tion of the centers in the brain or spinal cord from which this non-cholinergic innervation of the cutaneous vessels is derived was not sought in the present study.

Inasmuch as the neurotransmitter mediating sustained vasodilatation has not been delineated it remained to be determined whether the adrenergic nerves even after adrenergic neuronal blockade may have mediated the vasodilator effect. Results have been presented indicating that release of 'pseudo transmitter' in the form of the blocking agent itself did not mediate this response[7]. In the present work it is strongly suggested that sympathetic vasodilator fibres separate from the adrenergic nerves mediate sustained vasodilatation. Medullary vasomotor center stimulation and sympathetic nerve stimulation evoked comparable vasoconstrictor responses in the paw prior to adrenergic blockade, presumably by activation of a similar adrenergic nerve discharge. However, after adrenergic blockade medullary stimulation did not cause vasodilatation, whereas stimulation of the sympathetic trunk did. Stimulation of these sites in the medulla caused selective adrenergic stimulation which was shown to be separate from the sustained vasodilator innervation.

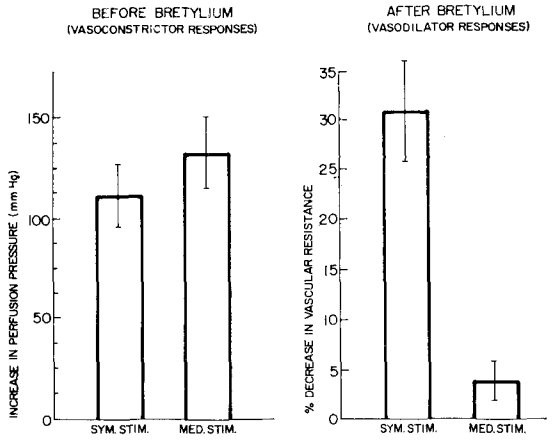

Fig. 3. Vasoconstrictor responses evoked by sympathetic stimulation (7–20 Hz) and medullary stimulation (240 Hz) before bretylium 10 mg/kg and vasodilator responses evoked after bretylium. Vasoconstrictor responses are indicated by increases in PP, in mm Hg and vasodilator responses as % decrease in vascular resistance. Values are mean of 10 experiments.

In previous studies it was reported that antihistamines were partially effective in blocking sustained vasodilatation, whereas in other reports no effect of antihistamines was obtained[3–6]. Certain antihistamines such as tripelenamine potentiate adrenergic responses[9]. If an adrenergic contribution to sympathetic stimulation remains after adrenergic neuronal blockade, the antihistamine may partially antagonize sustained vasodilatation by potentiating this residual adrenergic component rather than by a specific antihistaminic effect[6]. It is conceivable that this action of the antihistamine combination utilized in the present study accounts for the decrease in vasodilatation induced by LCS. We cannot rule out the possibility, however, that histamine release evoked by LCS may partially contribute to the vasodilator response and that the antihistamines may be acting specifically. The fact that a comparable vasodilator response elicited by sympathetic stimulation was not antagonized by the antihistamine would suggest involvement of histamine at least in some of the experiments involving LCS.

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Early Investigations on the Effect of Methyl Mercuric Chloride upon DMN-acute Hepatotoxicity[1]

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Summary. Acute toxicity induced by DMN was partially prevented by previously administering methyl mercuric chloride (MMC), a chemical inhibitor of the drug metabolizing enzyme system (DMES). We have studied the early changes occurring during the course of DMN-intoxication, namely disaggregation of polysomal profiles and necrosis, evaluated morphologically and by the release of S-GPT.

Mercury is an important environmental pollutant, largely used in industry and agriculture, and is very well known for its toxic effects on the peripheral and central nervous system[2]. Lucier et al.[3] and Alvares et al.[4] showed that low doses of mercury administered to rats inhibited the activity of the hepatic drug metabolizing enzyme system (DMES). Mercury does in fact reach high concentration in the microsomal fraction of the hepatocyte[5].

Pathophysiological alterations of the DMES may alter the course of toxicity induced by those drugs which act after their biochemical transformation occurring in the DMES. Carlson[6] has in fact found a protection by methyl mercury against CCl_4-poisoning. This haloalkane is a very well-known hepatotoxin which exerts its poisoning effects through a metabolite, the free radical $\cdot CCl_3$ formed in the DMES[7,8].

Dimethylnitrosamine (DMN), as CCl_4, is also metabolized within the DMES into derivatives which induce acute hepatic damage and carcinogenic effects as well[9].

The course of DMN toxicity is dependent on the rate of its metabolism. The inhibition of the DMES, obtained by feeding the rats with a protein-free diet, decreases DMN-

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Table I. Protection by MMC against the polysomal disaggregation induced by DMN

Treatment	Polysomes	
	Total ribosomes[a]	
a) Control	0.67 ± 0.004	(4)
b) MMC	0.77 ± 0.026	(4)
c) DMN	0.49 ± 0.013	(4)
d) MMC + DMN	0.61 ± 0.004	(4)

[a] Calculated by the areas of the polysomal patterns. Mean ± SE. Number of animals is given in parentheses. Rats were killed 90 min after DMN intoxication. MMC was injected s.c. at the doses of 15 mg/kg body wt. 48 and 24 h before intoxication. DMN was injected i.p. at the dose of 200 mg/kg b.w. Statistical significance of the differences by t-test: a–c, $p < 0.001$; a–b, $p < 0.005$; a–d, n.s.

toxicity[10,11]. Contrasting results have instead been obtained from experiments made on the liver of rats pretreated with inducers of the DMES, such as phenobarbital and 3-methylcholanthrene[12,13]. Up to now, a clear relationship between the activity of the DMES and DMN toxicity has not yet been established. The aim of our studies was to relate chemical inhibition of the DMES, induced by MMC, and toxicity by DMN. Our experimental data are referred in the initial stage of DMN-intoxication. Protein synthesis inhibition, probably due to a derangement of polyribosomes[14], is indicative of membrane alteration, occurring within 90 min of intoxication. Cell necrosis was also observed at 24 h after administration of DMN, and LD_{50} was seen in the animals after 4 days from intoxication.

Materials and methods. Male Wistar rats, weighing 200–250 g, were used in these experiments. They were fed a semi-synthetic diet, devoid of antioxidants. The animals were starved 16–18 h before intoxication. Methyl mercuric chloride, dissolved in olive oil, was injected s.c. at doses of 15 mg/kg body wt, 48 and 24 h before intoxication. Control rats received olive oil alone. 24 h after the second dose, dimethylnitrosamine, dissolved in 0.9% NaCl, was injected i.p, at different doses, as stated in Results. Control rats received saline. The determination of polysomal profiles was performed according to a method previously described[15].

Serum activity of glutamic-pyruvic transaminase (GPT) was determined according to a standard combination method provided by Boehringer (Mannheim, W. Germany).

For the histological examination, small portions of liver were immediately fixed in Bouin's solution, embedded in liquid paraffin and stained with hematoxylin-eosin. LD_{50} was calculated according to the method of WEIL[16], by using 4 groups of 5 animals each.

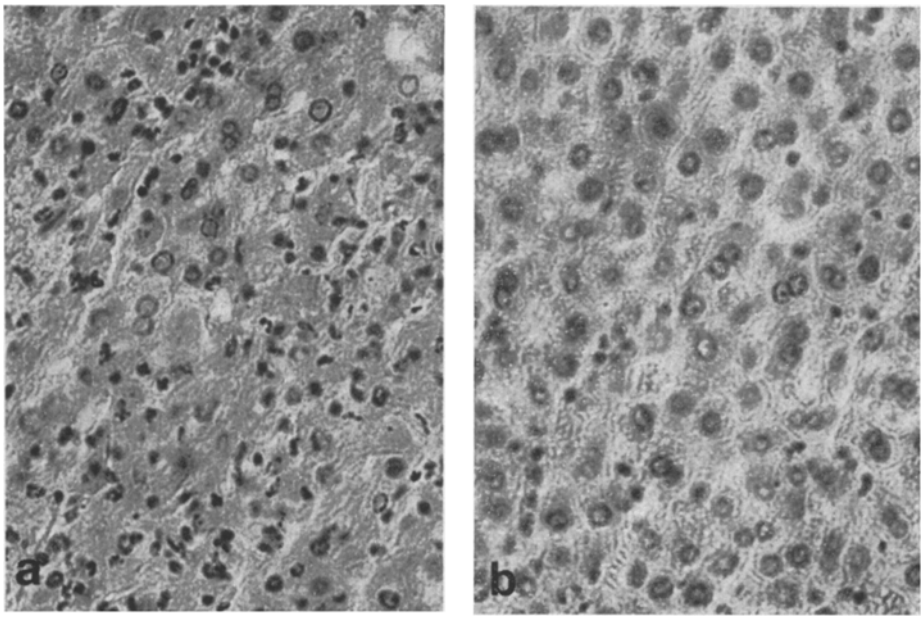
Results. LD_{50} for the rats treated with DMN alone was, at 4 days, 36.9 mg/100g body wt. and 50.4 mg for the animals pretreated with MMC and intoxicated with DMN.

The disaggregation of liver polysomal profiles was observed in animals poisoned with DMN alone in the early stage of intoxication, that is, at 90 min after the adminstration of the dialkylnitrosamine. The polysomal profiles from animals pretreated with MMC and then intoxicated with DMN did not differ from the control group. The administration of MMC alone caused per se an increase of the polymeric fractions. This datum is in agreement with that obtained by LUCIER et al.[3] (Table I).

Table II. Influence of MMC on serum activity of glutamic-pyruvic transaminase in DMN-poisoned rats

Treatment	S-GPT[a] (milliunits/ml)	
a) Control	14.00 ± 0.78	(5)
b) MMC	7.71 ± 2.09	(7)
c) DMN	1292.86 ± 80.61	(7)
d) MMC + DMN	33.66 ± 8.33	(6)

[a] Mean ± SE. Number of animals is given in parentheses. Rats were killed 24 h after DMN intoxication. MMC was injected s.c. at the doses 15 mg/kg body wt. 48 and 24 h before intoxication. DMN was injected i.p. at the dose of 100 mg/kg b.w. Statistical significance of the differences by t-test: a–c, c–d, $p < 0.001$.



Liver sections from a rat treated with DMN (100 mg/kg body wt.) and killed 24 h after intoxication (a) and from a rat pretreated with MMC at the dose of 15 mg/kg b.w. 48 and 24 h before intoxication with DMN at the dose of 100 mg/kg b.w. (b). Staining: hematoxylin-eosin.

Cell necrosis induced by DMN, evaluated by the release of S-GPT in the plasma, was completely prevented by the previous administration of the heavy metal (Table II). This datum is further supported by histological examination (Figures a and b). Livers taken from rats treated with DMN alone showed an intense necrosis, mainly centrolobular, with scattered cells showing ballooning and bordering the necrotic areas. Livers from rats pretreated with MMC had an almost normal histological appearance.

Discussion. DMN is a toxic and powerful carcinogen, which exerts its effect through the ultimate alkylating agent, a methyl carbonium ion[17].

The biochemical transformation of DMN to the ultimate toxic compound requires an NADPH-generating system, molecular oxygen and the microsomal fraction. However, the relation between the inducibility of the DMES and increase of toxicity it is not yet clear. It is unquestionable that for other models of hepatotoxicity there is a direct dependence on the availability of those enzymes which provide for their biochemical transformation. This is the case of CCl_4-intoxication: an enhancement of the DMES by phenobarbital causes a significant increase of toxicity, while an inhibition of the DMES effectively protects the animals against CCl_4[7,8]. On the other hand, pretreatment of the animals with typical inducers of the DMES is not always followed by an increase of DMN-toxicity. 3-Methylcholanthrene does, in fact, increase the LD_{50} of DMN-poisoned rats[18]. The toxicity of DMN has been investigated also in animals pretreated with inhibitors of the DMES. Either necrogenic doses of CCl_4[19] or feeding the animals with a protein free diet[10,11] are able to decrease DMN-toxicity with a parallel inhibition of the DMES. However, these models of inhibition may differently affect the physiological role of the detoxyfying mechanisms of the liver. MMC administered at low dose does not cause liver necrosis and does not affect the protein synthesis machinery. Moreover, MMC has a long-lasting effect compared to other commonly used inhibitors of the DMES, such as SKF 525-A. MMC has been administered, under our experimental conditions, 24 h before intoxication. The protection by MMC against DMN-toxicity has been shown in the early stage of intoxication. We have not followed the recovery and carcinogenicity. POUND et al.[20] have found an increase of liver hepatomas by DMN in CCl_4-pretreated rats. We do not rule out the possibility that MMC may affect in a different way the toxicity and carcinogenicity by DMN at a later stage.

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Influence of Endogenous Pyrogen on the Cerebral Prostaglandin-Synthetase System

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Summary. The biotransformation of arachidonic acid to prostaglandins in vitro is specifically augmented by endogenous pyrogen to a degree depending on the concentration applied, providing that the microsomal fraction of the cerebral cortex is used as prostaglandin-synthetase system. This effect is inhibited by non-steroidal anti-inflammatory agents. These findings are compatible with the hypothesis that prostaglandins might act as mediators of the febrile reaction induced by endogenous pyrogen.

The development of fever, above all in infections, is ascribed to the release of endogenous pyrogen (EP) from leucocytes when these cells are stimulated by endotoxin or lipid A[1,2]. The circulating EP induces the febrile reaction by way of thermoregulatory centres. It is thought that this effect of EP is mediated by certain prostaglandins (PG)[3,4]. This view is supported by the fact that the induction of fever by endotoxin or EP is accompanied with an increase in the concentration of PG in the cerebrospinal fluid[5-7]. It is also known that antipyretics suppress the biosynthesis of PG and that their antifebrile effect is associated with a concomitant reduction in the content of PG-like material in the cerebrospinal fluid[8-11]. In view of this close functional relation between EP and the biosynthesis of PG, we have investigated the effect of EP on the prostaglandin-synthetase system in vitro.

Material and methods. Isolation of endogenous pyrogen (EP). EP was isolated according to the methods of GANDER and GOODALE[12]. Anaesthetized rabbits were injected intraperitoneally with 400 ml of physiological

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