demethoxydaurorubicin; absence of the C-4 methoxyl substituent was associated with an increase in potency over daunorubicin. Therefore, it appears that the nature of the C-4 substituent on the chromophore substantially affects anthracycline pharmacology. The bearing of the enhanced lipophilic character of carminomycin on the nature and severity of adverse reactions to the drug remains to be established.

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GABA depletion and GABA-transaminase activity increase after intraventricular 6-hydroxydopamine

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Several studies have shown that intraventricular or intracerebral injection of 6-hydroxydopamine (6-OHDA) produce a rather selective, dose-dependent degeneration of catecholaminergic neurons [1–3] associated with a long-lasting depletion of brain catecholamines [4–6]. Similar results have been obtained in mammalian and avian species for adrenergic neurons in the periphery [7-11]. On the other hand, brain concentrations of serotonin [3, 4, 12], GABA [4, 13], acetylcholine, glycine, glutamic acid and other free amino acids [13] were not changed. In addition, low doses of 6-OHDA given intraventricularly (i.v.) do not seem to affect gross behaviour in rats [14], whereas after higher doses severe convulsions have been shown to occur [6, 13].

The aim of the present work was to study in chicks the effects of a single large intraventricular dose of 6-OHDA on GABA content, GAD and GABA-T activities in diencephalon and brain-stem.

Rhode Island Red chicks, 1 week old were used. For implanting cannulae the chickens were anaesthetized with halothane (Fluothane ICI) in oxygen delivered through a Vapor halothane vaporizer, according to Marley and Stephenson [15]. Chicks were tested at least 24 hr after implantation of cannulae when recovery was complete. The cannula position was verified by the outflow of clear cerebro-spinal fluid at the implantation time. Eight chicks received a single intraventricular dose (100 μg as free base) of 6-OHDA dissolved in 2.5 μl of distilled H_2O containing ascorbic acid (1 mg/ml); the pH was adjusted to 5.0 with hydrochloric acid.

The solution was made up just before use. Control animals ($n = 6$) received an equal volume of the vehicle solution. After decapitation the diencephalon and brain-stem were quickly dissected out within 30 sec and frozen in liquid nitrogen.

GABA was assayed by a C. Erba aminoacid analyzer with AMINEX A-5 (0.9 × 13 cm) ion exchange resin as previously described [16]

GAD-activity was assayed using the conditions of Beaven *et al.* [17], slightly modified. A 15 μl sample of the tissues homogenate (1:10) was mixed with 35 μl of reagent inside a 1.5 ml polypropylene Eppendorf vial. The vial was placed inside a 20 ml screw-cap liquid scintillation counting vial. A drop (20 μl) of 30% 2-phenylethylamine in methanol was adsorbed on a square (1 cm^2) of filter paper (Whatman 3MM), placed at the bottom of the counting vial away from the Eppendorf vial. The reagent consists of (i) L-[U-^{14}C]glutamic acid, 20 nCi; (ii) unlabeled L-glutamic acid to bring the concentration of aminoacid to 5×10^{-4}M; (iii) 1×10^{-5}M PLP; and (iv) 0.1 M sodium phosphate buffer, pH 6.8. Reaction blanks were prepared by substituting buffer for the sample. The counting vials were tightly capped and incubated at 37°C for 30 min; for deproteinization the vials were then placed on ice and uncapped one at a time and 20 μl of 2 N perchloric acid was added to the Eppendorf vials. The counting vials were quickly recapped and reincubated for

30 min. At the end of the second incubation, the Eppendorf vials were removed from the counting vials and discarded. Ten millilitres of liquid scintillation mixture (2.5 g PPO and 150 mg POPOP/1 toluene) was added in each counting vial and radioactivity was measured by a Nuclear Chicago liquid scintillator at room temperature. Preliminary experiments have shown that in our experimental conditions $^{14}CO_2$ loss was approx. 1.5%. In addition, under our experimental conditions the rate of enzyme reaction was linear as function of time (up to 30 min) as well as of protein (up to 0.150 mg).

GABA-T activity was assayed in 100 μl of the reaction mixture containing: GABA 5 μmoles; α-ketoglutarate 5 μmoles; PLP 0.4 μmole; 0.1 M phosphate buffer, pH 8.4 and homogenate (1:10). After 1 hr incubation at 37° the reaction was stopped by adding 0.4 ml of absolute ethanol. Controls were carried out by adding homogenate after ethanol. After centrifugation, the glutamic acid levels in the supernatants were determined by an aminoacid analyzer with 3AR/2/A/55 (0.9 × 55 cm) ion exchange resin.

Protein were determined by the procedure of Lowry *et al.* [18].

A single intraventricular dose of 6-OHDA (100 μg) produced 24 hr later, a significant decrease in GABA content accompanied by a sustained increase in GABA-transaminase activity in the diencephalon and brain-stem (Figs. 1 and 2). In addition, GAD activity was not significantly changed in presence of PLP (Fig. 3), although in absence of PLP an increase in brain-stem GAD activity was observed. Since two types of GAD, one dependent on and the other independent of free PLP, have been shown to exist in the brain [19, 20], the GAD increase reported to occur in the brain stem under our experimental conditions seems to reflect changes in GAD activity independent of free PLP. Recently, it has been reported that 4 days after 6-OHDA concomitantly to the degeneration of the nigro-striatal pathway, there was in the striatum in the presence of PLP an increase in GAD activity [21] which seems to be related to the destruction of specific neuronal pathways with consequent increase in absolute values in GAD per mg/protein.

In addition, immediately after the administration, 6-OHDA produced profound behavioural sedation, lethargy and squatting accompanied by a marked fall in body temperature, these effects lasting over 24 hr. In some chicks (4 out of 8) within 5 min from the injection, escape responses followed by convulsive episodes occurred. The depletion in GABA content may provide an explanation for aggressive behaviour [22] and convulsions reported to occur in approximately 50 per cent of treated chicks (present and unpublished experiments) and rats [13]. It is well known that an experimentally induced deficit in GABAergic inhibitory mechanisms is associated with epileptic seizures [23–25]. Interestingly the potential epileptogenic properties of 6-OHDA have been detected by showing that after intraventricular injection of this neurotoxic agent there is a significant lower electroconvulsive threshold [26].

The lack of effect on GABA content after i.v. injection of 6-OHDA in rats may be attributed to the different doses and

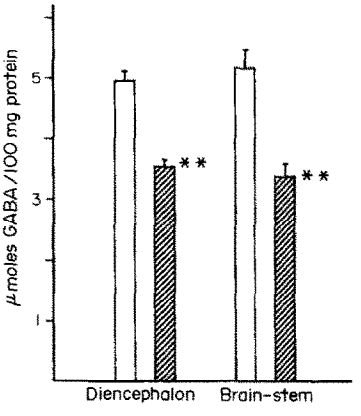

Fig. 1. Effect of a single intraventricular dose of 6-OHDA on diencephalon and brain-stem GABA content. The columns represent the mean values ± S.E.M. of 6–8 chicks; for each brain area the single values have been obtained from triplicate assays. **P < 0.001 in comparison to controls. ☐ controls ; ▨ treated.

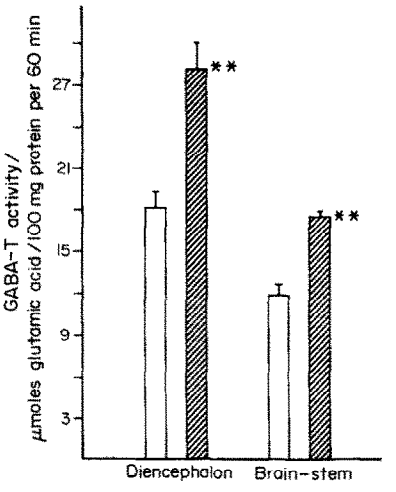

Fig. 2. Effect of a single intraventricular dose of 6-OHDA on diencephalon and brain-stem GABA-T activity. The columns represent the mean values ± S.E.M. of 6–8 chicks; for each brain area the single values have been obtained from triplicate assays. **P < 0.001 in comparison to controls. ☐ controls ; ▨ treated.

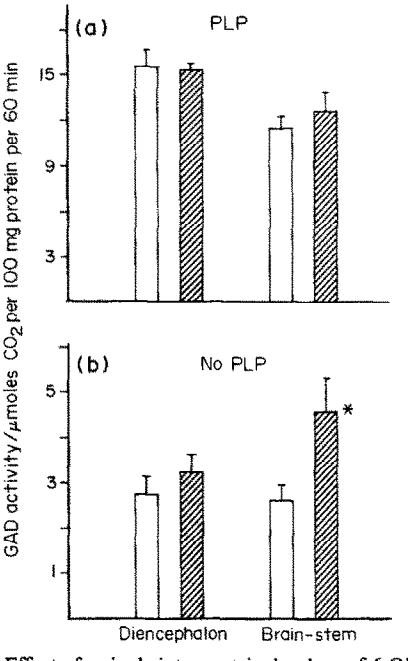

Fig. 3. Effect of a single intraventricular dose of 6-OHDA on diencephalon and brain-stem GAD activity. The columns represent the mean values ± S.E.M. of 6–8 chicks; for each brain area the single values have been obtained from triplicate assay. *P < 0.05 in comparison to controls. ☐ controls ; ▨ treated.

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).