EFFECTS OF HYDRAZINES ON THE METABOLISM OF CERTAIN AMINES AND AMINO ACIDS

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FOREWORD

This study was initiated by the Biomedical Laboratory of the Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio. The research was performed in support of Project No. 6302, "Toxic Hazards of Propellants and Materials," Task No. 630202, "Pharmacology and Biochemistry," under Contract No. AF 33(657)-11757, with the Science Research Institute, Oregon State University, Corvallis, Oregon. Dr. C. H. Wang was the principal investigator for Oregon State University and A. A. Thomas, MD and K. C. Back, PhD were contract monitors for the Toxic Hazards Branch, Physiology Division. Research was initiated 1 June 1963 and completed 30 June 1964.

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This technical report has been reviewed and is approved.

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ABSTRACT

Certain effects of simple hydrazines via i.p. administration upon the metabolism of amines and amino acids by rats were examined. Unsymmetrical dimethylhydrazine (UDMH), monomethylhydrazine (MMH) and hydrazine strongly inhibited oxidation of putrescine-1,4-C^{14} (1,4-diamino butane) and methylamine-C^{14} to $C^{14}O_2$ by intact rats. MMH caused a virtually complete inhibition of monoamine oxidase activity in vivo, but inhibition by UDMH and hydrazine was limited. In vivo and in vitro diamine oxidase activity was heavily suppressed by all three hydrazines. The inhibition duration of methylamine oxidase by UDMH and hydrazine was found to last several days. Inhibition of putrescine oxidation was reversed within 3 days, indicating a possible difference between the enzyme systems which metabolize methylamine and putrescine. The metabolism of varied oral and intraperitoneal doses of L-glutamic acid-1-C^{14} by rats was inhibited by hydrazine, but not by UDMH or MMH. Oxidation of large oral doses of L-alanine-1-C^{14} to respiratory $C^{14}O_2$ was slightly inhibited by UDMH, MMH and blocked to a greater extent by hydrazine. Similar results were found in the metabolism of low levels of γ-aminobutyric acid-1-C^{14} (GABA-1-C^{14}) except that hydrazine intoxication caused an almost complete inhibition of GABA-1-C^{14} conversion to $C^{14}O_2$. In vivo decarboxylation of trace amounts of 3,4-dihydroxyphenylalanine-1-C^{14} was not affected by any of the three hydrazines. A study of absorption of L-glutamate-U-C^{14} from the digestive tract of rats indicated that UDMH may cause a substantial loss of gastric motility.

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EFFECTS OF SIMPLE HYDRAZINES UPON AMINE METABOLISM IN THE RAT

SECTION I

INTRODUCTION

Inhibition of amine oxidases by hydrazines and hydrazides in vitro and in vivo is well documented (ref. 1, 2, 3, 4 & 5). Little work, however, has been reported on the effects of simple hydrazines on the metabolism of amines in intact animals. Werner and Seiler (ref. 6) have examined the metabolism of methylamine in intact animals after treatment by various inhibitors (including hydrazine compounds) particularly monoamine oxidase inhibitors, and found substantial inhibition. Davis and de Ropp (ref. 7) have reported increased urinary excretion of methylamine after administration of hydrazine derivatives. However, the significance of these findings is not certain, since the physiological role of aliphatic amines in the central nervous system is not well understood (ref. 5 & 8). The present work was undertaken in an effort to better understand the toxic effects of hydrazine, monomethylhydrazine (MMH) and unsymmetrical dimethylhydrazine (UDMH) upon this sector of mammalian metabolism. In this report the data in tables and the data presented graphically are all separate experiments and are not a duplication of presentation of data. All of our data on hydrazine effects is shown in the graphs only.

SECTION II

MATERIALS

Experimental Animals

All studies described in this report were conducted with male Sprague-Dawley rats obtained from Pacord Research, Inc., Portland, Oregon. The rats were maintained on Purina Laboratory Chow and water ad libitum until the time of experiments. Weight of the rats at the time of use was 240-260 grams.

Chemicals

UDMH (anhydrous 98-99%), hydrazine (anhydrous 95-100%) and MMH (BP 87-89°C) were obtained from the Matheson Company, Inc., Matheson Coleman & Bell Division, East Rutherford, New Jersey. To minimize air oxidation, these hydrazines were stored in the dark in nitrogen-flushed glass ampoules. All solutions of hydrazines were made fresh for each experiment with distilled water. Isopropyl isonicotinylhydrazide (Iproniazid) was supplied by Hoffman-la Roche, Inc., Nutley, New Jersey. 2-Phenyl-cyclopropylamine (SKF 385-B) was a gift from Smith Kline and French Laboratories, Philadelphia, Pennsylvania. Methylamine, 40% in water, was purchased from Matheson, Coleman & Bell, East Rutherford, New Jersey.

Radiochemicals

Methylamine-C^{14} hydrochloride (sp.act. 3.8 mc/mmole), putrescine-1,4-C^{14} dihydrochloride (sp.act. 3.1 mc/mmole) and tryptamine-2-C^{14} bisuccinate (sp.act. 1.3 mc/mmole) were purchased from the New England Nuclear Corporation, Boston, Massachusetts.

Radiorespirometry

The metabolism of methylamine-C^{14} and putrescine-1,4-C^{14} by intact normal and intoxicated rats was studied with a four-channel animal radiorespirometry system equipped with an analog-to-digital printout system. This apparatus has been described in detail elsewhere (ref. 9). In all experiments, the data presented represents two or more duplicate experiments.

Intoxication by Hydrazine, MMH and UDMH of Rats Used in Radiorespirometry

Administration of hydrazines to rats was by intraperitoneal injection of an aqueous solution of the respective hydrazines 30-45 minutes prior to administration of C^{14} labeled methylamine or putrescine. In some experiments, substrate oxidation was observed several days following administration of hydrazines to rats.

Administration of Amines

Methylamine-C^{14} and putrescine-1,4-C^{14} were administered intraperitoneally as aqueous solutions of the hydrochloride salts. In the case of methylamine-C^{14}, different concentrations were employed to determine effects, if any, of amine dose level upon the rate of catabolism to respiratory CO_2.

In Vivo Inhibition of Monoamine Oxidase (MAO)

In vivo inhibition of monoamine oxidase by hydrazines in intact rats was measured by an in vitro assay procedure, according to the method of Wurtman and Axelrod (ref. 10). Rats weighing 250-300 grams were administered hydrazine, UDMH or MMH 45 minutes prior to sacrificing. Brain and liver tissues were quickly removed, chilled on ice and assayed for MAO activity.

MAO Assay

Aliquots (300 mg) of the respective organs on a wet weight basis were added to 15 ml of chilled isotonic KCl solution (0.9%). The tissues were then homogenized for one minute in a Potter-Elvehjem homogenizer equipped with a Teflon pestle. Conical centrifugation tubes were used as assay reaction vessels. The order of addition of assay reagents was as follows: 0.5 M phosphate buffer, pH 7.2; the crude enzyme homogenate; and then the substrate tryptamine-2-C^{14} (5 μmmoles containing 10 μmc in 0.1 ml). Each reaction vessel was then incubated for 20 minutes at 37° C. After incubation, 0.2 ml of 2N HCl was added to terminate enzyme activity. The product of the

reaction, indoleacetaldehyde-2-C^{14} was then extracted with 6 ml of toluene and the entire mixture centrifuged to speed separation of the toluene and aqueous layers. For C^{14} assay, 4 ml aliquots of the toluene extract were added to counting vials containing 10 ml of terphenyl-POPOP-scintillation solution and counted in a Packard liquid scintillation spectrometer.

To insure a proportionality between the amount of product found and the amount of enzyme present, varying amounts of the crude homogenate were utilized in each of these assays. To check for the amount of tryptamine-2-C^{14} being extracted into toluene, a boiled enzyme preparation was also incubated with the control enzyme preparation. A typical assay contained 0.155 ml phosphate buffer, 0.02 ml crude enzyme preparation and 0.1 ml of substrate.

In Vitro Inhibition of Diamine Oxidase (DAO)

Fresh intestine tissues from rats weighing 250-300 grams were used for the in vitro assay of DAO inhibition. Other tissues, especially brain and liver, had no demonstrable DAO activity. The DAO assay procedure was according to the method of Okuyama and Kobayashi (ref. 11); and Burkard, Gey and Pletscher (ref. 12).

DAO Assay

A tissue sample (300 mg on a wet weight basis) was added to 15 ml of 0.08 M potassium phosphate buffer (pH 7.25). The solution was then saturated with n-octanol (0.2 ml to stop monoamine oxidase activity) and homogenized in a Potter-Elvehjem homogenizer for two minutes at 0° C. The homogenate was then transferred to a polypropylene centrifuge tube and centrifuged at 29,000 x G (15,000 rpm) for 30 minutes at 0° C. The supernatant fraction was used for enzyme assays. Conical glass centrifuge tubes were used as reaction vessels. The order of addition of the assay reagents for pre-incubation with inhibitor was as follows: phosphate buffer, enzyme preparation and finally the inhibitor. After addition of inhibitor, all vessels were pre-incubated for 15 minutes at 37° C, which was previously shown to provide maximum inhibition. Putrescine-1,4-C^{14} (0.158 μmole containing 0.1 μc in 0.1 ml) was added to both control and inhibited enzyme preparations and the complete assay mixture incubated for 60 minutes at 37° C. Upon completion of incubation, 100 mg of powdered sodium bicarbonate was added to each assay vessel. Toluene (6 ml) was then added and the reaction mixture extracted by shaking, followed by centrifugation of the mixture to separate the aqueous and toluene layers. To assay for the product of the reaction, Δ-1-pyrroline-C^{14}, a 4 ml aliquot of the toluene layer was placed in a counting vial containing 10 ml of toluene scintillation solution and counted in a liquid scintillation spectrometer.

To measure the amount of putrescine-1,4-C^{14} which can also be extracted from the reaction mixture, an enzyme control sample was boiled

prior to incubation. To insure that the reaction rate was dependent upon enzyme concentration and not upon substrate concentration, varying amounts of the enzyme preparation were utilized in these assays. At appropriate levels of enzyme concentration, the amount of product found was directly proportional to the amount of enzyme present in each assay. A typical assay mixture contained 0.5 ml of phosphate buffer, 0.5 ml of the enzyme preparation, 0.1 ml of inhibitor and 0.1 ml of substrate.

In Vivo Inhibition of Diamine Oxidase

In vivo inhibition of intestinal diamine oxidase (DAO) by hydrazines in intact rats was measured by the DAO assay procedure. Rats weighing 250-300 grams were administered hydrazine or UDMH 60 minutes prior to sacrificing. Tissues were quickly removed, chilled on ice and assayed for DAO activity.

The assay procedure was identical to that used for measuring the in vitro inhibition of DAO except that the inhibitor was not added and the pre-incubation omitted.

SECTION III

RESULTS AND DISCUSSION

Methylamine Metabolism by Normal Intact Rats

The rate and extent of methylamine catabolism to respiratory CO_2 by the rat was rapid and reproducible when the substrate level was 0.01 or 0.24 mmole/kg (table I, figures 1 & 2). It can be seen, however, that less than 50% of the C^{14} in the administered substrate appeared in the respiratory CO_2 prior to the virtual cessation of $C^{14}O_2$ production at about 5 hours. Since only small amounts of C^{14} were detected in the urine, it appears that a substantial portion of the administered methyl carbon may have entered an active C-1 pool and became incorporated into cellular constituents.

The nature of the amine oxidase which oxidizes methylamine to the corresponding aldehyde, formaldehyde, is not well established. Werner and Seiler (ref. 6) have shown that this enzyme may not be a typical mono- or diamine oxidase enzyme, but possibly a specific methylamine oxidase. Chung and McKenzie have demonstrated methylamine oxidation by a flavin system from rat liver (ref. 13).

Inhibition of Methylamine Catabolism by Hydrazines

The effect of hydrazine, UDMH, MMH and Iproniazid upon the catabolism of methylamine-C^{14} to respiratory $C^{14}O_2$ by the rat is shown in

figures 1 and 2 and table I. When the doses of hydrazine and UDMH given were 1.5 and 1.3 mmole/kg respectively, it can be seen that practically total inhibition of $C^{14}O_2$ formation by rats from methylamine-C^{14} occurred. In these experiments, UDMH and hydrazine were given 30 minutes prior to the administration of methylamine-C^{14} to obtain maximum inhibitory effects.

The inhibitory effect of UDMH on methylamine metabolism was found to persist for several days. Shown in figure 1 are the $C^{14}O_2$ recoveries when methylamine-C^{14} was administered to rats which had been treated with UDMH 6 days previously. A 30-35% inhibition of methylamine catabolism to $C^{14}O_2$ was observed even the sixth day after UDMH administration. Two days after treatment with hydrazine, $C^{14}O_2$ yield from methylamine-C^{14} was only 16-20% of that with the control animal (figure 2). Further study of the duration of the inhibition of methylamine oxidation has not been made. The inhibition of methylamine catabolism by low concentrations of MMH and UDMH was examined and the results are shown in table I. The inhibition was virtually complete at dose level of 0.1 mmole/kg of UDMH and 0.04 mmole/kg of MMH. In both instances these dose levels are less than 5% of the respective LD_{50}.

It is obvious tnat all three of the hydrazines examined were powerful in vivo inhibitors of the amine oxidases which catalyze the oxidation of methylamine.

Iproniazid is a well established inhibitor of amine oxidases (ref. 2, 6, 4,& 5), probably by virtue of its conversion to isopropyl hydrazine (ref. 3). The results shown in table I indicate that iproniazid effectively blocks the metabolism of methylamine-C^{14}

A few experiments were carried out at a higher substrate level of methylamine-C^{14}, 0.24 mmole/kg, to determine if a different degree of inhibition would be observed. However, as shown in table I, the results were very similar to that observed with the low substrate level. This fact suggests that, at the substrate levels employed, the inhibition was essentially independent of the methylamine concentration.

Methylamine metabolism and its inhibition by hydrazines appear to be mediated by a specific amine oxidase, since Werner and Seiler (ref. 6) have shown that hydrazine did not inhibit the catabolism of formaldehyde-C^{14} to respiratory $C^{14}O_2$. Werner and Seiler also found that the inhibitors of methylamine oxidation which are effective in intact animals (mouse, rat, guinea-pig) are also inhibitors in liver homogenates of ox, rabbit and rhesus monkey. Interestingly, they reported that methylamine was not oxidized by homogenates or mitochondrial preparations from organs of mouse, rat, guinea-pig and man; although these mammals can degrade methylamine extensively in vivo. Methylamine oxidase was distinguished by these workers from other amine oxidases by its response to various monoamine and diamine oxidase inhibitors.

Methylamine or its formation may play a physiological role in the

TABLE I

CONVERSION OF METHYLAMINE-C^{14} TO RESPIRATORY $C^{14}O_2$ IN RATS

Inhibitor	Cumulative $C^{14}O_2$ Yields Expressed as Percent of Administered C^{14}			
	Methylamine Level			
	.01 mmole/kg		0.24 mmole/kg	
	Time, min.		Time, min.	
	150	300	150	300
None	33.9 ± 1.2 (2)*		27.1 ± 4.2 (2)	38.4 ± 3.4 (2)
UDMH 1.0 mmole/kg	0.5 ± 0.2 (2)			
UDMH 0.1 mmole/kg			0.8 ± 0.1 (2)	
UDMH 0.01 mmole/kg	31.4 ± 1.0 (2)			
MMH 0.01 mmole/kg	0.9 ± 0.4 (2)	1.7 ± 0.7 (2)		
Iproniazid 0.56 mmole/kg	1.0 ± 0.3 (2)			

*Number in brackets denotes number of experimental rats per assay.

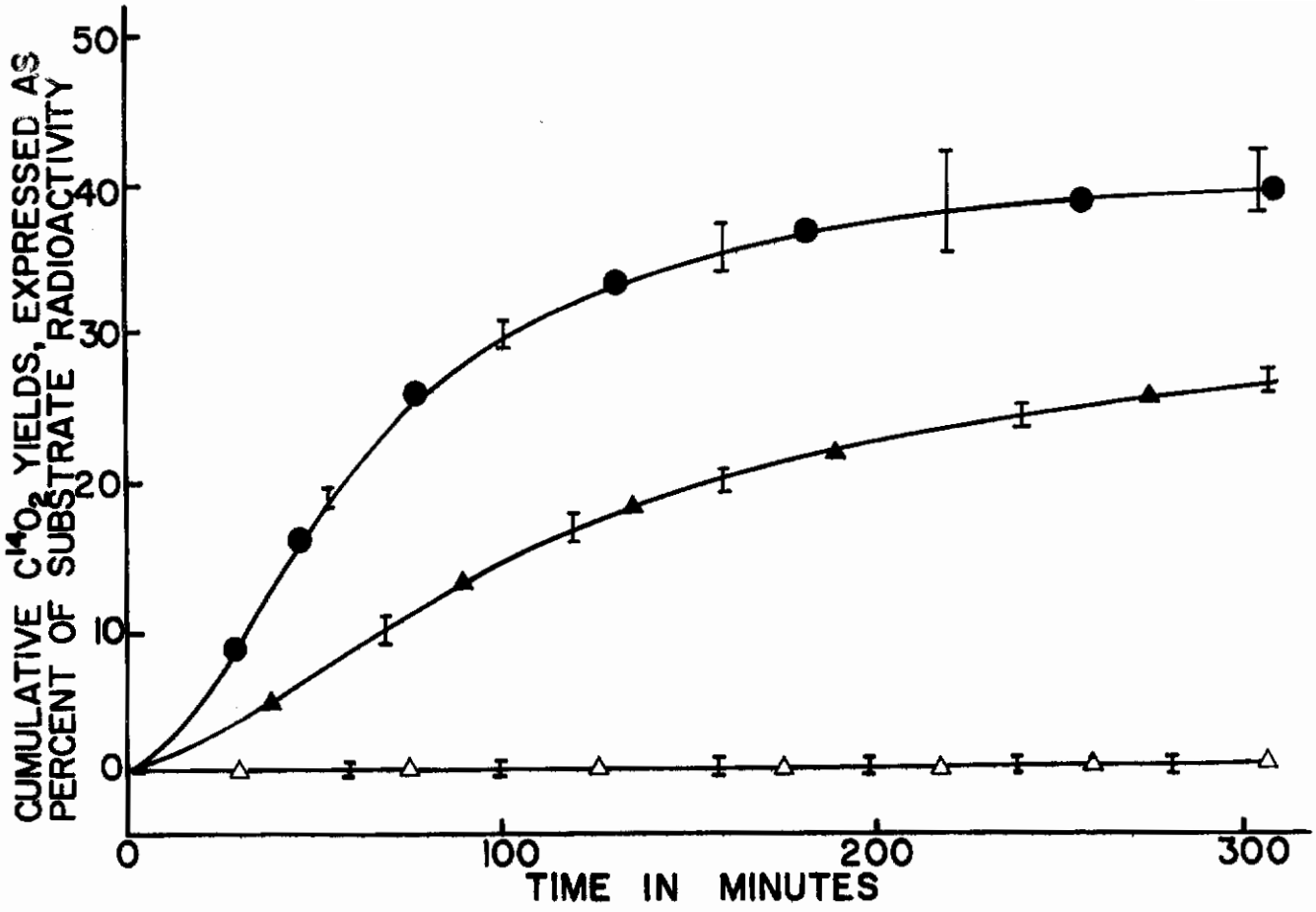

Figure 1. Effect of UDMH on the catabolism of methylamine-C^1 (0.01 mmole/kg) to $C^{14}O_2$ by the rat. Symbols are as follows: ● , control rats; △, rats given 1.33 mmole/kg of UDMH by i.p. injection 30 minutes prior to methylamine-C^{14}; ▲, rats given 1.33 mmole of UDMH/kg by i.p. injection 6 days prior to methylamine-C^{14}.

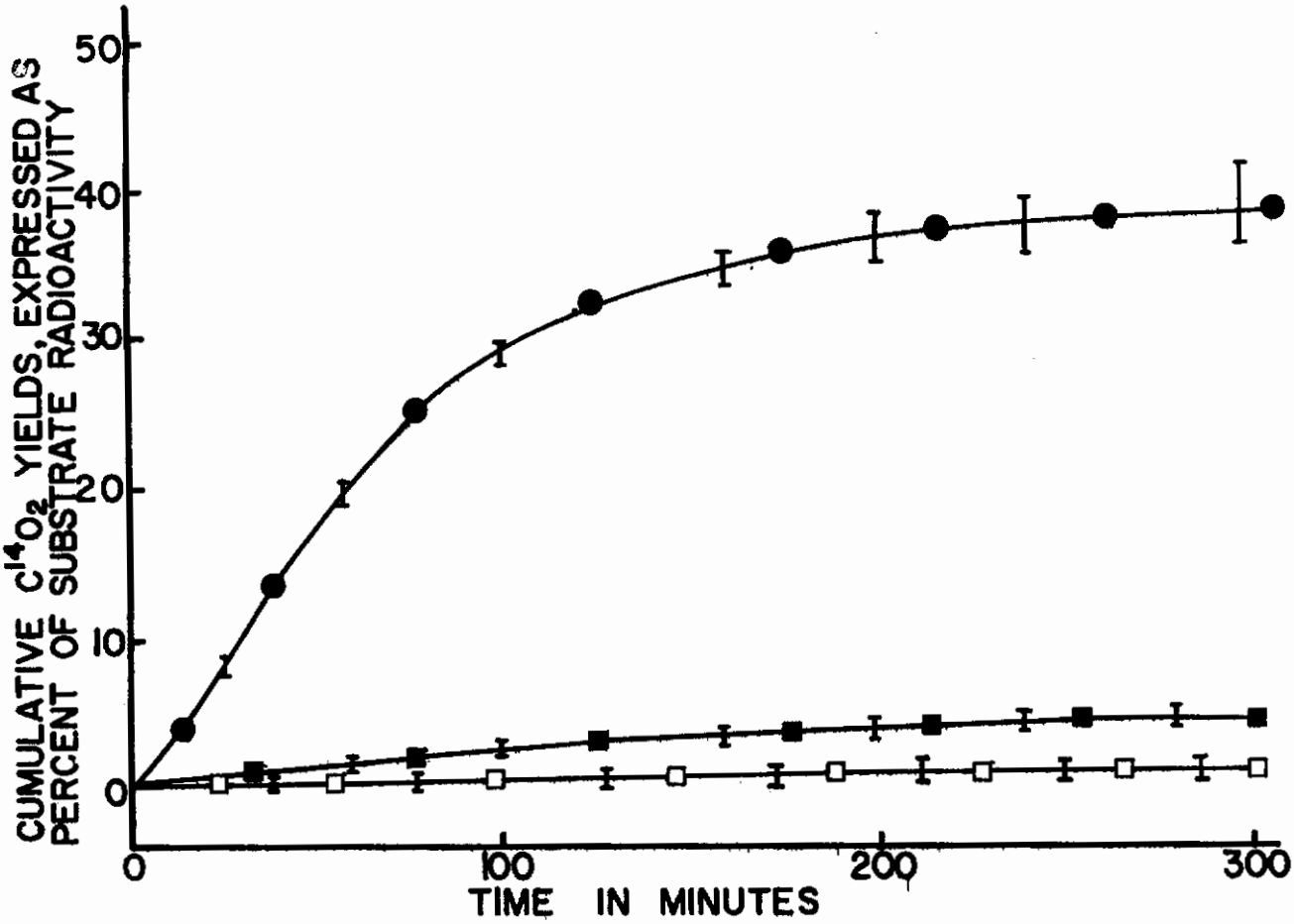

Figure 2. Effect of hydrazine on the catabolism of methylamine-C^{14} (0.01 mmole/kg) to $C^{14}O_2$ by the rat. Symbols are as follows: ● , control rats; □ , rats given 1.5 mmole/kg of hydrazine by i.p. injection 30 minutes prior to methylamine-C^{14}; ■ , rats given 1.5 mmole/kg of hydrazine by i.p. injection 2 days prior to methylamine-C^{14}.

central nervous system. Davis and de Ropp (ref. 7) have reported that hydrazines or substituted hydrazides are the only MAO inhibitors which could increase methylamine excretion and also show anti-depressant activity. These same workers have examined possible precursors of methylamine in rats (ref. 14) and have concluded that sarcosine and creatine appear to be the most likely precursors of methylamine. Other compounds studied as precursors were glycine, dimethylglycine, betaine and DL-methionine.

Metabolism of 1,4-Diamino Butane (Putrescine) by Normal Intact Rats

The conversion of putrescine-1,4-C^{14} (6.2 μmoles/kg) to respiratory $C^{14}O_2$ occurred very readily and extensively in the intact rat (table II and figure 3). The total C^{14} recovery after 18-20 hours was nearly 80% of the administered C^{14}. This extensive conversion of C-1 and C-4 of putrescine to CO_2 is somewhat surprising. It is assumed that this diamine is first oxidized by the enzyme diamine oxidase to γ-amino butyraldehyde, which is in turn oxidized to yield γ-amino butyric acid (GABA). GABA transaminase has been demonstrated in rat tissues (ref. 15). The product of the latter reaction would be succinic semialdehyde which in turn would be oxidized to succinic acid. Krebs cycle activity would readily convert the C-1 and C-4 of succinic acid to CO_2.

The extensive conversion of C-1 and C-4 of putrescine to respiratory CO_2 indicates that this diamine has a very rapid turnover in the intact rat.

Inhibition of Putrescine Catabolism by Hydrazines

The inhibition of putrescine catabolism in the intact rat by hydrazine, UDMH and MMH was examined and the results are shown in figure 3 and table II. All three hydrazines, even at doses as low as 5-8% of an LD_{50}, caused a virtually complete inhibition of the conversion of C-1 and C-4 of putrescine to respiratory CO_2. Since relatively little is known about the metabolism of putrescine, the more widely documented amine oxidase inhibitor, Iproniazid, was again used in a similar experiment to gain baseline information. The inhibition produced by Iproniazid was almost complete (table II). In addition, tranylcypromine (SKF 385B) a known effective non-hydrazide monoamine oxidase inhibitor which interferes only to a slight extent with diamine oxidase was also included in these experiments. The lack of effect by this compound is taken as a demonstration that putrescine was indeed metabolized via a diamine oxidase system. These results are shown in table II.

The persistency of inhibition of putrescine catabolism by hydrazines was examined (table III). It was found that only a slight inhibitory effect remained three days after treatment of rats with hydrazine, UDMH or MMH. The shorter duration of the inhibitory effect on putrescine catabolism as compared with that of methylamine suggests that separate amine oxidases may function for the metabolism of these two compounds.

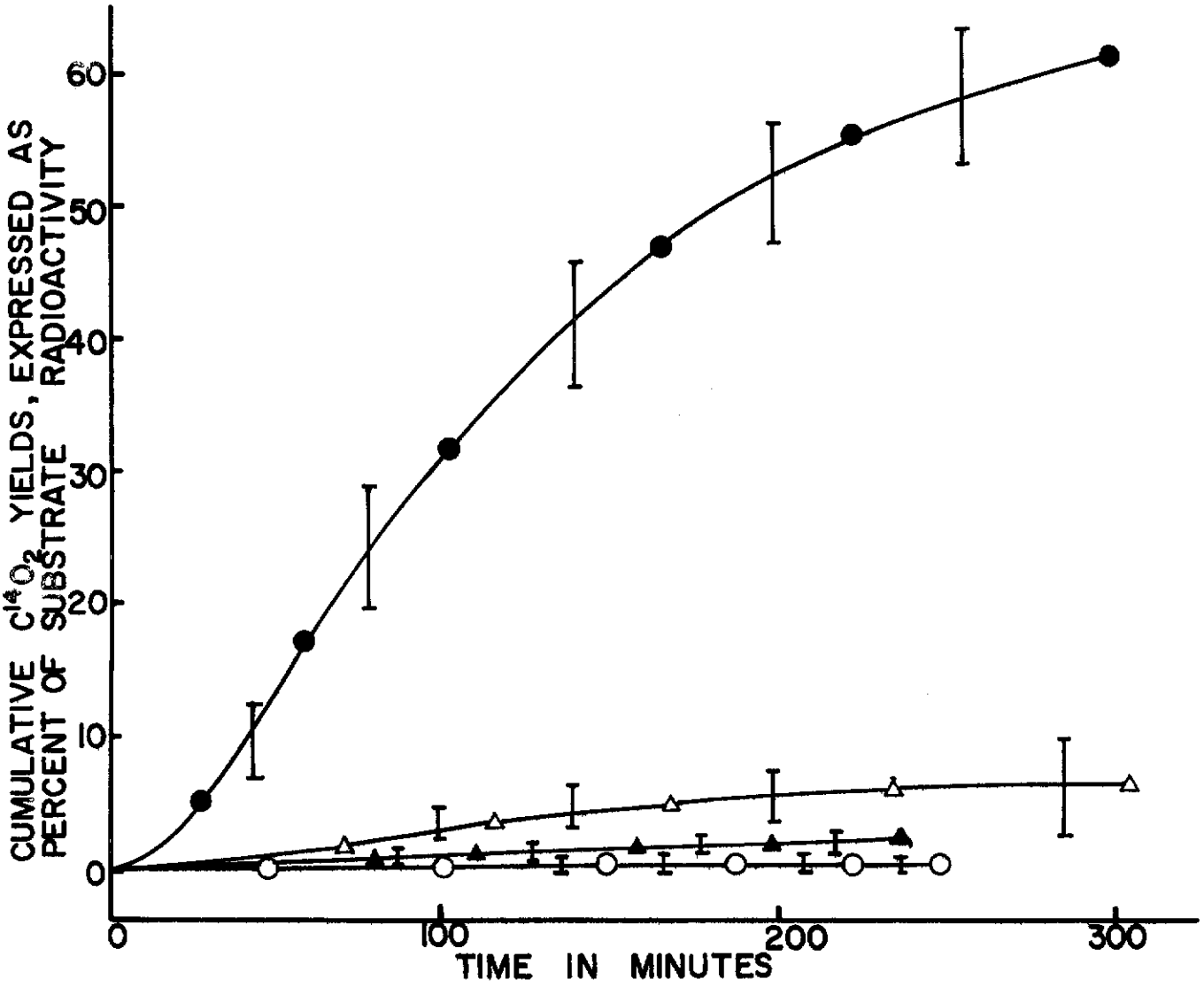

Figure 3. Effect of UDMH and MMH on the catabolism of putrescine-1,4-C^{14} (6.2 μmoles/kg) to $C^{14}O_2$ by rats. Symbols are as follows: ● , control rats; △, rats given 0.13 mmole/kg of UDMH by i.p. injection 30 minutes prior to putrescine-1,4-C^{14}; ▲, rats given 1.33 mmole/kg of UDMH by i.p. injection at the same time as putrescine-1,4-C^{14}; ○ , rats given 0.04 mmole/kg of MMH by i.p. injection 30 minutes prior to putrescine-1,4-C^{14}.

TABLE II

PUTRESCINE-1,4-C^{14} CATABOLISM TO RESPIRATORY $C^{14}O_2$ IN RATS+

PRETREATED WITH HYDRAZINES

Inhibitor#	Cumulative Recovery Expressed as Percent of Administered C^{14}*	
	Time in Minutes	
	150	300
None	36.1 ± 3.2 (8)	50.4 ± 4.1 (8)
UDMH 1.0 mmole/kg	0.5 ± 0.1 (2)	0.5 ± 0.1 (2)
UDMH 0.1 mmole/kg	5.3 ± 1.8 (2)	6.9 ± 2.9 (2)
UDMH 0.01 mmole/kg	32.7 ± 5.8 (2)	44.9 ± 12.6 (2)
MMH 0.5 mmole/kg	0.2 ± 0.1 (2)	0.4 ± 0.2 (2)
MMH 0.04 mmole/kg	0.5 ± 0.1 (2)	0.5 ± 0.1 (2)
Hydrazine 1.0 mmole/kg	0.3 ± 0.01 (2)	0.9 ± 0.01 (2)
Iproniazid (0.56 mmole/kg)		0.5 ± 0.1 (2)
Tranylcypromine 0.075 mmole/kg (SKF 385B)	34.1 ± 0.7 (2)	

*Numbers in brackets denotes number of experimental rats per assay.

#Hydrazines administered 30-40 minutes prior to putrescine-1,4-C^{14}.

+Chemical level of putrescine was 6.2 μmole/kg.

TABLE III

PUTRESCINE-1,4-C^{14} CATABOLISM TO RESPIRATORY $C^{14}O_2$ BY RATS AT THREE DAYS FOLLOWING PRETREATMENT WITH HYDRAZINES+

Inhibitor	Time of Inhibitor Admin. Prior To Substrate	Cumulative Recovery Expressed as Percent of Administered C^{14}* Time in Minutes 150	300
None		35.5 ± 2.8 (6)	50.1 ± 4.4 (6)
UDMH 1.0 mmole/kg	40 minutes	0.7 ± 0.2 (2)	2.6 ± 0.5 (2)
MMH 0.4 mmole/kg	40 minutes	0.2 ± 0.1 (2)	0.5 ± 0.2 (2)
Hydrazine 1.0 mmole/kg	40 minutes	0.3 ± 0.0 (2)	0.9 ± 0.1 (2)
UDMH 1.0 mmole/kg	3 days	35.0 ± 1.7 (2)	50.1 ± 0.2 (2)
MMH 0.4 mmole/kg	3 days	29.7 ± 1.3 (2)	38.1 ± 1.1 (2)
Hydrazine 1.0 mmole/kg	3 days	29.6 ± 2.6 (2)	48.3 ± 8.5 (2)

*Numbers in brackets denotes number of experimental rats per assay.

+Chemical level of putrescine was 6.2 μmole/kg.

The action and properties of diamine oxidases were reviewed recently by Zeller (ref. 5). Work reported by different groups (ref. 16) on diamine oxidases indicates that pyridoxal phosphate is a co-factor coupled with a flavin to catalyze diamine oxidations. It appears that the inhibition of diamine oxidases by hydrazines may be a reversible inhibition in contrast to the irreversible inhibition shown with monoamine oxidases (ref. 2, 17 & 4). Recent mechanisms proposed for the histaminase reaction (ref. 10) indicate that hydrazines may derive their inhibitory effect from their ability to replace the amino groups of substrate which interact with pyridoxal phosphate.

In Vivo Inhibition of MAO

The effects of simple hydrazines upon monoamine oxidase in rats were investigated. The inhibition of rat liver and brain MAO is shown in table IV. It was found that the oxidation of tryptamine-2-C^{14} to indoleacetaldehyde-2-C^{14} by MAO was rapidly and extensively inhibited by MMH. Hydrazine and UDMH were not as potent inhibitors of MAO as MMH. The inhibition of monoamine oxidases by hydrazine, MMH and isopropyl hydrazine have been investigated by other workers (ref. 2, 18 & 3). While the mechanism of inhibition is still unknown, a mechanism proposed earlier by Davison (ref. 2) has received considerable support recently (ref. 17). Work by Kory and Mingioli (ref. 18) indicates that hydrazine inhibition of the MAO reaction may result from the formation of a volatile material from hydrazines. The volatile substance is produced by a non-enzymatic reaction that requires oxygen and its rate can be greatly increased by cyanide or thiourea. This volatile substance has been shown to inhibit the liver and brain MAO reaction, both in vivo and in vitro.

In Vivo Inhibition of DAO

As shown in table V, the administration of UDMH or hydrazine at low doses to rats 60 minutes prior to sacrificing resulted in a complete inhibition of DAO. These experiments indicate that a good correlation exists between the ability of intact rats to convert putrescine-1,4-C^{14} to respiratory $C^{14}O_2$ and the DAO activity level in intestine tissues.

In Vitro Inhibition of DAO

The in vitro inhibition of DAO by hydrazine, MMH and UDMH is shown in table VI. These data indicate that the tested hydrazines display a more powerful inhibitory action upon DAO than that upon the MAO enzyme examined. MMH was found to be a slightly less effective inhibitor of DAO than hydrazine. UDMH, while still a potent inhibitor of this enzyme system, was found to be at least a hundred fold less effective than hydrazine.

These differences suggest that the ability of these hydrazines to inhibit DAO may be related to their degree of methylation. If these hydrazines are competing with amino groups for enzyme attachment, as speculated by

TABLE IV

IN VIVO INHIBITION OF MAO

Inhibitor	Dose* mmole/kg	% Inhibition Brain	Liver
Hydrazine	1.50	31 ± 5	9 ± 5
MMH	0.36	64 ± 10	94 ± 2
UDMH	1.33	38 ± 10	25 ± 10

*Each hydrazine was administered 45 minutes prior to sacrificing the rat for enzyme assay.

TABLE V

IN VIVO INHIBITION OF DAO*

Inhibitor	Concentration	Pre-treatment Period Before Sacrificing	Inhibition
UDMH	0.1 mmole/kg	60 minutes	94%
Hydrazine	0.2 mmole/kg	60 minutes	98%

*Two animals per experiment were employed.

TABLE VI

IN VITRO INHIBITION OF DAO

Inhibitor	Inhibitor Concentration Moles/Liter		Percent Inhibition Found
Hydrazine	From	5×10^{-7}	
	To	1×10^{-7}	95-98%
		5×10^{-8}	81-95
		3×10^{-8}	70-93
		9×10^{-9}	63-66
		3×10^{-9}	51-57
		1×10^{-9}	36-37
MMH	From	1×10^{-7}	
	To	9×10^{-8}	100
		1×10^{-8}	58-74
		9×10^{-9}	37-42
		5×10^{-9}	23-37
		1×10^{-9}	0-27
UDMH	From	7×10^{-5}	
	To	3×10^{-5}	98-100
		9×10^{-6}	72-74
		5×10^{-6}	48-64
		1×10^{-6}	25-27
		7×10^{-7}	0-7

Kapeller-Adler and Mac Farlane (ref. 16), the structural makeup of the hydrazines may have a considerable effect on their respective affinity to the enzyme system. This could possibly account in part for the extreme effectiveness of amino guanidine as an inhibitor of DAO systems (ref. 5).

SECTION IV

CONCLUSIONS

The significance of the profound inhibitory effects of simple hydrazines upon amine oxidases is not easily evaluated. It is clearly shown that the amine oxidases generally categorized as having diamine oxidase properties are drastically inhibited by hydrazine, MMH and UDMH. The physiological effects of virtually complete inhibition of the DAO enzyme system(s) do not appear to be a primary cause of lethality by hydrazines. However, the lack of knowledge concerning the physiological role of aliphatic amines, such as methylamine, accounts to some extent for such a conclusion.

The graded effectiveness of simple hydrazines in preventing the oxidation of a typical biogenic amine, tryptamine, by MAO was as one might predict from previously reported work on MAO enzymes. Thus, hydrazine and UDMH were not particularly effective inhibitors of MAO in vivo while MMH, a more potent inhibitor, was found to behave in a manner similar to isopropyl hydrazine (ref. 2 & 3) in its effect on MAO. MMH inhibition of MAO supports the contention that a potent hydrazine inhibitor of MAO must be able to undergo oxidation with ease and have an associated ability to form a free radical intermediate upon oxidation or degradation (ref. 4).

The inhibitory effects of simple hydrazines upon amine oxidases have not been shown in this work to be primary causes of lethality by hydrazines; yet central nervous system manifestations after intoxication by these compounds seem related to an essential role of amine oxidases in the mammalian body.

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THE EFFECT OF 1,1-DIMETHYLHYDRAZINE (UDMH), MONOMETHYLHYDRAZINE (MMH) AND HYDRAZINE UPON THE METABOLISM OF CERTAIN AMINO ACIDS

SECTION I

INTRODUCTION

The inhibition of amine metabolism by 1,1-dimethylhydrazine (UDMH), monomethylhydrazine (MMH) and hydrazine in intact rats has been described in Section I of this report. The present work includes similar experiments on the effect of these compounds upon the metabolism of certain amino acids which participate either in energy metabolism or regulatory functions of animals.

Numerous reports have shown that L-glutamic acid is one of the key metabolites in animal tissues, thus making it a desirable substrate for examining the effects of hydrazines on amino acid metabolism. Haslam and Krebs (ref. 1) reported that L-glutamate-1-C^{14} can be decarboxylated to form $C^{14}O_2$ extensively via the TCA cycle by brain slices and homogenates. Balazs (ref. 2) observed a similar fate for this amino acid in brain and liver mitochondria. It has also been shown that glutamic acid can be decarboxylated to give rise to γ-aminobutyric acid (GABA) in the brain (ref. 3 & 4). This alternate pathway of glutamic acid metabolism to GABA, and subsequently to succinate, may be an important source of energy for the brain (ref. 5). In addition, glutamic acid administered intracerebrally to rats has been demonstrated to result in an extremely rapid formation of glutamine in the brain (ref. 6).

The close metabolic relation between GABA and glutamic acid noted above, the relative abundance of GABA in the cellular gray matter areas of the central nervous system (ref. 3), and the abnormal nervous activity often associated with lowered GABA levels lend weight to the suggestion that cerebral energy metabolism may in part depend upon oxidative metabolism via the glutamic acid-GABA pathway (ref. 7).

Wilson et al (ref. 8) have reported on the metabolism of GABA-4-C^{14} in normal intact rats and concluded that intraperitoneally administered GABA is metabolized via succinate formation and Krebs cycle processes. Various hydrazines and hydrazides appear to alter the level of GABA in the brain of rodents. Among UDMH, MMH, SDMH (1,2-dimethylhydrazine) and hydrazine, only hydrazine intoxication caused elevation of the level of GABA in rat brain tissues (ref. 9).

L-alanine-1-C^{14} metabolism is of interest since this compound is metabolized primarily via the intermediary formation of pyruvate. Thus, pyruvate, a key metabolite in the glycolytic process, may serve as an indicator in tracing the effect of hydrazines, if any, upon the terminal processes

of glycolysis.

3,4-Dihydroxyphenylalanine (DOPA) is a precursor of norepinephrine and epinephrine and can be decarboxylated to form 3,4-dihydroxytyramine (dopamine) (ref. 10). In the original studies and in many conducted subsequently, the assay of DOPA decarboxylase in vivo involved administration of DOPA to experimental animals and measurement of urinary dopamine by bioassay of its pressor effect. Hansson and Clark (ref. 11), however, have recently reported an in vivo assay in which $C^{14}O_2$ production from 4 μg DOPA-1-C^{14} administered intravenously to mice was measured. The yields of $C^{14}O_2$ from DOPA-1-C^{14} over a 45 minute period were 36% of administered C^{14} from mice of one strain and 41% from those of another strain. The procedure was used to test the inhibitory effect of hydrazine analogs and various competitive inhibitors of DOPA metabolism, including methyl DOPA. They stated that hydrazine displays no effect upon $C^{14}O_2$ production. Dominguez and co-workers (ref. 12 & 13) have noted an inhibition of the rate of glycine and alanine metabolism to CO_2 by rats after intoxication with hydrazine, UDMH, SDMH and MMH.

The present work describes the effects of UDMH, MMH and hydrazine intoxication in rats upon the metabolism of administered L-glutamic acid-U-C^{14}, L-alanine-1-C^{14}, DOPA-1-C^{14} and GABA-1-C^{14}. The results suggest that the inhibition of the metabolism of these amino acids by hydrazines may be dependent upon the nature of the specific transaminase or decarboxylase and its association with the co-factor for these enzymes, pyridoxal phosphate. It was found that GABA is rapidly metabolized, presumably via succinate and the Krebs cycle. The effect of UDMH upon glutamate absorption from the gastrointestinal tract was briefly examined. The results indicate that stomach motility may be affected by UDMH intoxication.

SECTION II

MATERIALS

Radiochemicals

L-alanine-1-C^{14} (sp.act. 9.1 mc/mmole) and L-glutamic acid-1-C^{14} (8.0 mc/mmole) were obtained from the California Foundation for Biochemical Research, Los Angeles, California. L-glutamic acid-U-C^{14} (2.17 mc/mmole) 3,4-dihydroxyphenylalanine-1-C^{14} (2.17 mc/mmole) and γ-aminobutyric acid-1-C^{14} (3.72 mc/mmole) were supplied by the New England Nuclear Corporation, Boston, Massachusetts.

Experimental Animals

The experimental animals used were identical to those described in Section I of this report.

Radiorespirometry

The radiorespirometry apparatus was that used for the work described in Section I.

Chemicals

L-alanine was supplied by Nutritional Biochemical Corporation, Cleveland, Ohio. L-glutamic acid, sodium salt, was obtained from the California Foundation for Biochemical Research. γ-Aminobutyric acid was provided by Sigma Chemical Company, St. Louis, Missouri. The hydrazines employed in this work were stored and solutions prepared in a manner identical to that described in Section I.

SECTION III

METHODS

Standardization of Substrates

The radioactivity of each C^{14} labeled substrate was calibrated by counting a defined amount of the respective compound in 7 ml of ethanol-ethanolamine (2:1 v/v) and 10 ml of toluene containing 3 grams terphenyl and 30 mg POPOP per liter. Efficiency of counting was established by recounting each sample after addition of C^{14} labeled naphthalene as an internal standard. Counting was done with a Packard Model 314-EX liquid scintillation spectrometer. Sufficient counting times were employed to insure that the relative standard deviation of the counting data was no greater than 2%.

Administration of substrates

Substrate administration procedure has been described in Section I, excepting that for intragastric administration. In this case, a 5 ml syringe with PE 160 tubing was employed. The amount of C^{14} labeled substrate and method of administration is shown in text below each figure or table.

Administration of Hydrazines to Rats

The various hydrazines were made up in solution to provide the appropriate dose in a volume of less than 0.5 ml, and were prepared immediately prior to each use. The solution was administered intraperitoneally, approximately 40 minutes before C^{14} labeled substrate administration except when a different time or route of administration is noted.

Effect of UDMH on L-Glutamic Acid-U-C^{14}, Absorption from the Gastrointestinal Tract

Six fasted rats, weighing 240-260 grams, were used for this

experiment. Four of the rats were administered UDMH intraperitoneally, 1.0 mmole/kg, one hour prior to glutamate administration. All six rats were then administered sodium L-glutamate-U-C^{14} (4 mmole/kg) in a volume of 3 ml, by stomach tube. Two hours following glutamate-C^{14} administration, the six animals were sacrificed and the stomach, small intestine and large intestine together with the cecum were removed from each rat. Each organ with its contents was homogenized in a Waring blender and brought to a volume of 15 ml with distilled water. One ml of each homogenate was dried and transferred onto combustion paper. These samples were then burned in an atmosphere of oxygen to carbon dioxide and assayed for C^{14} content by a modified method of Kelly et al (ref. 14).

SECTION IV

RESULTS

Experiments on amino acid metabolism by normal and intoxicated rats were usually 8-12 hours in duration. In most experiments, where the inhibitory effects of hydrazines appeared early, data for the first four hours only of the experiment are presented.

L-Glutamic Acid-1-C^{14}

The production of $C^{14}O_2$ from normal rats after sodium L-glutamate-1-C^{14} (4 mmole/kg) was administered intraperitoneally is shown in figure 1. The conversion of this substrate to respiratory $C^{14}O_2$ reached a maximum rate 40 minutes after substrate administration and remained at the same rate for an additional 60 minutes. At the end of 100 minutes, approximately 70% of the administered radioactivity was recovered as respiratory $C^{14}O_2$.

Intoxication of rats with UDMH (1.5 mmole/kg) or MMH (0.5 mmole/kg) did not appreciably decrease their ability to metabolize carbon 1 of glutamic-1-C^{14} acid (4 mmole/kg intraperitoneally) to respiratory $C^{14}O_2$ (figure 1). In contrast, hydrazine (1 mmole/kg) administration to rats resulted in a virtually complete inhibition of $C^{14}O_2$ formation from L-glutamic acid-1-C^{14} administered in the same manner.

When sodium L-glutamate-1-C^{14} (20 mmole/kg) was administered by stomach tube, the percent yield of $C^{14}O_2$ was somewhat lower than was obtained from this substrate administered intraperitoneally (figures 2 and 1 respectively). However, the total amount of glutamate metabolized per unit of time was somewhat greater after oral administration of the larger oral dose than the intraperitoneally administered L-glutamate. UDMH intoxication (1.0 mmole/kg) had little or no effect upon the metabolism of orally administered L-glutamate-1-C^{14} (figure 2). Hydrazine intoxication (1.0 mmole/kg) of rats inhibited the rate at which orally administered L-glutamate-1-C^{14} was converted to $C^{14}O_2$ (figure 2). However, the inhibition was much less than that observed when the substrate was administered intraperitoneally and

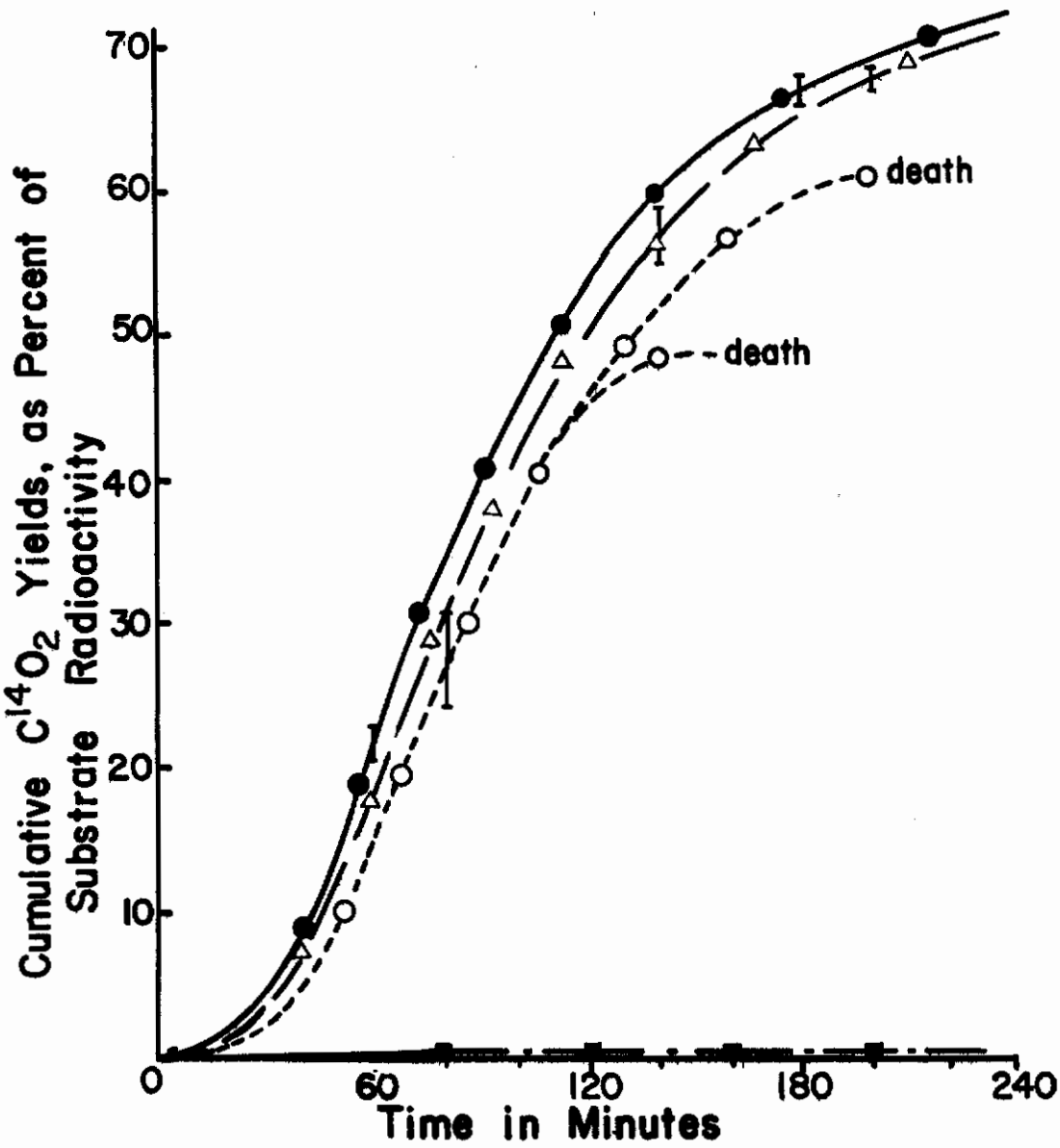

Figure 1. The effect of hydrazines upon the catabolism of sodium L-glutamate-1-C^{14} (4.0 mmole/kg by i.p. injection) to respiratory $C^{14}O_2$. Symbols are as follows: ● , control rats; Δ, rats given 1.5 mmole/kg of UDMH by i.p. injection 40 minutes prior to sodium L-glutamate-1-C^{14}; ○ , rats given 0.5 mmole/kg of MMH by i.p. injection 40 minutes prior to sodium L-glutamate-1-C^{14}; ■ , rats given 1.0 mmole/kg of hydrazine by i.p. injection 40 minutes prior to sodium L-glutamate-1-C^{14}.

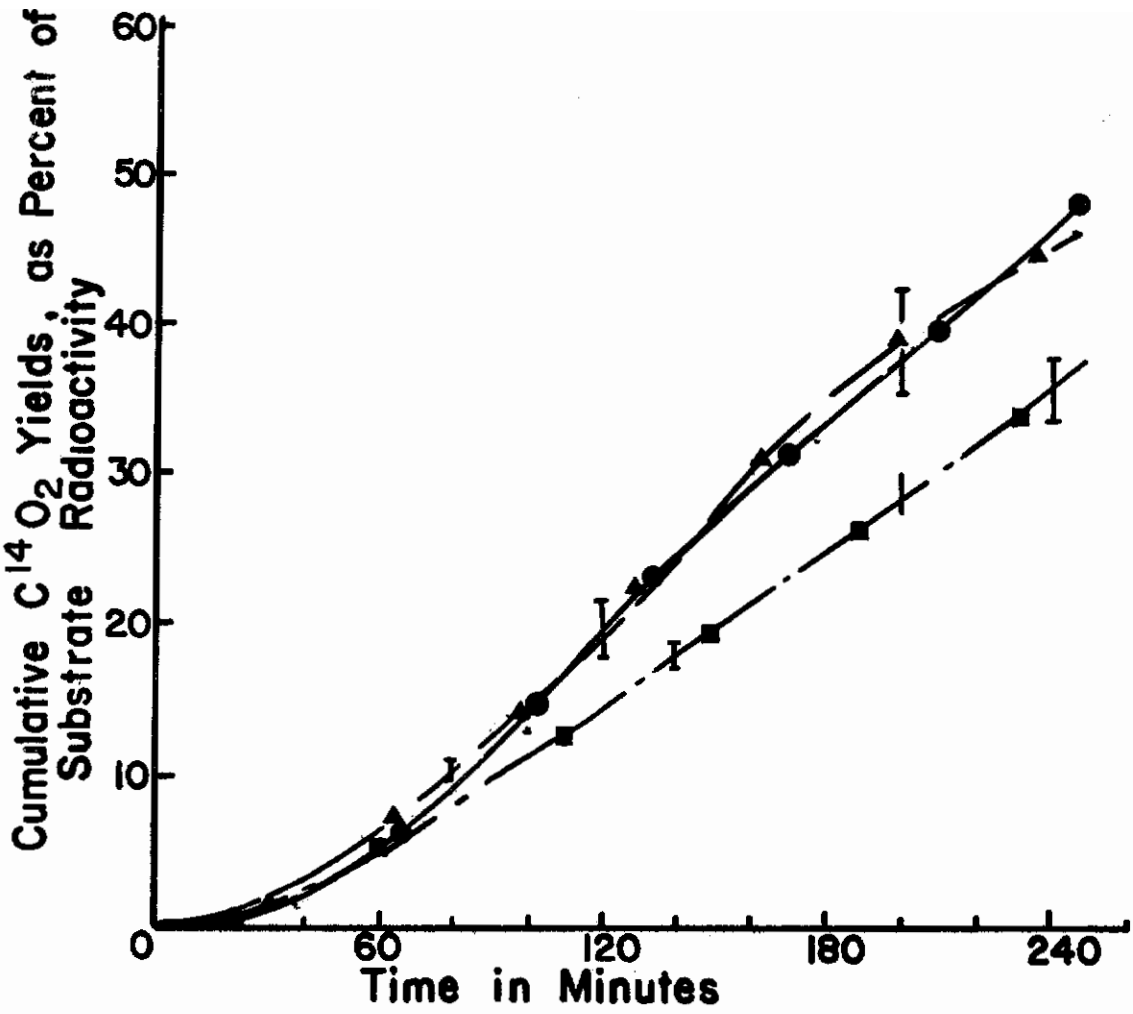

Figure 2. The effect of hydrazines upon the catabolism of sodium L-glutamate-1-C^{14} (20.0 mmole/kg per os) to respiratory $C^{14}O_2$. Symbols are as follows: ●, control rats; ▲, rats given 1.0 mmole/kg of UDMH by i.p. injection 40 minutes prior to sodium L-glutamate-1-C^{14}; ■, rats given 1.0 mmole/kg of hydrazine by i.p. injection 40 minutes prior to sodium L-glutamate-1-C^{14}.

at a lower dose level.

L-Glutamic Acid-U-C^{14}

UDMH intoxication (1 mmole/kg) of rats resulted in a definite delay or decrease in the production of $C^{14}O_2$ from orally administered L-glutamate-U-C^{14} (figure 3) after the initial hour of metabolism. This decrease in the $C^{14}O_2$ production rate from the intoxicated rat results either from inhibition of the rate of substrate absorption or the rate of metabolism after absorption. To help clarify this point, experiments designed to examine L-glutamate absorption were carried out.

It was found in a limited number of experiments that two hours after oral administration of 4 mmole/kg and 2 μc of sodium-L-glutamate-U-C^{14} that substantial amounts of radioactivity remained in the stomachs of UDMH treated animals, but not in those of control animals. The two control animals each retained 0.023 μc, or about 1% of the administered L-glutamate-U-C^{14} radioactivity. UDMH treated rats retained 0.52, 0.4, 0.27 and 0.39 μc, or approximately 20% of the administered radioactivity. No other areas of the gastrointestinal tracts of either treated or control animals were found to retain appreciable radioactivity.

GABA-1-C^{14} Metabolism

The radiorespirometric patterns for the conversion of GABA-1-C^{14} to respiratory $C^{14}O_2$ by normal and intoxicated rats are shown in figures 4 and 5. Normal rats were found to convert C-1 of GABA-1-C^{14} to respiratory $C^{14}O_2$ very rapidly and extensively at 0.004 mmole/kg substrate levels. These doses are both very low relative to effective therapeutic or toxic doses.

A slight inhibition of the conversion of intraperitoneally administered GABA-1-C^{14} to $C^{14}O_2$ at the 0.004 mmole/kg level was noted with rats intoxicated by either 1.5 mmole/kg of UDMH or 0.5 mmole/kg of MMH. In terms of total GABA metabolized at the end of 200 minute experiments, UDMH and MMH inhibited the rate of metabolism less than 10% at the 0.004 mmole/kg dose level of substrate (figure 4). UDMH inhibited GABA metabolism about 20% at the 0.4 mmole/kg level of substrate (figure 5). In sharp contrast, hydrazine at 1.0 mmole/kg almost completely inhibited the conversion of intraperitoneally administered GABA-1-C^{14} (0.004 mmole/kg) to respiratory $C^{14}O_2$ (figure 4). Hydrazine had essentially the same inhibitory effect when GABA-1-C^{14} was given via the same route at a higher dose level, 0.4 mmole/kg (figure 5).

L-Alanine-1-C^{14} Metabolism

The respirometric patterns for the conversion of orally administered L-alanine-1-C^{14} to respiratory $C^{14}O_2$ by normal and intoxicated rats are shown in figure 6. UDMH and MMH treatment of rats had some effect on their ability to metabolize carbon 1 or L-alanine to $C^{14}O_2$, but still allowed

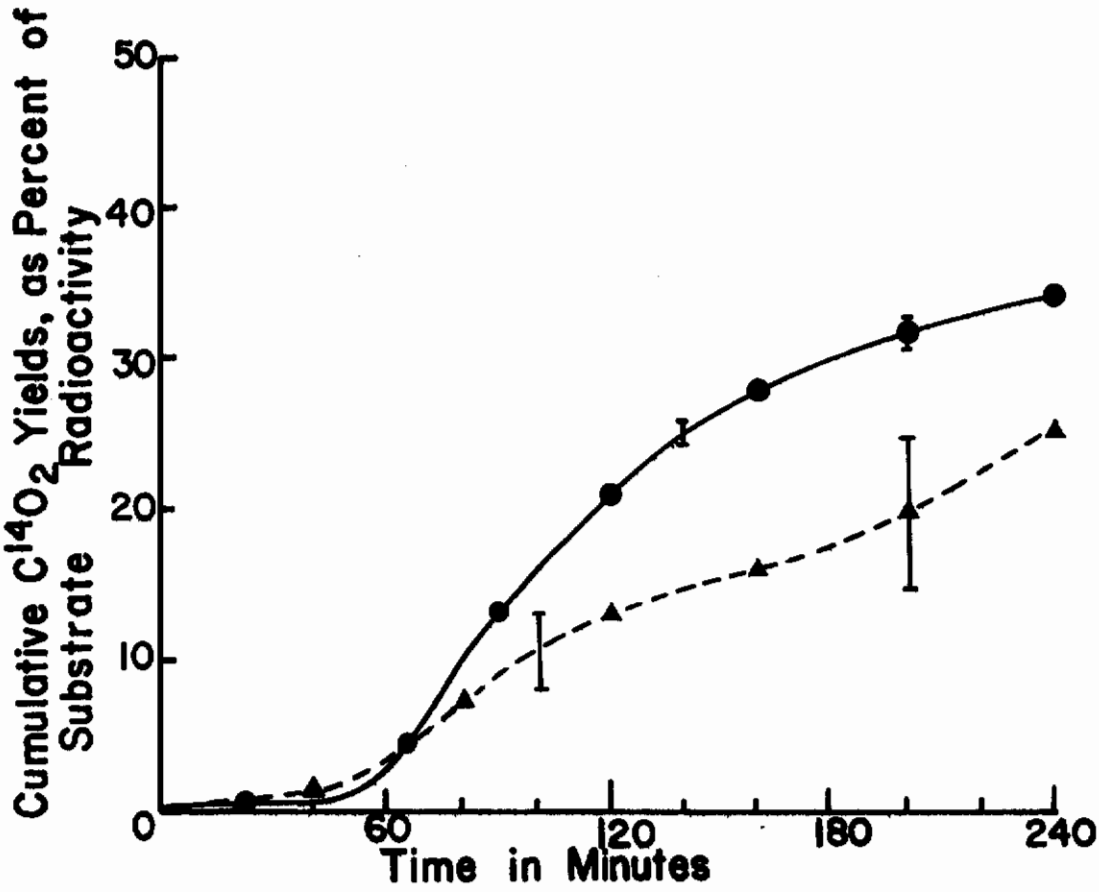

Figure 3. The effect of UDMH upon the catabolism of sodium L-glutamate-U-C^{14} (4.0 mmole/kg per os) to respiratory $C^{14}O_2$. Symbols are as follows: ●, control rats; ▲, rats given 1.0 mmole/kg of UDMH by i.p. injection 60 minutes prior to sodium L-glutamate-U-C^{14}.

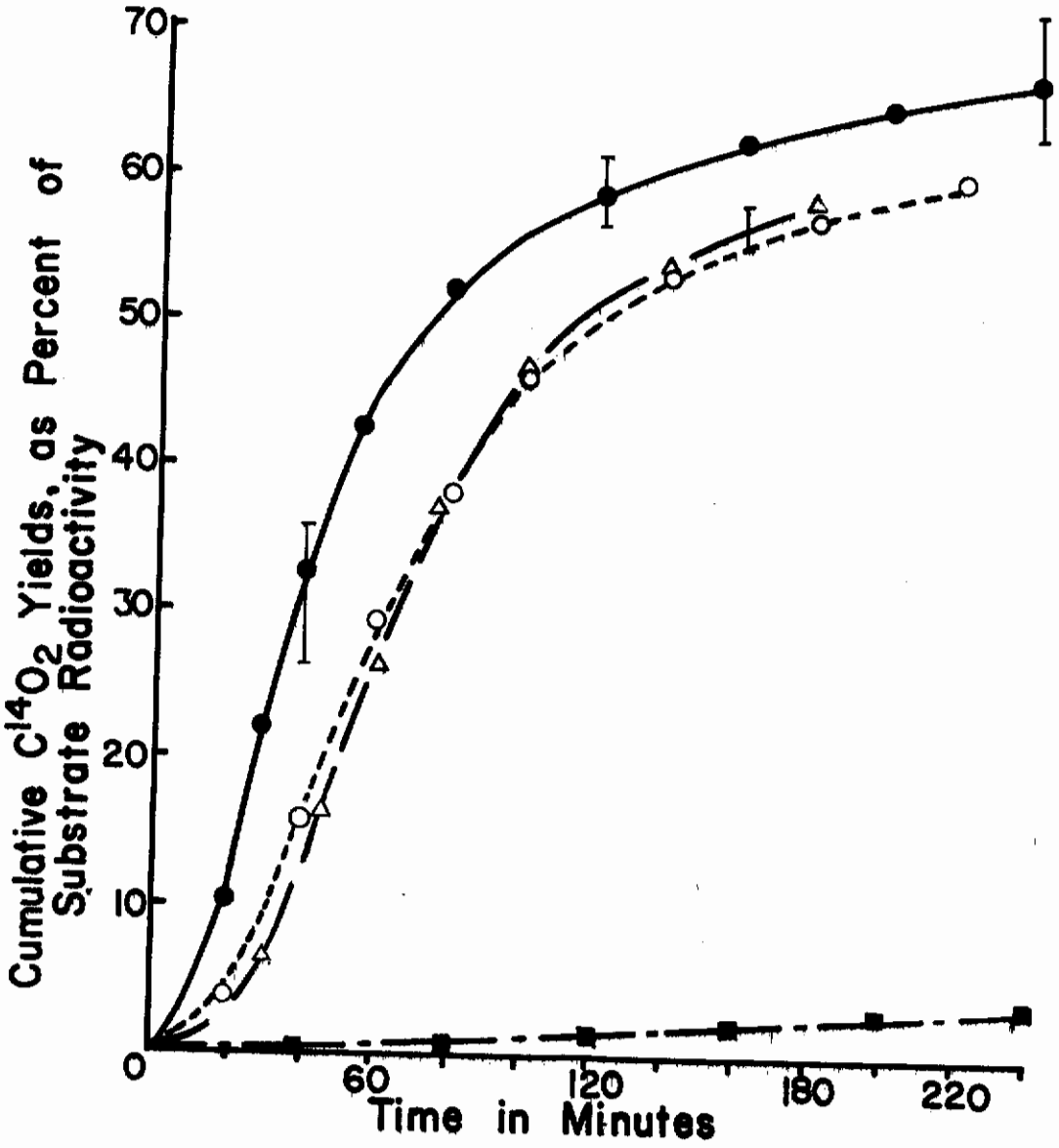

Figure 4. The effect of hydrazines upon the catabolism of GABA-1-C^{14} (0.004 mmole/kg by i.p. injection) to respiratory $C^{14}O_2$. Symbols are as follows: ●, control rats; Δ, rats given 1.5 mmole/kg of UDMH by i.p. injection 40 minutes prior to GABA-1-C^{14}; ○, rats given 0.5 mmole/kg of MMH by i.p. injection 40 minutes prior to GABA-1-C^{14}; ■, rats given 1.0 mmole/kg of hydrazine by i.p. injection 40 minutes prior to GABA-1-C^{14}.

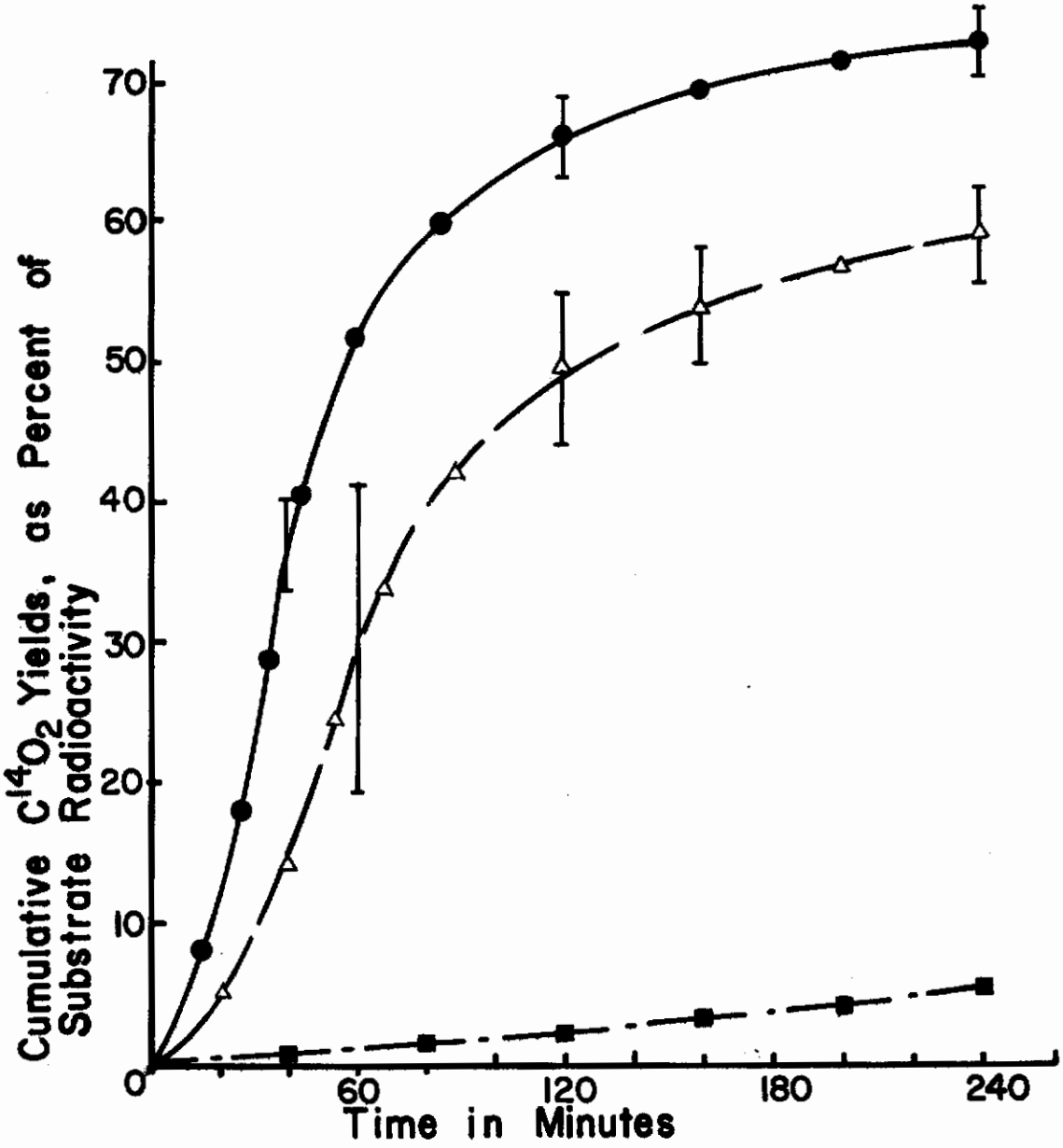

Figure 5. The effect of hydrazines upon the catabolism of GABA-1-C^{14} (0.4 mmole/kg by i.p. injection) to respiratory $C^{14}O_2$. Symbols are as follows: ●, control rats; Δ, rats given 1.5 mmole/kg of UDMH by i.p. injection 40 minutes prior to GABA-1-C^{14}; ■, rats given 1.0 mmole/kg of hydrazine by i.p. injection 40 minutes prior to GABA-1-C^{14}.

conversion of more than 70% of carbon 1 of alanine to respiratory CO_2. Hydrazine treatment of rats resulted in a considerable decrease in the rate and extent of $C^{14}O_2$ production from L-alanine-1-C^{14} (figure 6). These animals demonstrated a slow, constant rate of $C^{14}O_2$ output for several hours.

3,4-Dihydroxyphenylalanine-1-C^{14} (DOPA-1-C^{14}) Metabolism

Metabolism of trace amounts (0.9 mmole/kg) of DOPA-1-C^{14} by rats, after intraperitoneal administration was very rapid and extensive as shown by the $C^{14}O_2$ production data given in table I. Within two hours, 50% of the carboxyl carbon of DOPA-1-C^{14} was converted to respiratory $C^{14}O_2$ and during another four hours an additional 10% was recovered as $C^{14}O_2$. Treatment of rats with UDMH 1.5 mmole/kg dose levels or hydrazine at 1.0 mmole/kg resulted in no significant change in the capacity of these animals to metabolize DOPA-1-C^{14}, as shown in table I.

SECTION V

DISCUSSION

The metabolism of L-glutamic acid in intact rats is thought to occur largely via the Krebs cycle following conversion to α-ketoglutarate by transamination (ref. 15). An alternate pathway for glutamate presumably occurs in the central nervous system and consists of a C-1 decarboxylation by glutamic acid decarboxylase to form GABA, which is in turn metabolized to succinate (ref. 3, 8 & 16).

A definite variation of L-glutamate metabolism with respect to the route of administration has been reported by Wilson and co-workers (ref. 17). From their work, they concluded that after intraperitoneal administration, L-glutamate was metabolized largely via Krebs cycle. In contrast, they found that injection of trace levels of glutamic acid into the cecum of rats resulted in a microbial conversion of C-2 of glutamic acid to the methyl carbon of acetate which indicates that large oral doses may be partially metabolized by this alternate pathway.

The effect of intoxication by hydrazines upon L-glutamate metabolism in rats is complex and difficult to interpret. First, it appears that UDMH intoxication may reduce gastric motility since L-glutamate-U-C^{14} was cleared from the stomach of UDMH intoxicated rats at an appreciably slower rate than from control rats. It is doubtful that the decrease of movement of L-glutamate from the stomach resulted from absorption changes, since absorption normally occurs primarily from the intestinal tract rather than from the stomach.

The inhibition of L-glutamate metabolism by hydrazine in the rat and the lack of inhibition by UDMH or MMH intoxication was surprising, but

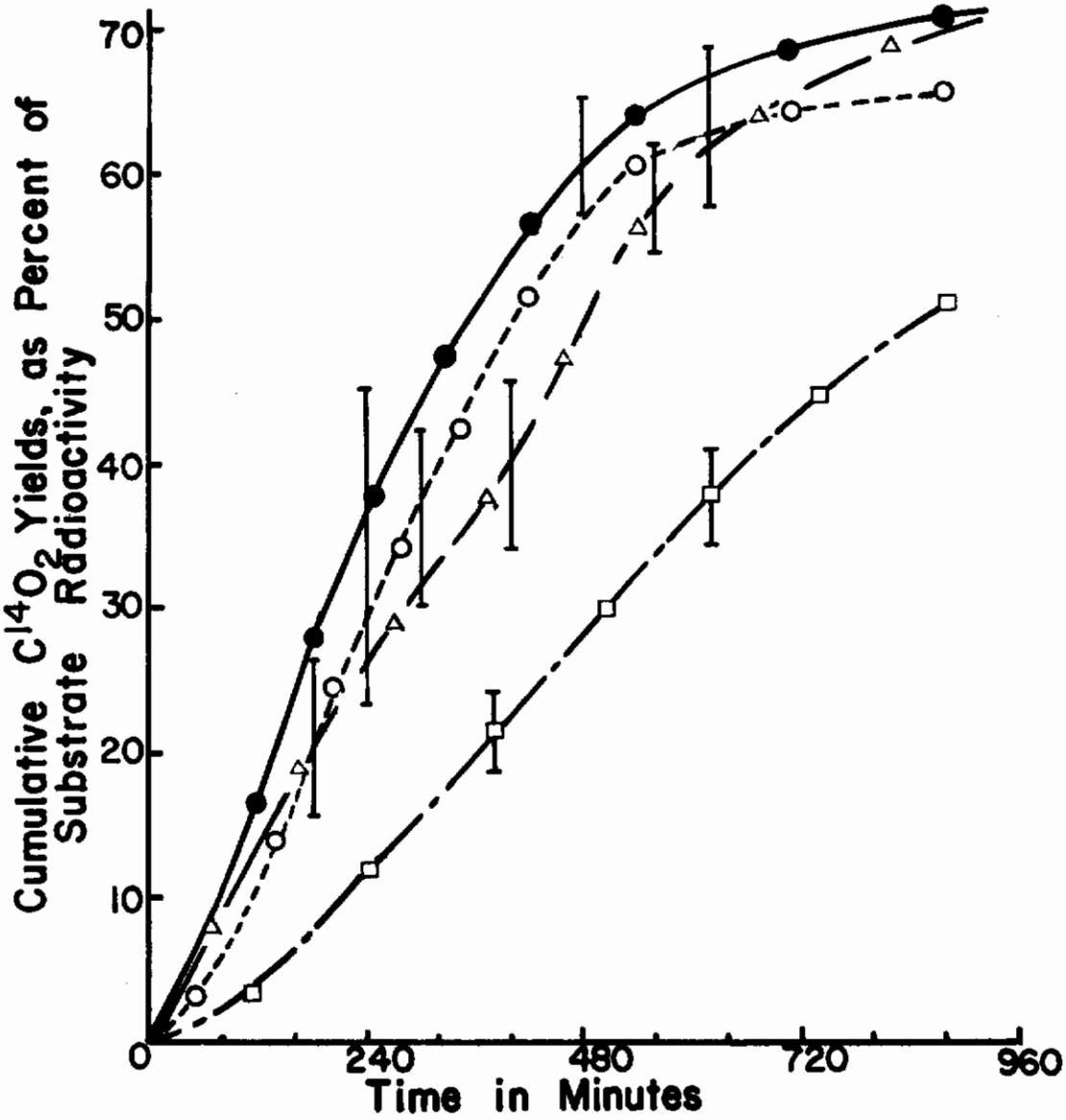

Figure 6. The effect of hydrazines upon the catabolism of L-alanine-1-C^{14}, (40 mmole/kg per os) to respiratory $C^{14}O_2$. Symbols are as follows: ●, control rats; △, rats given 1.5 mmole/kg of UDMH by i.p. injection 40 minutes prior to L-alanine-1-C^{14}; ■, rats given 1.5 mmole/kg of hydrazine by i.p. injection 40 minutes prior to L-alanine-1-C^{14}; ○, rats given 0.5 mmole/kg of MMH by i.p. injection 40 minutes prior to L-alanine-1-C^{14}.

TABLE I

CONVERSION OF 3,4-DIHYDROXYPHENYLALANINE-1-C^{14} (DOPA-1-C^{14}*) TO RESPIRATORY CO_2 IN NORMAL, UDMH AND HYDRAZINE TREATED RATS

	Cumulative Yield of $C^{14}O_2$ as Percent of Administered DOPA-1-C^{14}								
Time In Min.	Control Rats		Average	UDMH Treated Rats 1.5 mmole/kg		Average	Hydrazine Treated Rats 1.0 mmole/kg		Average
30	8.9	8.4	8.6	2.5	7.4	4.9	17.2	12.5	14.8
60	36.8	33.8	35.3	20.0	27.8	23.9	33.5	28.3	30.9
90	48.6	46.3	47.5	36.4	40.3	38.3	41.5	38.0	39.7
120	53.2	51.8	52.5	45.4	46.6	45.5	45.0	43.9	44.4
240	58.4	57.7	58.0	59.8	54.0	54.0	50.3	51.6	50.9

*DOPA-1-C^{14}, 0.9 μmole/kg, was administered intraperitoneally 40 minutes after intoxication with the respective hydrazine.

very similar to the inhibition of GABA metabolism. A considerable difference may therefore exist in the mechanism of inhibition of glutamate and GABA metabolism by hydrazine when compared to the inhibition by the alkyl-hydrazines, UDMH and MMH. While this difference is not understood, it may relate to the role of pyridoxal phosphate in these reactions.

Makino et al (ref. 18) and Davison (ref. 19) have reported experiments which indicate that various hydrazones of pyridoxal phosphate activate glutamic acid decarboxylase by functioning as co-enzymes per se. Patrick (ref. 20) reported a large increase in serum glutamic-oxalacetic transaminase of monkeys after hydrazine exposure but not after UDMH, which may also indicate activation rather than inhibition of this transaminase by hydrazine. Along a different line of thought, Medina and co-workers (ref. 21 & 22) have shown that UDMH inhibition of glutamate decarboxylase may relate to hydrazine inhibition of pyridoxal kinase.

At least two alternate pathways for GABA metabolism appear to exist. One pathway is via transamination and oxidation to succinic acid. The other pathway is an initial γ-β-dehydrogenation followed by a hydration to form γ-amino-β-hydroxybutyric acid (ref. 4).

Wilson et al (ref. 8) have examined the metabolism of GABA-4-C^{14} which was administered by a single intraperitoneal injection to intact rats. Total $C^{14}O_2$ recovery after four hours was 72% of the C^{14} administered. This value is in agreement with the $C^{14}O_2$ yield from GABA-1-C^{14} observed in our experiments. In addition, they found labeling patterns in isolated tissue glutamate, aspartate, alanine and glycogen which gave strong evidence that GABA undergoes metabolism via succinate.

The transamination of GABA to succinic semialdehyde is coupled with α-ketoglutarate and requires pyridoxal phosphate as a co-factor (ref. 23 & 24). Baxter and Roberts (ref. 25) have reported an inhibition of this transaminase in vivo with rats using hydroxylamine and aminooxyacetic acid without appreciably affecting the activity of glutamic acid decarboxylase. They also reported no detectable change in rat brain GABA levels 90 minutes after intraperitoneal administration of 180 mg/kg of hydrazine. Uchida and O'Brien (ref. 7) examined the effects of hydrazines on GABA levels in various regions of rat brain after intoxication by various hydrazines. They have shown that MMH, UDMH and SDMH lowered GABA levels by small amounts (17% maximum) in the medulla, cortex and mesencephalon-diencephalon positions of the brain, but effects on cerebellum GABA levels were small or absent. Hydrazine, however, was reported to raise GABA levels in all parts of the brain, especially in the mesencephalon-diencephalon region where the increase of GABA was 31%.

Thus, the results from our observations and work by others strongly indicate that hydrazine inhibits GABA metabolism by preventing the transamination of GABA. The relatively weak inhibition of this transaminase by

UDMH and MMH is in agreement with the inability of these hydrazines to cause an increase in brain GABA levels (ref. 9). Similarly, Sacktor and co-workers (ref. 4) found that a similar alkylhydrazine, phenylisopropyl-hydrazine, while being a powerful monoamine oxidase inhibitor, did not inhibit GABA metabolism by rat brain mitochondria. Since hydroxylamine has been shown to be a more powerful inhibitor of GABA metabolism (ref. 25) than even hydrazine, the size of the inhibitor molecule may be as important as its chemical properties.

The site of metabolism of exogenously administered GABA is of considerable interest. From previous work it appears that a majority of the exogenously administered GABA is metabolized in the liver and kidney (ref. 26 & 27). It also appears possible that a small portion of exogenously administered GABA may pass the blood-brain barrier (ref. 28) and be metabolized in nervous tissue even though a considerable amount of evidence has been reported to the contrary (ref. 29 & 30).

According to Greenberg, the principal pathway for the catabolism of L-alanine is through pyruvate (ref. 31). The effect of hydrazine, UDMH and MMH intoxication on the catabolism of large oral doses of L-alanine-1-C^{14} by rats is quite similar to the effect of these hydrazines on glutamate metabolism after per orum administration. In both instances, a greater inhibition was observed with hydrazine than the alkylhydrazines. The inhibition of L-alanine metabolism by hydrazine is presumably occurring at the transamination stage since acetate-1-C^{14} metabolism was only slightly inhibited by severe hydrazine intoxication.[1] A similar observation has been made by Dominguez and co-workers (ref. 13).

The findings that decarboxylation of DOPA-1-C^{14} to form $C^{14}O_2$ in intact rats occurred rapidly and extensively is in agreement with the work of Hansson and Clark (ref. 11). While hydrazine analogs of hydroxylated aromatic amino acids are very effective inhibitors of DOPA decarboxylase, we find that hydrazine or UDMH has little effect on this enzyme system in the intact rat. DOPA decarboxylase of guinea-pig kidney (ref. 32) and rat liver (ref. 33) have been partially purified and characterized. Pyridoxal phosphate has been shown to be a co-factor for this enzyme. It has been reported that pyridoxal phosphate is more easily dissociated from decarboxylases than from other pyridoxal phosphate dependent enzymes (ref. 31). Whether this difference is related to the peculiar lack of effect on decarboxylation of DOPA is not known.

In summary, the effect of the various hydrazines on the metabolism of GABA-1-C^{14} and L-glutamic acid-1-C^{14} is evidence that the mechanism of toxicity of hydrazine may be different than with alhylhydrazines such as

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[1]Wang, C. H., D. J. Reed and F. N. Dost, Unpublished Results.

UDMH or MMH. The ability of arginine and glutamate to partially reverse the effects of hydrazine intoxication (ref. 34) may suggest that the site of hydrazine intoxication is not necessarily in the CNS. However, it is difficult to visualize an immediate lethal response to hydrazine if the intoxication effects are primarily limited to the liver and kidneys. Considerably more information is needed on the role of GABA outside of the CNS.

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13. ABSTRACT Certain effects of simple hydrazines via i.p. administration upon the metabolism of amines and amino acids by rats were examined. Unsymmetrical dimethylhydrazine (UDMH), monomethylhydrazine (MMH) and hydrazine strongly inhibited oxidation of putrescine-1, 4-C^{14} (1,4-diamino butane) and methylamine-C^{14} to $C^{14}O_2$ by intact rats. MMH caused a virtually complete inhibition of monoamine oxidase activity in vivo, but inhibition by UDMH and hydrazine was limited. In vivo and in vitro diamine oxidase activity was heavily suppressed by all three hydrazines. The inhibition duration of methylamine oxidase by UDMH and hydrazine was found to last several days. Inhibition of putrescine oxidation was reversed within 3 days, indicating a possible difference between the enzyme systems which metabolize methylamine and putrescine. The metabolism of varied oral and intraperitoneal doses of L-glutamic acid-1-C^{14} by rats was inhibited by hydrazine, but not by UDMH or MMH. Oxidation of large oral doses of L-alanine-1-C^{14} to respiratory $C^{14}O_2$ was slightly inhibited by UDMH, MMH and blocked to a greater extent by hydrazine. Similar results were found in the metabolism of low levels of γ-aminobutyric acid-1-C^{14} (GABA-1-C^{14}) except that hydrazine intoxication caused an almost complete inhibition of GABA-1-C^{14} conversion to $C^{14}O_2$. In vivo decarboxylation of trace amounts of 3,4-dihydroxyphenylalanine-1-C^{14} was not affected by any of the three hydrazines. A study of absorption of L-glutamate-U-C^{14} from the digestive tract of rats indicated that UDMH may cause a substantial loss of gastric motility.

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