time of the assay and to the fact that whole brain analysis have been performed [4, 13].

Further studies are necessary in order to assess dose-related changes in GABAergic mechanisms as well as to ascertain whether the 6-OHDA effects of this system are permanent, as expression of an aspecific degeneration of GABAergic neurons.

In conclusion present experiments show that a high intraventricular dose of 6-OHDA is able to affect in the diencephalon and brain-stem GABAergic system in a way which can explain the occurrence of epileptic seizures and the lowering of convulsion threshold.

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Inhibition of 3′-methyl-4-dimethylaminoazobenzene (3′MeDAB)-induced hepatocarcinogenesis in the rat: chloramphenicol inhibits *N,N*-dimethylaniline *N*-oxidase and *in vitro* binding of [^{3}H]3′MeDAB to protein but not to RNA

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Metabolic activation prior to covalent binding of carcinogen in target tissues is a requisite step for tumour induction with most chemical carcinogens [1], and many inhibitors of carcinogenesis are considered to exert their protective effects by inhibiting metabolic activation and/or by increasing detoxification processes [2].

The antibiotic chloramphenicol (CAP) inhibits tumour induction by various carcinogens in liver and lung [3–5] and evidence in some reports suggests that it inhibits carcinogen activation [6, 7]. However, when CAP was administered in the same dose and by the same route as was necessary to prevent liver tumour induction by 3′MeDAB, there was no effect on the level of colorimetrically-determined protein-bound dye in the liver [8]. As the colorimetric assay, which depends on the presence of an intact azo linkage, may not estimate all bound derivatives of azo dyes, we decided to utilize a recently characterized *in vitro* system which catalyzes the macromolecular binding of [^{3}H]3′MeDAB metabolites * to further investigate the effects of CAP administration on the metabolic activation of 3′MeDAB. The effect of CAP on *N,N*-dimethylaniline (DMA) *N*-oxidase activity was also investigated, for this enzyme is implicated in the *N*-hydroxylation of azo dyes, an essential metabolic activation step [9].

Male, random-bred Sprague–Dawley rats (200–250 g) were pair-fed diets containing no supplements (control), 2% CAP, 0.06% 3′MeDAB or 2% CAP and 0.06% 3′MeDAB as

* Labuc and Blunck, *Biochem. Pharmac.* (1979).

described previously [8] for 4 days. The rats were exsanguinated under light ether anaesthesia, their livers removed and weighed, and microsomal or post-mitochondrial supernatant (PMS) preparations prepared. All tissue preparations were kept at 0–4° and used within 30 min.

Microsomal preparations were made by homogenizing liver samples (4 g) in 3 vol of 1.15% (w/v) KCl/0.01 M Na^+/K^+ phosphate (pH 7.4) using a Potter–Elvehjem homogenizer with a Teflon pestle; the homogenates were centrifuged at 10,000 g_{max} for 15 min and the resultant PMS centrifuged at 100,000 g_{av} for 1 hr. DMA *N*-oxidase activity was determined using microsomal preparations that had been washed once in homogenizing buffer and resuspended in 0.01 M Na^+/K^+ phosphate (pH 7.4). The incubation mixtures contained in a final volume of 1.5 ml of 0.01 M Na^+/K^+ phosphate (pH 7.4): $MgCl_2$ (7.5 μmoles), EDTA (0.3 μmole), glucose-6-phosphate (G-6-P, 7.5 μmoles), $NADP^+$ (0.6 μmole), NAD^+ (0.45 μmole), G-6-P dehydrogenase (1.5 i.u.), absolute ethanol (30 μl), DMA (4.5 μmoles), semicarbazide HCl (6 μmoles) and microsomes equivalent to 37.5 mg wet wt. of liver, and were incubated at 37° for 8 min under air. DMA *N*-oxide production was determined by the procedure of Ziegler and Pettit as modified by Arrhenius [10].

PMS was prepared by homogenizing whole livers in 3 vol of 0.25 M sucrose, 0.05 M [bis(2-hydroxyethyl)imino-Tris(hydroxymethyl)–methane] (BisTris), 0.025 M KCl and 0.005 M $MgCl_2$ (pH 7.4) and centrifuging the homogenate at 10,000 g_{max} for 15 min. PMS-mediated binding of [^{3}H]3′MeDAB metabolites to RNA and protein was assayed as described previously* in an incubation system similar to that of Kadlubar *et al.* [11], which contained in a final volume of 1.0 ml: BisTris (100 μmoles, pH 7.0 at 37°), $MgCl_2$ (5 μmoles), EDTA (0.5 μmole), $NADP^+$ (0.4 μmole), NAD^+ (0.3 μmole), G-6-P (5 μmoles), either ATP (10 μmoles) plus Na_2SO_4 (10 μmoles) or 3′-phosphoadenosine-5′-phosphosulphate (PAPS, 0.32 μmole), yeast RNA (3.75 mg), absolute ethanol (20 μl), [^{3}H]3′MeDAB (100 nmoles, sp. act. 119 mCi/mmole, labelled on the toluidine ring) and PMS equivalent to 50 mg wet wt. of liver. Incubations were carried out at 37° under air for 32 min, and RNA and protein were extracted by the modification of the method of Muramatsu [12] described previously*. Aliquots of RNA and protein were counted with a mean efficiency of 20% in a Packard Tri-Carb Liquid Scintillation Spectrophotometer (model C2425) using automatic external standardization. CAP (1.0 mM) was added where indicated, to some incubations of microsomal and PMS preparations from rats fed the control diet. 3′MeDAB (m.p. 119°) and [^{3}H]3′MeDAB (m.p. 119–120°) were synthesized by the method of Giese *et al.* [13]. The [^{3}H]3′MeDAB was purified by chromatography on alumina and had a radiochemical purity of greater than 98 per cent t.l.c. using solvents A and C as described by Kadlubar *et al.* [9]. PAPS was prepared by the method of Irving *et al.* [14] except that phosphate buffer (0.03 M, pH 7.6), which stabilizes ATP-sulphurylase [15], was substituted for Tris buffer. NAD^+ (free acid), $NADP^+$ (monosodium salt), G-6-P (disodium salt), and G-6-P dehydrogenase (yeast, specific activity 471 i.u./mg) were purchased from Calbiochem, while ATP (disodium salt) and BisTris were from Sigma Chemical Co., yeast RNA was from Boehringer Mannheim and CAP (D(−)*threo* isomer) was a gift from Parke, Davis & Co.

Microsomal DMA *N*-oxidase activity was significantly decreased by dietary CAP by 17.5%, from 2.06 nmoles/mg protein/min to 1.70 nmoles/mg/min ($P < 0.05$, mean of values obtained from 6 rats). Dietary 3′MeDAB had a similar inhibitory effect (18.9 per cent inhibition) and the concurrent feeding of 3′MeDAB and CAP produced a partially additive effect (26.2% inhibition). This trend was also obtained if the data were expressed as DMA oxidized/g liver or /total liver.

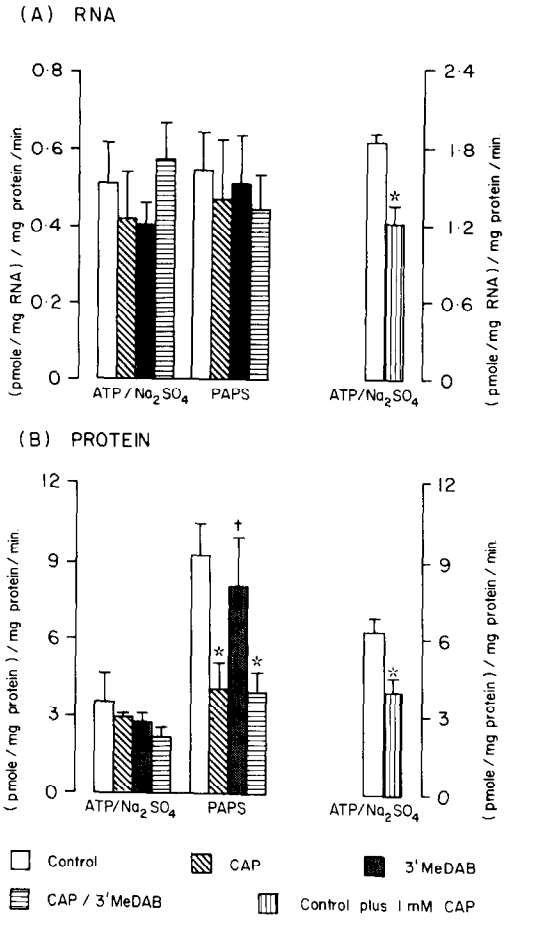

Fig. 1. Rats were pair-fed a control diet or diets containing 2% CAP and/or 0.06% 3′MeDAB for 4 days. For the studies on the effect of *in vitro* addition of CAP, rats were fed unrestricted control diet, then starved for 16 hr. The PMS-mediated binding of [^{3}H]3′MeDAB to exogenous yeast RNA and endogenous PMS protein using either ATP/Na_2SO_4 or PAPS as co-factor, were determined as described in the text. Binding is expressed as pmole [^{3}H]-3′MeDAB/mg RNA or protein/min/mg PMS protein. The results represent the mean ± S.E.M. values obtained from 6 rats fed each diet and from 3 control rats for the *in vitro* CAP experiments. *significantly different from control, $P < 0.05$ (Student's *t*-test). † significantly different from 3′MeDAB/CAP, $P < 0.05$ (Student's *t*-test)

Addition of 1 mM CAP to the microsomal preparation *in vitro* also significantly decreased DMA *N*-oxidase activity by 19.9% ($P < 0.05$, mean of values obtained from 3 rats). Similar trends in results were obtained when microsomal 3′MeDAB reductase, 3′MeDAB *N*-demethylase, aminopyrine *N*-demethylase and DMA *N*-demethylase activities were measured in rats pair-fed 3′MeDAB and/or CAP (data not shown). Thus, liver microsomal preparations from rats fed 3′MeDAB and CAP, which inhibits carcinogenesis, generally exhibit a lower capacity to metabolize xenobiotics *in vitro* than do microsomes from rats fed only 3′MeDAB.

Binding of [^{3}H]3′MeDAB metabolites to both PMS protein and exogenous yeast RNA was measured in the presence of an activated sulphate donor (PAPS or a PAPS generating system containing ATP and Na_2SO_4). In agreement with the effects of 3′MeDAB and CAP on microsomal xenobiotic metabolizing capacity, the level of [^{3}H]3′MeDAB metabolite binding to PMS protein was significantly decreased in rats fed

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CAP or 3′MeDAB plus CAP (Fig. 1); however this effect was only noted if PAPS, rather than ATP and Na_2SO_4, was included in the incubation medium. In contrast, binding of [^{3}H]3′MeDAB metabolites to yeast RNA was not significantly altered by either CAP and/or 3′MeDAB in the diet under any of the assay conditions. The different effects of feeding 3′MeDAB and/or CAP to rats on the binding of [^{3}H]3′MeDAB metabolites to PMS protein and yeast RNA *in vitro* may be a consequence of the involvement of different 3′MeDAB metabolites in RNA and protein binding*; alternatively the accessibility of RNA and protein to metabolites of 3′MeDAB may differ, for metabolites of CAP also bind to tissue proteins [16, 17].

Addition of CAP to control microsomes *in vitro* decreased both RNA and protein binding by 3′MeDAB metabolites to an approximately equal extent; while only protein binding of [^{3}H]3′MeDAB metabolites *in vitro* was decreased if CAP was fed (Fig. 1). The differences in the *in vivo* and *in vitro* effects of CAP on [^{3}H]3′MeDAB binding might be due either to activation of CAP *in vitro* to metabolites that bind to both RNA and protein in competition with 3′MeDAB metabolites, to some direct effect of CAP on 3′MeDAB activation *in vitro*, or to differences in the concentration of CAP present *in vivo* and *in vitro*. The first alternative is a distinct possibility because PMS protein may contain pre-existing bound metabolites of CAP when CAP is administered *in vivo*, whereas exogenous yeast RNA would not. Regardless of the interpretation placed on them, our data nevertheless confirm that compounds known to inhibit xenobiotic metablizing activity *in vitro* often do not produce the same effect *in vivo* [18, 19].

In conclusion, both CAP and/or 3′MeDAB in the diet produced a general decrease in the capacity of liver microsomal preparations to metabolize xenobiotics *in vitro*, as exemplified by a decrease in the *N*-oxidation of DMA. Binding of [^{3}H]3′MeDAB metabolites to PMS protein *in vitro* was likewise decreased when rats were fed CAP or 3′MeDAB plus CAP, but only if PAPS was included in the incubation medium. In contrast, there were no changes in the activation of [^{3}H]3′MeDAB to metabolites that bind to yeast RNA *in vitro* when either 3′MeDAB and/or CAP were fed. The latter finding supports the results of an earlier study of the levels of colorimetrically-determined protein-bound 3′MeDAB metabolites produced *in vivo* [8] and suggests that *in vitro* binding of 3′MeDAB metabolites to yeast RNA, which appears to involve the *N*-sulphate ester of the azo dyes*, is a more relevant system for studying the metabolic activation of amionazo dye carcinogens than is *in vitro* binding of 3′MeDAB metabolites to protein. However, the possibility exists that PMS protein from rats fed the CAP-containing diets could contain bound CAP metabolites that may prevent subsequent binding of any [^{3}H]3′MeDAB metabolites generated *in vitro*. The effect of dietary CAP on liver uptake, subcellular distribution and covalent binding of [^{3}H]3′MeDAB metabolites to DNA, RNA and protein *in vivo* is therefore under investigation.

* Labuc and Blunck, *Biochem. Pharmac.* (1979).

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