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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Table I. Effect of endogenous pyrogen on the activity of the prostaglandin-synthetase system[a]

Incubation conditions	Formation of PG (cpm $\pm s_{\bar{x}}$)	Increase in formation of PG (%)	No. of experiments
Control	3789 ± 181		
194[b]	4546 ± 192	20	2
Control	3789 ± 181		
388[b]	5251 ± 204	39	2
Control	4465 ± 324		
775[b]	7744 ± 577	73	10

[a] Microsomal fraction of bovine cerebral cortex.
[b] Addition of endogenous pyrogen (protein content in µg/ml).

saline solution, to which glycogen 0.1 g, penicillin 200,000 IU and streptomycin 250 mg had been added. After 16 h, the exudate was withdrawn and the cell count determined (4×10^6 to 1×10^7/ml). The cells were then separated from the exudation fluid by centrifugation (Fraction I). The sediment was resuspended in physiological saline, heated to 37 °C for 1 min and then removed from the washing fluid by centrifugation (Fraction II). It was resuspended again and EP liberated by incubation at 37 °C for 16 h. The EP was isolated by centrifugation, and the protein contents of the various fractions determined according to LOWRY's method[13].

Isolation of the PG-synthetase system (PGSS). PGSS was isolated from bovine cerebral cortex or seminal vesicles[14]. Cerebral PGSS was obtained from the grey matter of the cerebral cortex. The microsomal fractions were isolated by differential centrifugation, suspended in a buffer solution containing saccharose and stored at − 28 °C under nitrogen.

Influence on the PGSS. The enzymatic micro-assay was carried out according to WHITE and GLASSMAN[15]. The incubation medium contained the microsomal fractions of cerebral cortex or seminal vesicle as enzyme, ^{14}C-labelled and unlabelled arachidonic acid as substrate and the co-factors glutathion and 1-noradrenalin. The PG formed were isolated by column chromatography and the yield determined radiometrically.

Results. In agreement with published observations[12,16,17] the pyrogenic response to i.v. injection of EP showed a rapid increase in rectal temperature by a maximum of 2 °C. followed by a relatively rapid decline within 3 h.

Incubation of the cerebral PGSS with EP resulted in an increase in the biotransformation of arachidonic acid into PG, varying in degree according to the concentration applied (Table I). However, the EP had no effect on the biotransformation of arachidonic acid, when the microsomal fraction of bovine seminal vesicle was used as enzyme.

The increase in the rate of synthesis of PG observed upon incubation of cerebral PGSS with EP proved to be specific, since the enzymatic reaction was not affected by Fractions I and II obtained during the isolation of EP (Table II). These fractions also displayed no pyrogenic effect after i.v. administration. Lipopolysaccharide isolated from *E. coli* and ovalbumin were equally devoid of any influence on the PGSS.

The increase in the activity of cerebral PGSS induced by EP could be inhibited by non-steroidal anti-inflammatory agents. Diclofenac sodium, for instance, which possesses marked antipyretic properties[18], exerted a dose-dependent suppressant effect on the augmented activity of the PGSS induced by EP.

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Table II. Effect of proteins of different origins on the activity of the prostaglandin-synthetase system[a]

Incubation conditions[b]	Protein content (µg/ml $\pm s_{\bar{x}}$)	Formation of PG (cpm $\pm s_{\bar{x}}$)	Increase in formation of PG (%)	No. of experiments
Control		4465 ± 324		
Endogenous pyrogen	775 ± 124	7744 ± 577	73 ± 4	10
Control		5342 ± 585		
Fraction I[c]	10030 ± 1261	5001 ± 542	−6 ± 2	3
Control		5403 ± 566		
Fraction II[c]	784 ± 118	5014 ± 595	−7 ± 6	3
Control		5376 ± 220		
Lipopolysaccharide[d]	0,1–1000[e]	5087 ± 213	−5 ± 6	3
Control		5194 ± 302		
Ovalbumin	0,1–1000[e]	5578 ± 146	+7 ± 4	3

[a] Microsomal fraction of bovine cerebral cortex. [b] Addition of 20 µl to incubation system. [c] Isolation conditions as described in Methods. [d] Isolated from *E. coli* (Difco). [e] Range of concentrations tested.

Discussion. The findings reported explain on the one hand why elevated concentrations of PG are demonstrable in the cerebrospinal fluid in the course of febrile reactions due to the release of EP[5,19]. On the other hand, in so far as PG can be considered to serve as mediators of the pyrogenic effects of EP, the observed activation of cerebral PGSS by EP would explain this mechanism of action. It must be borne in mind, however, that the concentration of arachidonic acid is a rate-limiting step in the chain of enzymatic reactions leading to the biosynthesis of PG. The question thus arises whether the pyretic effect of EP is solely the result of its influence on cerebral PGSS, or whether other mechanisms may also be involved[20].

In contrast to the activity of cerebral PGSS, that of PGSS isolated from seminal vesicles was not affected by EP. This disparity could be connected with the fact that the PGSS in various tissues are isoenzymes[21]. The possibility cannot, however, be excluded that the different susceptibilities of the PGSS isolated from the cerebral cortex and the seminal vesicle may only be due to variations in the isolation conditions. The finding that the cerebral PGSS displays less enzymatic activity than PGSS from seminal vesicles favours this assumption.

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Mescaline: its Effects on Learning Rate and Dopamine Metabolism in Goldfish (*Carassius auratus*)[1]

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Summary. The pharmacological action of mescaline on goldfish was studied with the Bitterman-Agranoff shock-avoidance test. In short term experiments with high mescaline doses an increase in learning rates was observed. Similar results were obtained with apomorphine and L-dopa. However, when the fish were exposed to smaller mescaline doses (or to fluphenazine) for 3 days, their ability to avoid electric shock was reduced. Apparently, mescaline induced a release of dopamine which stimulated central dopaminergic systems. Subsequently, MAO destroys the liberated dopamine. Thus, the ensuing dopamine deficit appears to be responsible for the marked changes in behavior in the chronic experiment.

It has been reported that the clinical symptoms in man and animals induced by mescaline can be reversed by injection of chlorpromazine[2] and that mescaline is capable of releasing cerebral dopamine[3]. These and other observations suggest the involvement of a dopaminergic system in mescaline action. If this were true, monoamine oxidase (MAO, EC 1.4.3.4), should be a part of this reaction by destroying the liberated dopamine. To test this conjecture we exposed goldfish to mescaline. We already had extensively studied the influence of modulators of amine metabolism on the behavior and cerebral amine metabolism of goldfish[4]. As in the past, we used the modified Bitterman-Agranoff shock avoidance test[5]. The fish are electrically shocked when they do not clear a submerged barrier within 10 sec after a light signal is turned on. This response is recorded as an error. 30 trials, given every 30 sec, form 1 training period which is repeated on 2 consecutive days. Each set of controls or test animals was comprised of at least 6 fish. When we plotted the logarithm of the number of errors for 5 consecutive trials, for all fish, against the number of trials (1st to 5th, 6th to 10th, etc.), a linear function characterized by high correlation coefficients ($r \leq 0.9$) emerged[4]. This observation makes it possible to tabulate the results numerically and to gain simple and strictly operationally defined behavioral parameters, e.g. slope (SL) of straight lines serving as a convenient indicator of learning rate. Here, the term of learning pertains to the totality of physiological processes which are responsible for behavioral changes in repeat performances.

In contrast to the outcome of experiments with hundreds of control animals, the slope of the linear function obtained for mescaline-treated fish always became positive for the second and more so for the third trial period (Table). The animals were increasingly unable to initiate movements in order to avoid electric shock although their capacity to move appeared to be completely intact. The pharmacologically inactive analogues of mescaline, viz. 3,4,5-trimethoxybenzylamine and 2,3,4-trimethoxy-β-phenylethylamine did not react in our system. The mescaline effect seems to be due to a lack of cerebral dopamine as indicated by the following observations reported in the Table: a) the behavioral changes are accompanied by a marked drop in brain dopamine levels while the content of norepinephrine and serotonin remained constant; b) application of several inhibitors of MAO, e.g. deprenyl, or of L-dopa abolished both the behavioral and metabolic mescaline effects; c) the dopaminergic antagonist, fluphenazine, evoked the same behavioral response as mescaline; again L-dopa administration prevented this reaction. In contrast, the treatment of the animals with L-dopa or with the dopaminergic agonist apomorphine led to higher learning rates. These responses made it pos-

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