cytochrome P-450 produces characteristic difference spectra of three principal types [13, 14] which can usually be quantified by determination of the spectral binding dissociation (K_s) constant. Alkylbenzoxazoles interacted with PB-induced rat liver microsomes to yield weak difference spectra which coupled with a low solubility in the binding mixture did not allow for the reliable determination of K_s values. The observed peak/trough wavelengths for the cytochrome P-450 difference spectra of compounds I, IX and X were 389/418 nm (type RI), 389/419 nm (type RI) and 384/414 nm (unclassified) respectively. Studies with benzimidazole derivatives [23] have shown that 2-methyl substitution alters binding from type II (benzimidazole) to type RI (2-methylbenzimidazole). 2-Methyl substitution of 5-methylbenzoxazole produced a similar red shift in the absorption maximum of the cytochrome, however in this latter case the difference spectrum became uncharacteristic.

In summary, all benzoxazoles tested inhibited APDM activity in rat liver microsomes and there was an apparent relationship between inhibitory potency and partition coefficient which was best described by a second order equation in log P ($pI_{50} = 1.273 \log P - 0.130 (\log P)^2 + 1.13$, $n = 11$, $r = 0.985$). The maximum inhibitory activity for a 2-alkylbenzoxazole series was lower than that of an arylimidazole reference compound and lower than that demonstrated previously for a 2-alkylbenzimidazole series. Eleven of twelve benzoxazoles enhanced AH activity in rat liver microsomes (and zoxazolamine was inhibitory), however, no relationship was apparent between physico-chemical properties and degree of enhancement of AH activity. Alkylbenzoxazoles represent the first series of compounds to enhance AH activity and may be a useful series for a more detailed study of the mechanism of enhancement of hepatic microsomal aniline hydroxylation.

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Toxic effect of chlorisondamine in neonatal rat liver*

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Chlorisondamine (CHLOR) is a long-lasting ganglionic blocker that has been used both clinically, in treatment of hypertension, and experimentally, in studies of ganglionic transmission. The physiological and biochemical effects of CHLOR administration are typical of ganglionic blockade: CHLOR decreases blood pressure, inhibits salivary secretion, decreases plasma catecholamines and prevents reflex sympathetic stimulation of a number of a peripheral tissues including the adrenal, heart and blood vessels [1–10]. Although CHLOR is an effective ganglionic blocker, some evidence suggests that this drug possesses other actions which can both oppose and potentiate its ganglionic blocking activity. Acute CHLOR administration causes a transient sympathetic stimulation that induces adrenal tyrosine hydroxylase [7], and chronic CHLOR administration stimulates adrenal catecholamine-synthesizing enzymes by a

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combination of direct and neural actions [10]. While this drug lacks overt toxic effects [3], these non-ganglionic actions limit its clinical usefulness. In the present study, we demonstrate a direct toxic effect of CHLOR on liver of suckling rats that further limits the use of this drug in developmental studies.

Materials and methods

Animals. Lactating rats with litters of ten pups and adult male rats were obtained from Zivic Miller Laboratories (Allison Park, PA). Rats were housed in a vivarium with a 12-hr light and dark cycle and an ambient temperature of 22°. Rats were transferred to the laboratory the day before each experiment. Pups used in this study were 8 days old. All drugs were administered subcutaneously in sterile saline (1 μl/g body wt) except growth hormone, which was dissolved in 0.01 N $NaHCO_3$. Control animals were injected with appropriate vehicle at the same time as experimental animals.

ODC activity. Animals were decapitated 4 hr after drug administration, when ornithine decarboxylase (ODC, EC 4.1.1.17) activity following administration of each of these drugs is maximal. Tissues were removed and homogenized 20:1 (w/v) in ice-cold Tris buffer (10 mM, pH 7.2) with a Polytron homogenizer and then centrifuged at 27,000 *g* for 20 min. ODC activity in the supernatant fraction was determined by measuring the release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine as described previously [11].

Materials. Chlorisondamine chloride was obtained from CIBA Pharmaceuticals (Summit, NJ) and dexamethasone sodium phosphate from Merck, Sharpe & Dohme (Rahway, NJ). Phenylephrine and dibutyryl cAMP (sodium salt) were obtained from the Sigma Chemical Co. (St. Louis, MO). Growth hormone (NIH S11) was a gift of the Hormone Distribution Program of the NIAMDD. Ornithine monohydrochloride, DL-[1-^{14}C], specific activity 30–50 mCi/mmole, was purchased from the New England Nuclear Corp. (Boston, MA).

Statistics. Student's two-tailed unpaired *t*-test was used for statistical analysis of the data. Statistics were calculated on the raw data.

Results and discussion

CHLOR has been used in this laboratory to investigate neurally-mediated induction of the enzyme ornithine decarboxylase in heart and blood vessels [5]. In preliminary studies of liver ODC induction by adrenergic agents, we evaluated possible toxic effects of CHLOR by determining its effect on liver ODC induction by several hormones that act directly on the liver. These responses should be altered by hepatotoxic, but not by ganglionic blocking actions of CHLOR. Eight-day-old rat pups were injected with saline or CHLOR (2.5 mg/kg), and 30 min later were injected with vehicle, dexamethasone, dibutyryl cAMP, phenylephrine or growth hormone. Four hours after hormone administration, pups were killed, and liver ODC activity was determined as described in Materials and Methods. The results of this experiment are shown in Table 1. CHLOR decreased basal ODC activity and either significantly diminished (dexamethasone) or completely eliminated (cAMP, phenylephrine, growth hormone) ODC induction. These effects probably reflect an action on enzyme synthesis *in vivo*, as adding CHLOR directly to the ODC assay did not affect enzyme activity in liver homogenates (data not shown).

ODC activity is a sensitive index of tissue growth and differentiation, and its decrease in liver following CHLOR administration probably indicates a significant impairment of tissue growth and/or metabolism. Such an effect should be more dramatic in growing tissues. To test this possibility, adult male rats were injected with saline or CHLOR, followed by vehicle, growth hormone, phenylephrine or dexamethasone. As shown in Table 1, CHLOR had no effect on basal ODC activity and did not prevent its induction by any of these hormones. The slight difference between dexamethasone-stimulated ODC activity in CHLOR-injected rats and controls was not statistically significant and was well within the normal range of variability for the response to this hormone.

To determine if the apparent toxicity of CHLOR was characteristic of all ganglionic blocking agents, rat pups were injected with one of two other ganglionic blockers, mecamylamine or hexamethonium, followed by growth hormone or vehicle. These pups were killed 4 hr after the second injection. The results of this experiment are shown in Table 2. The effects of CHLOR shown in Table 1 are included in this table for comparison. Neither hexamethonium nor mecamylamine decreased basal ODC activity (in fact, mecamylamine increased basal ODC activity) or its induction by growth hormone, while CHLOR decreased both. This finding suggests that the toxic effects of CHLOR are not a characteristic of all ganglionic antagonists.

Table 1. Effect of chlorisondamine on liver ODC induction*

	Control	Chlorisondamine
Eight-day-old pups		
Vehicle	100 ± 24 (40)	16 ± 3† (40)
Dexamethasone, 5 mg/kg	325 ± 72† (10)	94 ± 34‡ (10)
Dibutyryl cAMP, 40 mg/kg	668 ± 67† (5)	8 ± 1†‡ (5)
Phenylephrine, 5 mg/kg	284 ± 82† (15)	13 ± 3†‡ (15)
Growth hormone, 100 μg/pup	594 ± 197† (15)	73 ± 21‡ (10)
Adult males		
Vehicle	100 ± 28 (8)	92 ± 19 (10)
Dexamethasone, 5 mg/kg	621 ± 134† (5)	451 ± 75† (5)
Phenylephrine, 5 mg/kg	372 ± 38† (5)	337 ± 79† (5)
Growth hormone 100 μg/rat	1198 ± 134† (5)	1351 ± 194† (5)

* Animals were injected with saline or CHLOR (2.5 mg/kg). Thirty minutes later, animals were injected again with vehicle or hormone. Four hours after the second injection, animals were killed, and liver ODC activity was determined as described in Materials and Methods. Results are expressed as per cent control ± S.E.M.; N is indicated in parentheses. Control ODC activity = 0.626 nmole·g^{-1}·hr^{-1} for pups and 0.264 nmole·g^{-1}·hr^{-1} for adults.

† $P < 0.05$ or better relative to vehicle-injected control.

‡ $P < 0.05$ or better relative to drug-injected animals.

Table 2. Effect of ganglionic blockers on liver ODC activity*

	Control	Growth hormone
Vehicle	100 ± 20 (40)	323 ± 60† (10)
Hexamethonium, 15 mg/kg	77 ± 21 (20)	318 ± 71† (5)
Mecamylamine, 2.5 mg/kg	222 ± 47† (20)	429 ± 57† (10)
Chlorisondamine, 2.5 mg/kg	16 ± 3† (40)	73 ± 21‡ (10)

* Eight-day-old rat pups were injected with saline, hexamethonium or mecamylamine. Thirty minutes later, pups were injected with vehicle or growth hormone. Animals were killed 4 hr after the second injection, and liver ODC activity was determined as described in Materials and Methods. Results are expressed as per cent control ± S.E.M.; N is indicated in parentheses. Control ODC activity = 0.547 nmole·g^{-1}·hr^{-1}.

† $P < 0.05$ or better relative to vehicle-injected control.

‡ $P < 0.05$ or better relative to growth-hormone injected animals.

Finally, to determine if CHLOR had similar toxic effects on other tissues, rat pups were injected with saline or CHLOR, and both liver and heart ODC activities were measured 4 hr after drug administration. As shown in Table 3, CHLOR decreased liver ODC significantly, but it had no effect on heart ODC activity. This finding corroborates previous studies demonstrating that CHLOR lacks direct cardiac action [3].

Table 3. Effect of chlorisondamine on heart and liver ODC activity*

	ODC activity	
	Saline	Chlorisondamine
Liver	100 ± 28 (5)	27 ± 1† (5)
Heart	100 ± 31 (5)	115 ± 25 (5)

* Eight-day-old rat pups were injected with saline or chlorisondamine (2.5 mg/kg). Animals were killed 4 hr after injection, and liver ODC activity was determined as described in Materials and Methods. Results are expressed as per cent control ± S.E.M.; N is indicated in parentheses. Control liver ODC activity = 0.287 nmole·g^{-1}·hr^{-1}; control heart ODC activity = 0.987 nmole·g^{-1}·hr^{-1}.

† $P < 0.05$ or better relative to control.

The results of this study suggest that CHLOR has a toxic effect on the liver of developing rats. CHLOR decreased basal ODC activity and prevented its induction by several hormones. As the hormones tested are not thought to act neurally, it is unlikely that the ganglionic-blocking activity of CHLOR is responsible for this effect. Furthermore, effective doses of other ganglionic blockers did not have a similar action. Although it is possible that higher doses of mecamylamine or hexamethonium might affect liver ODC activity, these data show that doses which are reported to block ganglionic transmission have no effect on liver ODC activity, while a ganglionic blocking dose of CHLOR markedly suppressed liver ODC activity.

The physiological mechanism mediating the decrease in ODC activity caused by CHLOR is not clear, although inhibition of RNA and/or protein synthesis is a likely possibility. Almost all drugs which impair liver ODC induction by hormones are inhibitors of one or both of these processes. These drugs include classical inhibitors like actinomycin D and cycloheximide as well as other agents such as ethanol [12–17]. The one difference between the effects of the latter agents and CHLOR is that CHLOR effects were restricted to the liver, while these agents affect ODC induction in other tissues. Perhaps liver protein synthesis is more sensitive to inhibition by CHLOR. Alternatively, CHLOR might be sequestered in the liver of suckling rats to a greater extent that these other drugs.

The hepatotoxic action of CHLOR clearly is restricted to developing animals, as neither basal nor stimulated ODC activity was affected in adult rats by CHLOR administration. This difference could reflect higher tissue drug levels in neonates or a greater sensitivity of rapidly growing tissues to the effects of CHLOR. The insensitivity of liver ODC to CHLOR that was observed in adult rats is consistent with previous reports that CHLOR lacks obvious toxic effects in adult animals [3].

In summary, we have shown that CHLOR both decreases basal ODC activity and prevents its induction by hormones in liver of suckling rats. Other ganglionic blockers do not have similar effects, and CHLOR does not affect liver ODC activity in adult rats. The action of CHLOR in developing liver is characteristic of agents which impair protein or RNA synthesis. These findings suggest that CHLOR has a direct toxic effect on the liver in developing rats.

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Effect of diacetylmonoxime and atropine on malathion-induced changes in blood glucose level and glycogen content of certain brain structures of rats

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The organophosphorous compounds inhibit cholinesterase and increase the level of acetylcholine, which stimulates the central nervous system and causes hyperactivity or tremors and induces convulsions in excessive amounts [1–4]. Hyperglycaemia is another manifestation of toxicity induced by certain organophosphorous compounds [5, 6]. The oximes have been reported to control the toxic effects induced by organophosphate compounds by reactivating the phosphorylated (inhibited) cholinesterase [7–9]. The effect of oximes on the hyperglycaemia induced by organophosphorous compounds has not been determined. The aim of the present study was to determine the effect of diacetylmonoxime (DAM) and atropine on the level of blood glucose in malathion-treated rats.

Certain tremorigenic agents, for example oxotremorine [10], have been reported to reduce the level of cerebral glycogen. Since tremors appear early during the course of poisoning induced by organophosphorous compounds, we also determined the effect of malathion and other drugs on the glycogen content of various brain structures including the corpus striatum (which is mainly involved in the production of tremors [11, 12]).

Adult male albino rats, 175 ± 15 g, were used. The animals were fasted for 18 hr before use since preliminary experiments indicated that more uniform results were obtained in this manner. The animals were injected with malathion, 500 mg/kg, i.p. Some of the malathion-treated animals were injected with DAM (100 mg/kg, i.p.) or atropine (25 mg/kg, i.p.) immediately or 30 min after the administration of malathion. Controls received normal saline. Malathion was also administered to rats that had received reserpine (1 mg/kg, i.p.) daily for 3 days. The animals were decapitated 1 hr after treatment with malathion. The skull was opened immediately, and various brain regions were quickly separated [13]. The blood glucose level was determined by the method of Nelson [14]. The glycogen was extracted according to the method of Lebaron [15] and estimated colorimetrically as described by Montgomery [16].

The data were analysed statistically using Student's *t*-test, and significant differences between the means were determined.

The values of blood glucose and of glycogen in various brain structures of malathion-treated rats are given in Table 1. The level of blood glucose was raised, and glycogen in various brain structures was reduced after treatment with malathion (Table 1). The changes in the levels of blood glucose and cerebral glycogen were not modified by DAM or atropine given 30 min after the administration of malathion; treatment with these drugs immediatley after malathion prevented the increase in blood glucose and the depletion of glycogen in various brain structures (Table 1). Pretreatment with reserpine did not modify the induced changes in blood glucose and cerebral glycogen in malathion-treated animals.

It was reported previously that organophosphorous compounds induced hyperglycaemia through the release of catecholamines which have a known hyperglycaemic effect [17]. Our results indicate that treatment with reserpine, a known depletor of catecholamines [18], failed to prevent the increase in blood glucose level in malathion-treated rats (Table 1). Other workers have also reported that α-adrenergic blockade prevented the catecholamine-induced hyperglycaemia [19] but did not abolish the organophosphate-induced hyperglycaemia [6]. Thus, other mechanisms may be involved in the production of hyperglycaemia in malathion-treated animals.

It was reported previously that DAM readily crossed the blood–brain barrier and was more effective than other oximes in reactivating the cholinesterase in the brain [1, 20]. Accordingly, DAM was used in the present study to obtain quick reactivation of cholinesterase activity in the brain. The results presented indicate that DAM given 30 min after the administration of malathion did not restore the induced hyperglycaemia to normal; however, DAM or atropine given immediately after malathion prevented the hyperglycaemia and depletion in the level of glycogen in various brain structures of malathion-treated animals (Table 1). The drugs may have been less effective when administered 30 min after malathion treatment since, at that time, the rise in blood glucose, possibly mediated through acetylcholine, would most likely have been fully established.

Certain organophosphorous compounds (e.g. Soman) have been reported to increase the level of cyclic AMP in the brain [21]. Cyclic AMP is believed to regulate the storage of glycogen which is hydrolysed or reduced with the rise in blood glucose level [22]. According to certain workers, the level of cerebral glycogen is increased after anesthesia and the administration of certain barbiturates and sedatives [23, 24]. Thus, the level of cerebral glycogen seems to be influenced by the state of activity of the brain. The reduction in the level of glycogen in various brain structures (Table 1) may have been related to the stimulatory effects associated with an increase in the concentration of acetylcholine [1–4] or of cyclic AMP [21], induced by organophosphorous compounds. It is also possible that