Table 1. Effects of amphetamine and its hydroxylated derivatives on the specific radioactivity (SR: mCi/m-mole) of norepinephrine (in tissue and medium)

	Hypothalamic SR	Medium SR	R = Hypothalamic SR / Medium SR
Controls	10·3 ± 1·4	11·0 ± 2·2	0·93 ± 0·14
Amphetamine	6·3 ± 1·0*	22·5 ± 3·8*	0·28 ± 0·04*
Hydroxyamphetamine	9·1 ± 1·7	34·3 ± 3·6*	0·26 ± 0·03*
α-Methyloctopamine	6·4 ± 0·3*	15·5 ± 0·7	0·41 ± 0·02*

* $P \leqslant 0{\cdot}01$.

increase in the specific radioactivity of the norepinephrine in the supernatant, but there is no effect in the tissue. These apparently contradictory results can be explained by an accelerated synthesis of ^{3}H-norepinephrine in the tissue.

In conclusion, the three amines studied, having different mechanisms of activity, produce the same results, preferential release of newly synthesized norepinephrine.

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Inhibition of beef plasma amine oxidase by clorgyline

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The antidepressant drug Clorgyline® [*N*-methyl-*N*-propargyl-3(2,4 dichlorophenoxy) propylamine HCl] was first described by Johnston [1] who showed it to be capable of selectively and irreversibly inhibiting the activity of the FAD^+-linked mitochondrial monoamine oxidase toward certain substrates at lower concentrations than were required to inhibit the oxidation of others. Graphs of percentage inhibition vs log. Clorgyline® concentration gave biphasic curves under certain conditions, and several workers now use this as a criterion of multiplicity [2–4]. Recently we have proposed a model for multiplicity of FAD^+-linked monoamine oxidase in rat liver mitochondria [5]. However in a large number of studies in which clorgyline has been used, either crude homogenates of tissue [see e.g. refs 6–10] or a "high speed" (approx. 375,000 *g* min) pellet derived from such an homogenate [see e.g. refs 1–3, 11–13] have been used as the enzyme source. It is possible that certain of these preparations may well be contaminated with the soluble pyridoxal phosphate-Cu^{2+}-dependant monoamine oxidase present in plasma, as it has been shown that this enzyme is capable of adhering to membranes [14] (platelet plasma membranes). Thus it was decided to investigate the effect of Clorgyline® on a purified preparation of beef plasma monoamine oxidase.

Purification of the enzyme was basically by a published procedure [15], up until the calcium phosphate gel step whence ammonium sulphate fractionation was carried out and the activity in the 45–50 per cent (saturation) precipitate was used for assay after dialysis against 3mM potassium phosphate buffer, pH 7·2. The resultant preparation showed a single activity band on continuous polyacrylamide gel (5 per cent) electrophoresis [16] with benzylamine as substrate. The gel being stained for activity using a novel method that has previously been used for staining other

hydrogen peroxide producing enzymes [17]. 0·001 Units of enzyme were loaded onto the gel in 10 per cent sucrose, and after electrophoresis the gel was stained at room temperature in a solution of 0·05 M potassium phosphate buffer, pH 7·2 containing 0·25 mg/ml of diaminobenzidine and a final concn of 5 mM benzylamine hydrochloride. A single band of activity was observed, that did not occur if benzylamine was not included in the staining medium or if the enzyme had been pretreated with 1mM potassium cyanide. The enzyme was assayed directly by a spectrophotometric assay using benzylamine as substrate [18], in 120 mM potassium phosphate buffer, pH 7·2, 30° as previously described in full [19]. Routinely assays contained protein at a final concentration of 0·3 mg/ml and reaction velocity was found to be proportional to protein concentration over the range examined (0–0·5 mg/ml), whether the spectrophotometric assay was used or oxygen consumption was followed. The enzyme was shown to be irreversibly inhibited by incubation with 1 mM potassium cyanide. Initial rates were found to be linear for at least 5 min under these assay conditions.

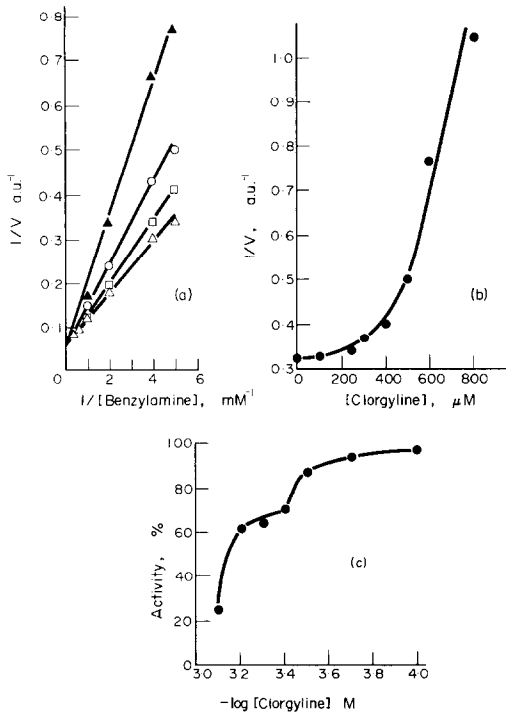

Fig. 1. Inhibition of plasma amine oxidase by Clorgyline®. Assays were carried out as described in text. (a) Lineweaver–Burk plot. The Clorgyline® concentrations were 0·4 mM (□), 0·5 mM (○), 0·6 mM (▲) and zero (△). (b) Dixon plot. The benzylamine concentration was held constant at 0·25 mM. (c) "Dose–response curve". A replot of the data shown in (b). The initial rate observed in the presence of 0·25 mM benzylamine alone was taken as 100 per cent. Arbitrary units are defined as a change in extinction of 0·001 extinction units/min. This is equivalent to the production of 724 pmoles of benzaldehyde/min.

A Lineweaver–Burk plot [20] gave a K_m of 0·9 mM for benzylamine (oxygen concentration was air saturating, 0·23 mM), and a maximum velocity of 0·034 μmoles of benzylamine oxidised/min per mg of protein.

Clorgyline® was found to be a reversible inhibitor of the enzyme; activity being restored by dilution or dialysis. An initial rate investigation by Lineweaver–Burke analysis [20] showed that Clorgyline® was a competitive inhibitor of benzylamine oxidation (Fig. 1a). However Dixon analysis [21] yielded a parabolic result, and the curve obtained when the benzylamine concentration was held constant at 0·25 mM is shown in Fig. 1b. Such a result is not consistent with the presence of two-enzymes with different affinities for Clorgyline® which would yield a hyperbolic result [see e.g. ref 5], but could be rationalised by supposing that more than one molecule of inhibitor could bind to the same enzyme species as that which would bind substrate [22]. This result is especially interesting if the data in Fig. 1b is plotted as a semi-log. "dose–response" curve as in Fig. 1c. The curve obtained is remarkably similar to that given by preparations of monoamine oxidase that are believed to contain two enzyme species differing in their affinities for Clorgyline® [1]. However in the case described above with the plasma amine oxidase, the kinetics are of reversible inhibition whereas in the case of mitochondrial monoamine oxidase the inhibition is irreversible.

This result exemplifies the fact that great care must be taken in examining tissue for potential "multiplicity" using Clorgyline®. This is more so the case when the enzyme source is crude and likely to be contaminated with blood viz. liver, heart and spleen, and if the assay concentrations of substrates are low. We would recommend that tissues being investigated for multiplicity of mitochondrial monoamine oxidase be carefully washed; the effect of cyanide investigated, it being an irreversible inhibitor of plasma amine oxidase [see e.g. ref. 14] and reversible for mitochondrial monoamine oxidase [19]; the assays being preferably carried out at saturating substrate concentrations (× 10 K_m concn), and that a check is made on the irreversibility of Clorgyline® inhibition.

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Acute alcoholic fatty liver—Metabolism or stress

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Mallov and Bloch [1] first reported that a large single dose of ethanol, given to the rat either by stomach tube or by intraperitoneal injection, provoked a significant accumulation of triglycerides (TG) within 12–16 hr. Numerous investigators have confirmed this observation (for references, see Hawkins and Kalant [2]), but the mechanism has not yet been clarified. Since the effect is abolished by hypophysectomy or adrenalectomy [1], the accumulation of hepatic TG was first attributed to stimulation of mobilization of fatty acids from peripheral adipose tissue to the liver, under the influence of adrenalin released as a consequence of intoxication. Others have pointed out, however, that in the fasting animals the only significant source of fatty acids would be the peripheral adipose tissue. The accumulation of TG in the liver was attributed to impairment of oxidation of the mobilized fatty acids as a consequence of the increased NADH/NAD ratio secondary to the oxidation of ethanol [3].

One method of distinguishing between these two possibilities appeared to be the use of pyrazole, an inhibitor of alcohol dehydrogenase activity [4–6]. If the accumulation of hepatic TG required the active metabolism of ethanol, it should be prevented by a dose of pyrazole which drastically reduced alcohol oxidation during the experimental period. In contrast, if the lipid accumulation were due to a direct effect of ethanol *per se*, rather than to its metabolism, the administration of pyrazole should have no effect upon it.

Unfortunately, the results of such experiments have been contradictory. Morgan and DiLuzio [7] found that pyrazole completely prevented the alcohol-induced acute fatty liver. Bustos *et al.* [8] found that it had no effect whatever on the TG accumulation measured at 16 hr after the alcohol. Numerous other groups [9–12] have obtained intermediate results with reduction of TG accumulation under some conditions and not under others. The effects of the pyrazoles appear to be complex, and a more detailed analysis of their effects upon the acute alcoholic fatty liver has been presented elsewhere [13].

In order to avoid the complications introduced by the inhibitors of alcohol oxidation, a simple expedient has been adopted. Since alcohol oxidation reaches maximal velocity at quite low blood alcohol concentrations, any effects on fat metabolism due to the metabolism of ethanol should be fully demonstrated at sustained low concentrations of alcohol. In contrast, any effects secondary to intoxication should be demonstrable only at higher alcohol levels, and should be proportional to the level. In the present study, hepatic TG accumulation has been examined in rats given the same total dose of ethanol, but in one case as a single dose producing marked intoxication, and in the other as divided doses which did not produce gross intoxication.

The subjects used were adult male Wistar rats purchased from High Oak Farms, Guelph, Ontario. The mean body weights of the animals in different experiments varied from 135 to 265 g, but within any given experiment the weight range was not more than ± 15 g difference from the mean. The animals were housed in group cages, and fed standard Purina Rat Chow and water *ad lib.* up to the time of their selection for the experiment.

In the first experiment, animals were selected at random and distributed among four matched groups. Chow was removed at 4:00 p.m. on the day preceding the experiment, but water remained available *ad lib.* throughout the experiment. Treatments were begun at 9:00 a.m. the next day according to the following schedule. Group 1 received a dose of ethanol (4·8 g/kg) given by stomach tube as a 20% (v/v) solution in water. Group 2 received an equal volume of an equicaloric solution of glucose in water, also as a single dose by intubation. Group 3 received the same total dose of alcohol as group 1, but divided into four doses of