sensitive process which is completed by the 4th day following pupation. Treatment with 5FU during the first 3 dasy is inhibitory to the realization of this program, the level of ADH activity being locked at the time of treatment. MUTH[2] has shown that the sensitivity of pupal development to 5FU treatment changes drastically between the 3rd and 4th days after pupation. Pupae treated during the first 3 days show severe inhibition of the development of most adult structures, and they die after the 13th pupal day. However, treatment from the 4th day permits development of a viable adult emerging on the 13th day after pupation (on schedule with normal development), although a few adult structures may still be abnormal[3,4]. Therefore, the establishment of the pattern of change in ADH activity may be dependent on the same process(es) necessary for the establishment of the major portion of the program of metamorphic changes in the *Ephestia* pupa. This process is quite likely the synthesis of specific nucleic acids during early pupa.



Effect of 5FU on developmental changes in ADH activity. Solid lines indicate changes in control animals wounded 1 day after pupation. The pattern is essentially the same as described for normal development[5]. Dashed lines a) b) and c) represent animals treated 1, 3 and 4 days after pupation, respectively. The first points for a), b) and c) are control values, since the earliest assays for ADH activity were at 2, 4 and 5 days, respectively. Each point represents a determination on 5–10 individuals.

The effect of 5FU on nucleic acid metabolism has been extensively studied[6]. As an analog of uracil, 5FU can be incorporated into all classes of RNA molecule, and such 5FU-RNA can result in miscoding, or in inhibition of translation. 5FU can also inhibit DNA synthesis, presumably by being converted to 5-fluorodeoxyuridylic acid, an inhibitor of thymidylate synthetase[7]. Thus, these effects could be inhibitory to any developmental program which requires the synthesis of specific nucleic acid(s) during a specific time period.

Zusammenfassung. Behandlung von Puppen der Mehlmotte *Ephestia kühniella* mit 5-Fluorourazil während der ersten 3 Puppentage unterdrückt die charakteristischen Schwankungen der ADH-Aktivität während der weiteren Entwicklung. Behandlung am 4. Tag oder später hat keinen Einfluss auf das Enzym. Die Wirkungen von 5-Fluorourazil auf ADH decken sich mit früher beobachteten Wirkungen auf morphogenetische Prozesse.

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Inhibition of Mouse Liver Protein and Nuclear RNA Synthesis Following Combined Oral Treatment with Sodium Nitrite and Dimethylamine or Methylbenzylamine

Nitrosamines appear to be a major class of carcinogens that are likely to be causally releated to human cancer in industrialized society[1]. High concentrations of nitrosamines have been found in nitrite-preserved fish meal[2] and in fermented liquors[3]; trace concentrations of nitrosamines have also been reported in tobacco smoke, cheese, meat, grains and alcoholic beverages[4–7]. Even more widespread and of possibly greater significance are precursors of nitrosamines-nitrites and secondary amines[1]. Secondary amines can be nitrosated in vitro[8–11] and in vivo[12] to form corresponding nitrosamines. Chronic feeding of rats with nitrite combined with secondary amines produced synergistic carcinogenicity[13]. More recently, combined oral administration of nitrite and secondary amines produced synergistic acute toxicity and liver necrosis in mice[14]. We report here acute inhibition of liver protein and nuclear RNA synthesis following combined oral administration of nitrite and secondary amines to mice.

Male Swiss albino mice (ICR/Ha) weighing between 23 and 28 g were housed in porcelainized tubs and given food and water ad libitum. Single doses of dimethylamine-hydrochloride (DMA) and methylbenzylamine-hydrochloride (MBA) were administered to mice by gavage at concentrations of 2000 and 1200 mg/kg either alone or in combination with sodium nitrite ($NaNO_2$) at concentrations of 150 or 100 mg/kg, respectively (Tables I–III).

For the ^{3}H-leucine uptake studies, mice were injected with 1.0 µc of ^{3}H-leucine (55 mC/µmoles; New England

Table I. Synergistic effects of dimethylamine-hydrochloride and sodium nitrite on incorporation of ^{3}H-cytidine into mouse liver nuclear RNA

Groups	^{3}H-Cytidine incorporation[a]					
	At 2 h		At 6 h		At 18 h	
	No. of replicates	Mean ± S.E. (% of control values)	No. of replicates	Mean ± S.E. (% of control values)	No. of replicates	Mean ± S.E. (% of control values)
Control	4	3.06 ± 0.32	4	10.0 ± 0.8	4	13.3 ± 13
Sodium nitrite (150 mg/kg)	4	2.88 ± 0.48 (94%)	4	7.9 ± 0.8 (79%)	4	10.3 ± 0.8 (77%)
Dimethylamine (2000 mg/kg)	4	2.98 ± 0.10 (97%)	4	10.5 ± 0.7 (105%)	2	14.4 ± 0.5 (108%)
Sodium nitrite and Dimethylamine	4	2.06 ± 0.20 (67%)	4	3.8 ± 1.4[b] (38%)	4	8.1 ± 1.4 (60%)

[a] Expressed as cpm/μg DNA; [b] Statistically significant interaction, $p < 0.01$.

Table II. Synergistic effects of dimethylamine-hydrochloride and sodium nitrite on incorporation ^{4}H-leucine into mouse liver protein

Group	Mean ^{3}H-leucine incorporation[a]			
	At 2 h		At 18 h	
	No. of replicates	Mean ± S.E. (% of control values)	No. of replicates	Mean ± S.E. (% of control values)
Control	9	39.7 ± 4.1	5	41.7 ± 2.9
Sodium nitrite (150 mg/kg)	9	39.7 ± 4.1 (100%)	9	39.5 ± 3.7 (95%)
Dimethylamine-hydrochloride (2000 mg/kg)	10	54.4 ± 10.2 (137%)	10	41.6 ± 2.2 (100%)
Sodium nitrite and dimethylamine-hydrochloride	10	15.0 ± 3.5[b] (38%)	10	27.9 ± 5.2 (67%)

[a] Expressed as cpm/mgP; [b] Statistically significant interaction, $p < 0.01$.

Nuclear) 30 min prior to sacrifice; mice were sacrificed 6 or 18 h after administration of DMA alone or with nitrite and 6 h after administration of MBA alone or with nitrite. Uptake of ^{3}H-leucine into whole liver protein was determined with aliquots of liver homogenates from individual mice. Following precipitation with 10% TCA, protein pellets were washed consecutively twice with 10% TCA, with 10% TCA at 90 °C for 15 min, with 95% ethanol containing 10% potassium acetate, with absolute ethanol, twice with ethanol-ether (3:1), and twice with ether[15]. Duplicate aliquots of dried protein were dissolved in Protosol and counted in a Beckman liquid scintillation counter.

For nuclear RNA studies, mice were injected with 2.5 C of ^{3}H-cytidine (5.9 μC/mMole, New England Nuclear) 45 min prior to sacrifice; mice were sacrificed 2, 6 or 18 h after administration of DMA alone or with nitrite. Highly purified liver nuclei were isolated from 2 pooled livers by sedimentation through 2.1 M sucrose, containing 1 mM $MgCl_2$ and 3.5 mM potassium phosphate at pH 6.8[9]. Nuclei were lysed with 0.1% sodium lauryl sulfate and aliquots were washed consecutively with 4% PCA, twice with 2% PCA, with absolute ethanol, and twice chloroform-methanol (1:1). After drying, the nucleic acids were hydrolyzed with 5% PCA at 90 °C for 30 min; aliquots were counted for radioactivity and analyzed for DNA, by the diphenylamine procedure[16].

The effects of combined administration of DMA and $NaNO_2$ on nuclear RNA synthesis, as measured by incorporation of ^{3}H-cytidine into nuclear RNA, are shown in Table I. Incorporation of ^{3}H-cytidine into nuclei of control mice and mice treated with either DMA or $NaNO_2$ were similar. However, combined administration of DMA and $NaNO_2$ inhibited cytidine incorporation by 33, 62 and 40% at 2, 6 and 18 h following treatment, respectively (Table I).

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Table III. Synergistic effects of methylbenzylamine-hydrochloride and sodium nitrite on mouse liver protein synthesis

Treatment	No. of replicates	Mean ^{3}H-leucine incorporation ± S.E.[a] (% of control values)
Control	8	53.4 ± 4.8
Sodium nitrite (100 mg/kg)	8	52.6 ± 2.3 (99%)
Methylbenzylamine-hydrochloride (1200 mg/kg)	8	56.3 ± 4.8 (105%)
Sodium nitrite and methylbenzylamine-hydrochloride	9	40.4 ± 4.2[b] (76%)

[a] Expressed as cpm/mgP; [b] Statistically significant interaction, $p < 0.01$.

The effects of DMA and $NaNO_2$ on uptake of ^{3}H-leucine into mouse liver protein are shown in Table II. Neither DMA nor $NaNO_2$ alone induced any statistically significant inhibition. However, DMA and $NaNO_2$ together produced 63 and 33% inhibition of leucine uptake at 6 and 18 h after treatment, respectively (Table II). Comparable inhibition of leucine incorporation was produced by combined administration of MBA and $NaNO_2$ (Table III).

In the present experiments, DMA or MBA when administered in combination with $NaNO_2$ produced synergistic acute inhibition of liver protein and nuclear RNA synthesis; similar inhibitory effects are also induced by dimethylnitrosamine[17] and methylbenzylnitrosamine[18]. These data confirm and extend findings of previous studies on induction of synergistic acute toxicity and hepatic nerosis in mice following oral administration of DMA or MBA together with $NaNO_2$[14] and afford strong presumptive evidence of in vivo nitrosamine synthesis from nitrite and amine precursors. The relevance of these findings to potential human hazards from continued use of nitrate and nitrite as food additives and from drinking of water with elevated nitrate merits further consideration.

Zusammenfassung. Orale Gabe von Dimethylamin oder Methylbenzylamin zusammen mit Natriumnitrit bewirkte eine synergistische Hemmung der Protein-Synthese in der Leber von Mäusen. Ausserdem hemmte die kombinierte Gabe von Dimethylamin und Nitrit synergistisch die Synthese nuklearer RNS in der Leber.

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Encephalitogenic Protein: a β-Pleated Sheet Conformation (102–120) Yields a Possible Molecular Form of a Serotonin Receptor

Carnegie[1] has recently suggested that a specified segment of the basic A1 protein composes one of the serotonin receptors in the central nervous system. The conformation suggested by Carnegie is based on a previous model of ours[2]. We wish to suggest an alternative model that accounts for the mode of action of the unusual aminoacid methyl-arginine. The model suggested by Carnegie is based on a coil conformation for the polypeptide and the role of methyl arginine is not accounted for. An α-helical conformation would be unlikely owing to the two glycine moieties in the sequence and the protein itself contains no α-helix[1]: but a β-conformation is possible. The conformation is illustrated in Figure 1 for this segment of the protein itself with 3 turns and 11 cross C=O... HN hydrogen bonds. This locates the 3 key aminoacids —Trp, Arg (Lys) and Gln—in a conformation capable of binding 5HT (in its preferred conformation)[3] in the manner shown in Figure 2. The β-structure gives a rigid molecule and the binding groups for 5HT are relatively fixed. As may be seen in Figure 2, the methyl-Arg and Phe moieties and the Gln (120), Arg (121) and Lysine (104) hydrocarbons form a lipophilic 'bed' at the bottom of which lies the indole ring of the Trp moiety, fixed by lipophilic interactions and steric hindrance. If the 5HT molecule in its preferred conformation is bound by π-π stacking to the Trp molecule, it attains the following additional contacts: 1. lipophilic: β-hydrocarbon to Trp π cloud and β-hydrocarbon and Arg γ-hydrocarbon: α-hydrocarbon to Arg

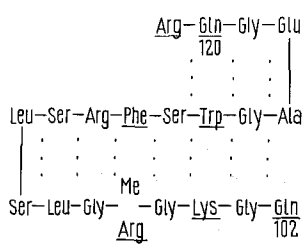

Fig. 1. The suggested conformation of the encephalitogenic polypeptide. The dots indicate CO..HN hydrogen bonds.

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