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4-Aminobutyrate in Mammalian Putrescine Catabolism

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The effects of inhibitors of diamine oxidase (EC 1.4.3.6), monoamine oxidase (EC 1.4.3.4) and 4-aminobutyrate aminotransferase (EC 2.6.1.19) on the catabolism of putrescine in mice *in vivo* were studied. Diamine oxidase inhibitors and carboxymethoxylamine (aminooxyacetate) markedly inhibit the metabolism of [^{14}C]putrescine to $^{14}CO_2$, but affect different enzymes. Aminoguanidine specifically inhibits the mitochondrial and non-mitochondrial diamine oxidases, whereas carboxymethoxylamine specifically inhibits 4-aminobutyrate transamination by the mitochondrial pathway. Hydrazine inhibits at both sites, and results in increased concentrations of 4-aminobutyrate in brain and liver. Pretreatment of mice with carboxymethoxylamine and [^{14}C]putrescine leads to the urinary excretion of amino[^{14}C]butyrate. Carboxymethoxylamine does not affect the non-mitochondrial pathway of putrescine catabolism, as the product of oxidative deamination of putrescine in the extramitochondrial compartment is not further oxidized but is excreted in the urine as derivatives of 4-aminobutyraldehyde. Another catabolic pathway of putrescine involves monoamine oxidase, and the monoamine oxidase inhibitor, pargyline, decreases the metabolism of [^{14}C]putrescine to $^{14}CO_2$ *in vivo*. Catabolism of putrescine to CO_2 *in vivo* occurs along different pathways, both of which have 4-aminobutyrate as a common intermediate, in contrast with the non-mitochondrial catabolism of putrescine, which terminates in the excretion of 4-aminobutyraldehyde derivatives. The significance of the different pathways is discussed.

Putrescine (1,4-diaminobutane), the precursor of the ubiquitous polycations spermidine and spermine (Cohen, 1971; Bachrach, 1973), is attracting increasing interest because of its growth-promoting effects on bacterial (Inouye & Pardee, 1970; Cohen, 1971) and mammalian cell cultures (Ham, 1964; Duffy *et al.*, 1971; Pohjanpelto & Raina, 1972; Pohjanpelto, 1973), and its stimulation of RNA synthesis (Reynolds & Russell, 1973). High concentrations of putrescine were observed in regenerating liver (Russell *et al.*, 1970; Heby & Lewan, 1971), embryonic brain (Seiler & Lamberty, 1975*a*,*b*), tumours (Kremzner, 1973; Seiler *et al.*, 1975) and the cerebrospinal fluid of patients with malignant tumours (Marton *et al.*, 1974). The short biological half-life of putrescine compared with that of spermidine and spermine (Snyder *et al.*, 1970; Seiler, 1973; Al-Therib, 1974) and the rapid turnover of ornithine decarboxylase (EC 4.1.1.17) (Snyder *et al.*, 1970) makes putrescine especially suitable as a growth-regulating compound (Russell, 1973).

Putrescine is a typical substrate of diamine oxidase [amine–oxygen oxidoreductase (deaminating) (pyridoxal-containing), EC 1.4.3.6] (Kapeller-Adler, 1970). It was therefore generally assumed that its oxidative deamination by this enzyme was the first step of putrescine catabolism (Zeller, 1942; Tabor & Tabor, 1964; Kapeller-Adler, 1970). In mammalian brain, however, diamine oxidase activity is low (Burkard *et al.*, 1963). It was shown that in mouse brain putrescine is acetylated first to monoacetylputrescine (Seiler & Al-Therib, 1974*a*), a substrate of monoamine oxidase [amine–oxygen oxidoreductase (deaminating) (flavin-containing), EC 1.4.3.4]. Acetylputrescine can be oxidized to *N*-acetyl-4-aminobutyrate, which is converted *in vivo* into 4-aminobutyrate (Seiler & Al-Therib, 1974*a*), and further degraded via succinic semialdehyde and succinate to CO_2 (Baxter, 1970).

The polyamines spermidine and spermine can be degraded to putrescine (Fischer *et al.*, 1972; Seiler, 1973; Hölttä *et al.*, 1973). Further, putrescine is not only a substrate of spermidine synthesis, but is also an activator of *S*-adenosylmethionine decarboxylase (EC 4.1.1.50) of eukaryotic organisms (Pegg & Williams-Ashman, 1969; Williams-Ashman *et al.*, 1971, 1972). The control of putrescine catabolism is therefore of fundamental importance. The idea that diamine oxidase is involved in this process is not new (Kapeller-Adler, 1970). However, the influence of diamine oxidase inhibitors and of inhibitors of other enzymes of putrescine catabolism on the concentrations of endogenous putrescine and putrescine catabolites seems not to have been studied. The present work is an attempt to close this gap in our knowledge.

Materials and Methods

Materials

Reagents. Laboratory chemicals of A grade were purchased from E. Merck, Darmstadt, Germany. Aminoguanidine sulphate and putrescine dihydrochloride (Th. Schuchardt, München, Germany), spermidine phosphate and spermine phosphate (Fluka A.G., Buchs, Switzerland), 4-aminobutyric acid (Serva A.G., Heidelberg, Germany), hydrazine sulphate (Riedel de Haen, Seelze, Germany), pargyline hydrochloride (*N*-benzyl-*N*-methylprop-2-ynylamine hydrochloride) (Abbott Laboratories, North Chicago, Ill., U.S.A.), marsilid phosphate (isonicotinic acid 2-isopropylhydrazide phosphate) (Deutsche Hoffmann–La Roche, Grenzach, Germany), carboxymethoxylamine (amino-oxyacetic acid) (Mann Research Laboratories, New York, N.Y., U.S.A.), PPO (2,5-diphenyloxazole) (Koch–Light Laboratories, Colnbrook, Bucks., U.K.) and diamine oxidase (B grade, pig kidney) (Calbiochem, San Diego, Calif., U.S.A.) were purchased. Dansyl chloride (5-dimethylaminonaphthalene-1-sulphonyl chloride) was prepared in our laboratory (Seiler, 1970).

Radiochemicals. [1,4-^{14}C]Putrescine dihydrochloride (sp. radioactivity 15.6 Ci/mol) and DL-[3-^{3}H]-glutamic acid (sp. radioactivity 2.8 Ci/mmol) were purchased from New England Nuclear Corp., Boston, Mass., U.S.A.

Mass spectrometer. This was a Varian model CH5 MAT (Bremen, Germany); electron beam energy 70 eV; temperature of the ion source 250°C.

Liquid-scintillation spectrometer. There was an SL 30 Intertechnique with a Multi-8 computer, from Plaisir, France.

Experimental animals. Male albino mice (NMRI, Gesellschaft fur Versuchstierzucht, Hannover, Germany) weighing 35±2 g were used. Groups of ten animals were housed in standard cages (20 cm × 40 cm), having access to standard diet (Altromin, Lage, Germany) and water *ad libitum*.

Methods

Injection technique. All compounds were administered in freshly prepared solutions in 0.9% NaCl by intraperitoneal injection of 0.2 ml volumes. With carboxymethoxylamine, solutions were neutralized with $NaHCO_3$ before administration.

Determination of polyamines. Determination of the polyamines in brain and liver was done by dansylation as described previously (Seiler, 1970; Seiler & Lamberty, 1973). Quantitative determination of the compounds separated by t.l.c. was performed by fluorimetry for spermidine and spermine, and by quantitative mass spectrometry for putrescine (Seiler & Knödgen, 1973; Seiler & Schmidt-Glenewinkel, 1975). Specific radioactivities of spermidine, spermine and putrescine were measured by mixing 4 ml of the dioxan solutions that were used for quantitative fluorimetry with 10 ml of PPO–toluene scintillant (5 g/litre) and then counting by liquid scintillation. For putrescine a ten times larger amount of tissue extract (corresponding to 8 mg of tissue) than was normally used for quantitative determination was worked up. The fluorescing spot of the dansyl derivative of putrescine was extracted from the one-dimensional chromatogram and purified by rechromatography, by using cyclohexane–ethyl acetate (7:5, v/v) and then cyclohexane–ethyl acetate (1:1, v/v).

Determination of 4-aminobutyric acid. This was done by fluorimetry of Dns-2-oxopyrrolidine, the reaction product of 4-aminobutyric acid with excess of dansyl chloride (Seiler & Wiechmann, 1968). As liver concentrations of 4-aminobutyric acid are low, it was necessary first to separate Dns-2-oxopyrrolidine from the bulk of the other fluorescing dansyl derivatives. This was done by hydrolytic cleavage of Dns-2-oxopyrrolidine to Dns-4-aminobutyrate, extraction of dansyl amides from the alkaline solution with toluene, reaction of Dns-4-aminobutyrate with acetic anhydride to Dns-2-oxopyrrolidine and purification of this derivative by two-dimensional t.l.c. (Seiler & Wiechmann, 1969). In contrast with the previously described separations six two-dimensional chromatograms were prepared on a single 20 cm × 20 cm plate (with a 200 μm thick layer of silica gel G) by using porous polyethylene supports (Seiler, 1971). On each plate two standard samples, two tissue samples and two tissue samples with added amounts of 4-aminobutyrate were separated simultaneously in the following solvents: first dimension, benzene–cyclohexane–methanol (75:15:2, by vol.) (two runs); second dimension, diethyl ether–cyclohexane (3:1, v/v) (two runs). For a detailed description of the procedure see Seiler (1975).

Determination of specific radioactivity of 4-aminobutyrate and glutamate. For determination of the specific radioactivities the entire dansyl derivatives of a whole mouse liver were applied in a 15 cm-long streak to a silica gel plate (20 cm × 20 cm; 200 μm-thick layer of silica gel G) and developed in ethyl acetate – chloroform – methanol – acetic acid (50:30:20:1, by vol.). The zones corresponding to Dns-2-oxopyrrolidine and Dns-5-oxo-1-pyrrolidine-carboxylic acid (the dansyl derivatives of 4-aminobutyrate and glutamate respectively; Seiler *et al.*, 1971) were scraped off and eluates of the material of these zones were rechromatographed. The 4-aminobutyrate derivative was first purified by chromatography with cyclohexane–ethyl acetate (7:5, v/v) and then in a further one-dimensional separation with benzene–cyclohexane–methanol (75:15:2, by vol.). The dansyl derivative of glutamate was purified by rechromatography first in chloroform–methanol–acetic acid (17:2:1, by vol.) and then, after reappli-

cation of the Dns-pyrrolidonecarboxylic acid zone to another plate, in benzene–acetic acid–water (13:7:2, by vol.). The fluorescent spots were eluted with 5ml of dioxan. Scintillation counting was performed after addition of 10ml of PPO–toluene scintillant.

Determination of respiratory $^{14}CO_2$. Mice were injected with 5μCi (51μg) of [1,4-^{14}C]putrescine dihydrochloride. Pretreatment with certain inhibitors (see under 'Injection technique') was made 2h before the administration of the [^{14}C]putrescine. Immediately after administration of the radioactive material the mice were placed into a glass tube, through which a constant stream of air (25ml/min) was drawn. CO_2 was absorbed in a glass tube (3cm×50cm) containing 200ml of methanol–ethanolamine–water (25:5:1, by vol.). Complete absorption of CO_2 was ensured by bubbling the air through the absorption mixture, by using a cube of pumice as end-piece. At 10min intervals 2ml samples of the absorption mixture were taken for radioactivity measurements.

After 2h the animals were removed from the tube. Urine samples (collected during the respiratory experiment) were collected for a further 22h in vessels containing 5ml of ethanol.

Determination of radioactive metabolites of [^{14}C]-putrescine in urine. The centrifuged urine samples were evaporated to dryness, the residue was taken up in 0.5ml of ethanol–water (1:1, v/v) and again centrifuged. Some of the samples were hydrolysed with 0.5ml of 6M-HCl at 110°C for 16h. The residues of these samples were also dissolved in 0.5ml of ethanol–water. These solutions were applied in 3cm-long streaks to t.l.c. plates (with 200μm-thick layers of silica gel G). The plates were sprayed with pyridine–acetic acid buffer, pH4.8 (Fischer & Bohn, 1957). Electrophoretic separation was performed by using the same buffer in the electrode chambers at 20V/cm for 45min at 2°C. Radioactivity was determined along the length of the electrophoretic path by removing 5mm×40mm zones, eluting the adsorbent of each zone with 3ml of methanol–phenethylamine (4:1, v/v) and determining the radioactivity by liquid-scintillation counting (see Seiler & Al-Therib, 1974*a*).

Assay of 4-aminobutyrate–2-oxoglutarate aminotransferase (EC 2.6.1.19). The method of Baxter & Roberts (1958) was applied to 1ml samples of brain and liver homogenates containing 60–80mg of tissue in 0.1M-sodium borate buffer, pH8.1.

Results

Respiratory excretion of $^{14}CO_2$ from [1,4-^{14}C]putrescine

In accordance with previous findings (Jänne, 1967) a large proportion of putrescine is excreted as CO_2 by rodents. In the present work 43±10% of the intraperitoneally injected [1,4-^{14}C]putrescine was found in the expired air within 2h (Fig. 1). Pretreatment of the animals with pargyline (*N*-benzyl-*N*-methylprop-2-ynylamine), a potent inhibitor of monoamine oxidase (Taylor *et al.*, 1960), decreased the expiration of $^{14}CO_2$ to about one-half of the control. An even more pronounced effect was elicited by inhibitors of diamine oxidase. As shown in Fig. 1, the $^{14}CO_2$ expiration was decreased to about 2% of the injected radioactivity if hydrazine sulphate or aminoguanidine sulphate was administered.

An important aspect of putrescine catabolism was revealed by the action of carboxymethoxylamine, a well-known inhibitor of 4-aminobutyrate aminotransferase (Wallach, 1961; Baxter & Roberts, 1961). Although even toxic doses (100mg/kg) of carboxymethoxylamine effected only an 80% inhibition of the enzyme in brain and liver (Fig. 2), $^{14}CO_2$ expiration was nevertheless markedly decreased. Animals pretreated with 50mg of carboxymethoxylamine/kg 2h before administration of [^{14}C]putrescine excreted

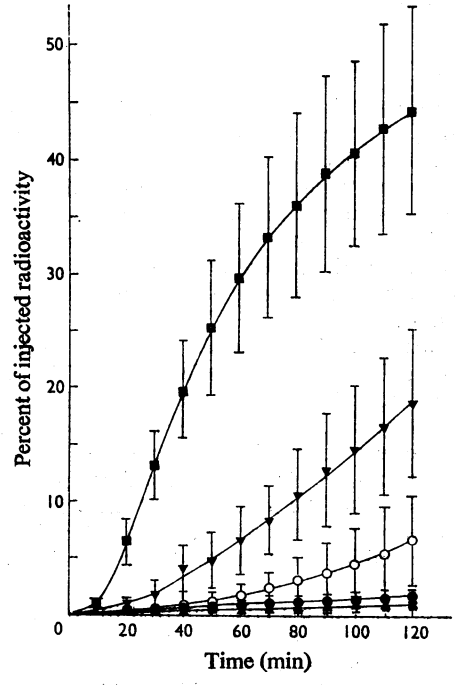


Fig. 1. *Influence of certain metabolic inhibitors on expiration of $^{14}CO_2$ by [^{14}C]putrescine-injected mice*

Pretreatment with the inhibitors was 2h before the intraperitoneal administration of 5μCi of [1,4-^{14}C]putrescine dihydrochloride. The standard deviation of the mean of four determinations is indicated by the vertical bars. ■, Control; ▼, pargyline (60mg/kg); ○, carboxymethoxylamine (50mg/kg); ●, aminoguanidine sulphate (60mg/kg); ▲, hydrazine sulphate (60mg/kg).

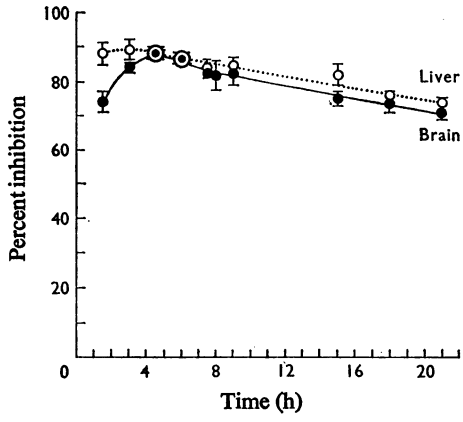


Fig. 2. *Time-dependence of 4-aminobutyrate-2-oxoglutarate aminotransferase inhibition in brain (●) and liver (○) of mice, pretreated with 100 mg of carboxymethoxylamine/kg*

For further details see the text.

only 5% of the radioactivity as $^{14}CO_2$ in the first 2h after dosage.

Urinary excretion of ^{14}C from [1,4-^{14}C]putrescine

Electrophoresis of the 24h urine after administration of [^{14}C]putrescine revealed two major radioactive zones: putrescine and an electrophoretically immobile spot (Fig. 3). A small proportion of the radioactive material moved electrophoretically like spermidine, spermine or monoacetylputrescine. It should be noted, however, that the last two compounds were not completely separated under these conditions.

Pretreatment of the animals with 60mg of hydrazine sulphate/kg inhibited the formation of the radioactive compounds situated at the origin of the electrophoretogram, and radioactivity at the position of putrescine was concomitantly increased. The same effects were observed with aminoguanidine-pretreated animals (Fig. 3). The proportion of the urine radioactivity corresponding to monoacetylputrescine was also increased in animals treated with diamine oxidase inhibitors.

Hydrolysis with 6M-HCl removed the radioactive material situated at the origin, without considerably increasing the amount of putrescine. From this, and from the effects of diamine oxidase inhibitors, it was assumed that the material at the origin of the electrophoretogram represents mainly derivatives of 4-aminobutyraldehyde. It is known that 4-aminobutyraldehyde formed from putrescine by diamine oxidase is spontaneously converted mainly into Δ^1-pyrroline (Okuyama & Kobayashi, 1961), although other products of the reaction of diamine oxidase with putrescine were also observed. Experiments *in vitro* with hog kidney diamine oxidase and [1,4-^{14}C]putrescine, showed that the excretion products in the urine were not identical with Δ^1-pyrroline. Since the urinary excretion products were chromatographically heterogeneous it is assumed that different derivatives are formed within the organism, most probably by spontaneous reaction of the highly reactive 4-aminobutyraldehyde or of Δ^1-pyrroline.

Pargyline administration slightly decreased the urinary excretion of radioactive putrescine and also of the electrophoretically immobile putrescine-degradation products. A relatively large proportion of radioactivity was observed in the spermidine zone (Fig. 3), which is consistent with the enhanced spermidine synthesis in chick embryos after marsilid administration (Caldarera *et al.*, 1965).

The proportion of radioactive putrescine in the urine of the animals treated with carboxymethoxylamine was less than that of controls. In addition to the electrophoretically immobile derivatives radioactive 4-aminobutyrate was observed in a considerable proportion. 4-Aminobutyrate was unambiguously identified in this experiment by dansylation, two-dimensional t.l.c. and mass spectrometry. Carboxymethoxylamine has apparently no significant inhibitory effects on diamine oxidase, although it has a hydroxylamine moiety, but it does inhibit 4-aminobutyrate aminotransferase in the intact animals, and consequently the further degradation of 4-aminobutyrate via succinic semialdehyde, succinate and the citrate cycle does not occur.

Influence of inhibition of amine oxidase and 4-aminobutyrate aminotransferase on brain and liver concentrations of putrescine and 4-aminobutyrate

The effects of some inhibitors of diamine oxidase, monoamine oxidase and 4-aminobutyrate aminotransferase on tissue concentrations of putrescine and 4-aminobutyrate are summarized in Table 1. The concentrations of the polyamines spermidine and spermine were not significantly altered. The effects elicited by the different compounds showed considerable variation and the standard deviations were significantly greater than those of untreated control animals. Nevertheless some of the changes in putrescine and 4-aminobutyrate concentrations effected by the inhibitors were significant.

Aminoguanidine sulphate, a potent inhibitor of diamine oxidase (Schuler, 1952; Schayer *et al.*, 1954; Waton, 1956; Kobayashi, 1957), does not significantly affect the concentrations of putrescine or 4-aminobutyrate in brain. The liver concentration of 4-aminobutyrate was slightly decreased, whereas the liver concentration of putrescine was more than doubled within 4h. Decreased 4-aminobutyrate concentrations in liver were found after marsilid administration, or after repeated injections of pargyline.

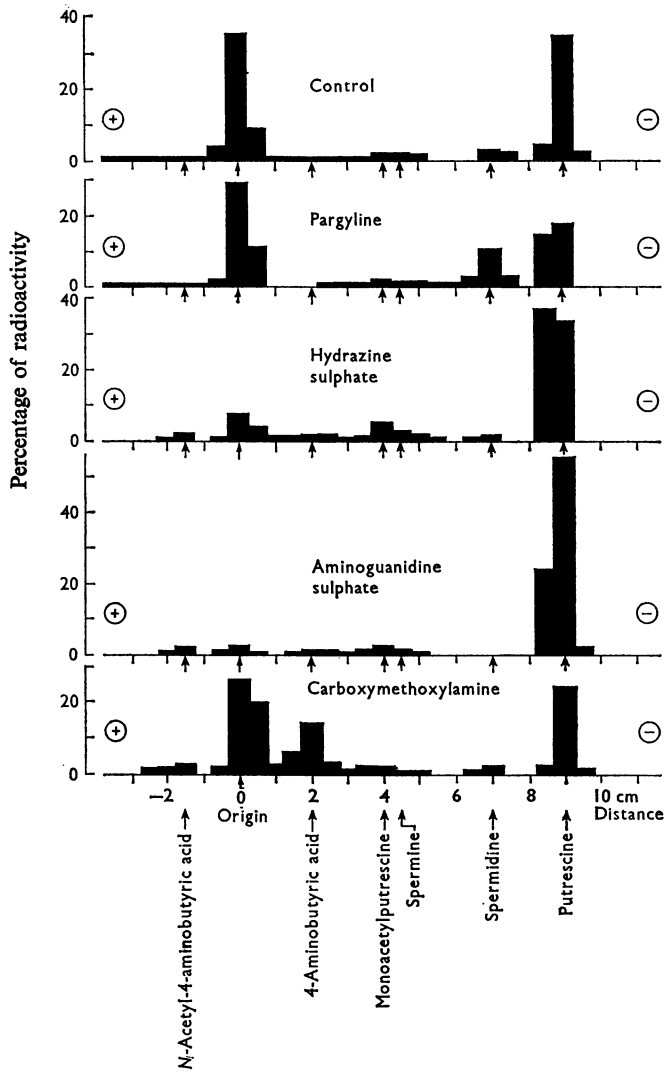


Fig. 3. *Distribution patterns along the length of electrophoretograms of the radioactive material in the urine of* [^{14}C]*putrescine-injected mice*

The animals were the same as those used for the determination of expiration of $^{14}CO_2$ (Fig. 1). Urine samples were gathered, however, for 24h after the injection of 5μCi of [1,4-^{14}C]putrescine dihydrochloride. The positions of reference compounds are indicated by arrows. Electrophoresis was performed on silica-gel plates, in pyridine–acetate buffer, pH4.8, at 20V/cm, for 45min.

Hydrazine sulphate, another well-known inhibitor of diamine oxidase (Schuler, 1952; Waton, 1956), caused much greater enhancement of liver putrescine concentration than any other of the tested compounds. Its effects on brain concentrations of putrescine were, however, not significant. It had a more pronounced effect on liver 4-aminobutyrate concentration than did aminoguanidine, and this effect was of about the same magnitude as that produced by carboxymethoxylamine (Table 1).

By intraperitoneal injection of large amounts of 4-aminobutyrate a significant increase in putrescine

Table 1. *Influence of intraperitoneally administered carboxymethoxylamine, pargyline, marsilid, hydrazine, aminoguanidine, or of large doses of 4-aminobutyric acid, on brain and liver concentrations of putrescine and 4-aminobutyric acid*

Results are expressed as percentage of the control, ±S.D.; $n \geq 3$.

Pretreatment	Putrescine		4-Aminobutyric acid	
	Brain	Liver	Brain	Liver
Aminoguanidine sulphate (60mg/kg) 4h after administration	95±118	249±95	100±30	77±16
Hydrazine sulphate (60mg/kg) 4h after administration	118±98	435±285	260±44	530±130
Marsilid (60mg/kg) 4h after administration	112±84	154±125	92±38	57±13
Pargyline (60mg/kg) 4h after administration	98±79	195±154		
10×60mg/kg on 5 consecutive days	101±13	111±13	90±33	71±22
11×60mg/kg on 11 consecutive days	159±111	151±70	74±26	76±18
Carboxymethoxylamine				
100 mg/kg; 5.5h after administration	180±66	61±21	344±71	851±172
50mg/kg; 2h after administration				282±90
3h after administration				415±94
5h after administration				482±285
4-Aminobutyric acid (2.5g/kg) (injected in three portions during 3h); 1h after administration	159±40	332±217		
Control (nmol/g fresh wt)	8.5±1	18.4±3	2600±26	31.5±3

Table 2. *Influence of intraperitoneally administered carboxymethoxylamine on the metabolism of putrescine into 4-aminobutyric acid in mouse liver*

For this, 42μCi of [1,4-^{14}C]putrescine dihydrochloride was given 1h after the administration of carboxymethoxylamine. The values are means±S.D.; $n \geq 4$.

Treatment	Time after [^{14}C]putrescine injection (h)	4-Aminobutyric acid concentration (nmol/g)	Specific radioactivity (d.p.m/nmol)		Specific radioactivity ratio 4-aminobutyric acid/putrescine
			4-Aminobutyric acid	Putrescine	
0.2ml of 0.9% NaCl intraperitoneally	1	26.6± 4	3200± 880	135900± 6500	0.023±0.007
	2	30.3± 3	1670± 600	56600±13000	0.030±0.02
	4	34.2±11	912± 380	24700±11000	0.037±0.02
50mg of carboxy-methoxylamine/kg intraperitoneally	1	75.0± 8	9140±3900	166200± 1700	0.054±0.02
	2	126.0±13	12400±1850	148200±11800	0.083±0.02
	4	165.2±13	6515±2300	109600±44000	0.059±0.03

concentration is elicited, both in brain and liver. The relative increase in liver is about twice that of brain (Table 1).

Metabolism of putrescine and glutamate to 4-aminobutyrate in mouse liver

The metabolism of intraventricularly administered [^{3}H]glutamate and [^{14}C]putrescine into 4-aminobutyrate in mouse brain has been studied previously (Seiler & Knödgen, 1971). Analogous experiments were carried out with mouse liver. A mixture of 42μCi (0.43mg) of [1,4-^{14}C]putrescine dihydrochloride and 110μCi (5.7μg) of DL-[3-^{3}H]glutamate was administered intraperitoneally to mice. Glutamate and 4-aminobutyrate were isolated from the liver and purified as their dansyl derivatives. The specific radioactivities of these compounds were measured with respect to ^{3}H and ^{14}C. The effect of carboxymethoxylamine on the metabolic processes was studied, and the results are summarized in Tables 2 and 3. Incorporation of radioactivity from [^{3}H]-glutamate into 4-aminobutyrate was insignificant, one reason for this being the dilution of the [^{3}H]glutamate by the large glutamate pool of liver and its rapid conversion into glutamine. Indeed the specific radioactivities of glutamate and putrescine differed by a factor of 10^{4}, 1h after the administration of the radioactive precursors (see Tables 2 and 3). [^{14}C]Glutamate derived from [^{14}C]putrescine had about the same specific radioactivity as that labelled directly by administration of [^{3}H]glutamate. The specific radioactivity of 4-aminobutyrate with respect to ^{14}C was much higher than that of [^{14}C]glutamate. This is in accordance with previous findings (Seiler *et al.*, 1971) and with the view that 4-aminobutyrate is

Table 3. *Influence of intraperitoneally administered carboxymethoxylamine on glutamate metabolism in mouse liver*

A mixture of 42μCi of [1,4-^{14}C]putrescine dihydrochloride and 110μCi of DL-[3-^{3}H]glutamic acid was given 1h after the administration of carboxymethoxylamine. The values are means±S.D.; $n \geq 3$.

Treatment	Time after administration of the radioactive compounds (h)	Specific radioactivity of glutamic acid (d.p.m./μmol)	
		^{3}H	^{14}C
0.2ml of 0.9% NaCl	1	15000±1800	8030±2500
	2	3760±1800	2130± 780
	4	2770±1100	1140± 400
50mg of carboxymethoxylamine/kg	1	19400±1600	11600±2900
	2	8330±1300	7080±1300
	4	4100±1000	4580± 670

formed from putrescine along a direct pathway that does not include glutamate as intermediate.

As can be deduced from Table 2, pretreatment of the animals with carboxymethoxylamine increases the specific radioactivities of 4-aminobutyrate and of putrescine. However, as the ratios of the specific radioactivities show, that of 4-aminobutyrate is more enhanced. An effect of carboxymethoxylamine treatment on glutamate metabolism is apparent from the results in Table 3. The decrease in the specific radioactivities of [^{3}H]- and of [^{14}C]-glutamate in mouse liver is less rapid in the treated animals than in the controls.

Discussion

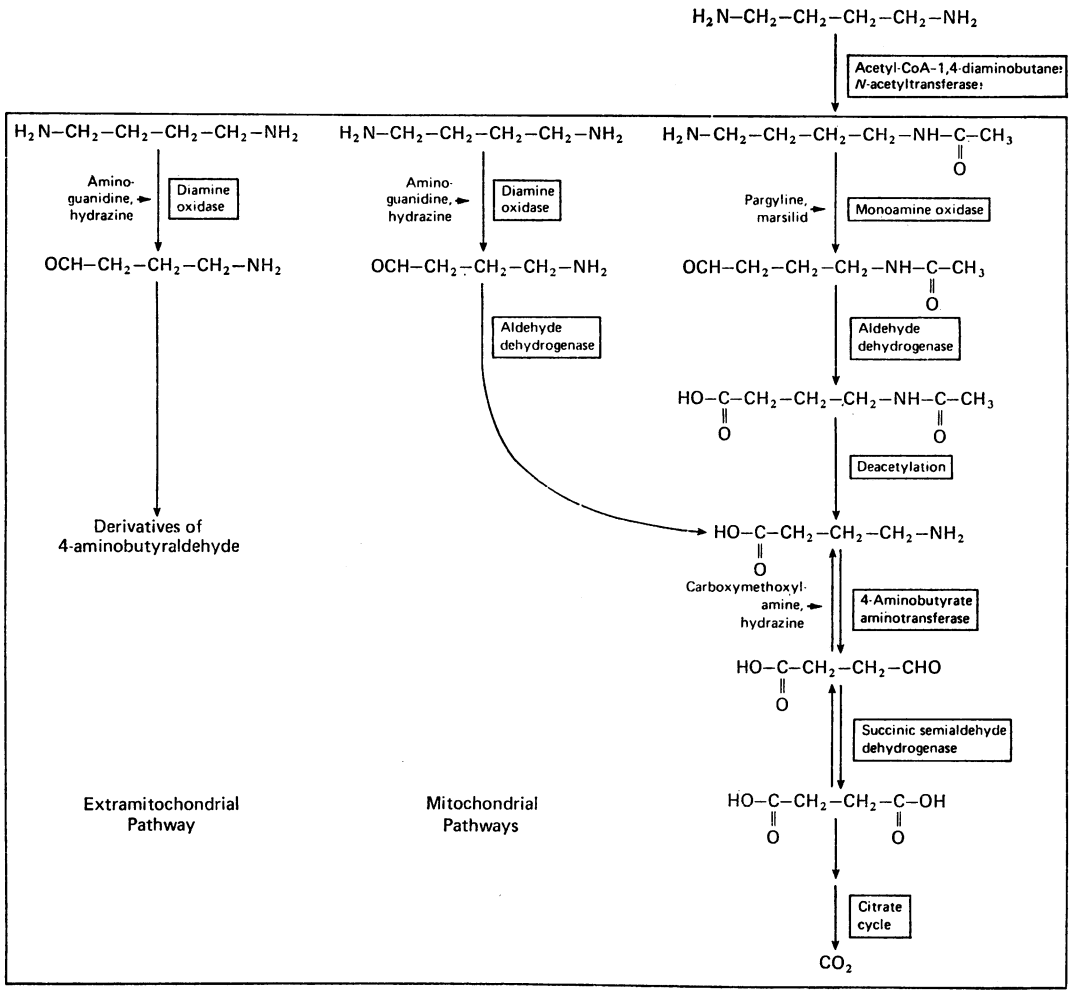
These results, together with previously published observations (Seiler & Al-Therib, 1974*a*), allow the construction of a scheme for mammalian putrescine catabolism, where 4-aminobutyrate plays a central role (Scheme 1).

The nearly complete inhibition of [^{14}C]putrescine catabolism and the increase in putrescine concentration in liver caused by injection of 60mg of aminoguanidine sulphate or hydrazine sulphate/kg is consistent with the important role of diamine oxidase in putrescine degradation. Two reaction sequences, both starting with the oxidative deamination of putrescine, appear to exist. In the mitochondrial pathway the oxidative deamination is coupled with an aldehyde dehydrogenase, which forms 4-aminobutyrate. In those metabolic compartments that do not contain an appropriate aldehyde dehydrogenase, as for instance the circulation system, 4-aminobutyraldehyde, the reaction product of diamine oxidase, is transformed into urinary excretion products, probably by non-enzymic reactions.

The existence of these two pathways is clearly shown by the effects of carboxymethoxylamine. Carboxymethoxylamine is known to inhibit almost exclusively the mitochondrial 4-aminobutyrate aminotransferase, at least *in vivo*, whereas its inhibitory qualities are less specific *in vitro* (Baxter, 1970). A considerable increase in endogenous 4-aminobutyrate concentration results from this inhibition as well as the urinary excretion of 4-aminobutyrate. The concomitant decrease in the formation of $^{14}CO_2$ from radioactive putrescine *in vivo* and the inhibition of 4-aminobutyrate aminotransferase in brain and liver suggest that the total amount of $^{14}CO_2$ is produced by the mitochondrial pathway that includes 4-aminobutyrate as an intermediate. On the other hand the urinary excretion of 4-aminobutyraldehyde derivatives was not influenced by pretreatment with carboxymethoxylamine, in agreement with the proposed reaction sequence, and in agreement with the fact that carboxymethoxylamine has no inhibitory activity towards diamine oxidase.

Hydrazine is not only an inhibitor of diamine oxidase but also an inhibitor of 4-aminobutyrate aminotransferase (Medina, 1963). The effect on 4-aminobutyrate transamination is shown by the considerable increase in 4-aminobutyrate concentrations in brain and liver in hydrazine-treated animals. Since glutamate decarboxylase is not inhibited by hydrazine (Baxter, 1970), we may assume that 4-aminobutyrate formation occurs by glutamate decarboxylation, despite the low percentage of incorporation of radioactivity from [3-^{3}H]glutamate into liver 4-aminobutyrate.

In carboxymethoxylamine-treated animals the specific radioactivity of 4-aminobutyrate increased significantly more than that of putrescine (Table 2), indicating that dilution of radioactive 4-aminobutyrate (from [^{14}C]putrescine) by 4-aminobutyrate formed from endogenous precursor pools was diminished. An NAD^{+}-dependent carboxymethoxylamine-sensitive pathway of 4-aminobutyrate formation from glutamate has been recently detected (Seiler & Wagner). The inhibition of this pathway could account for the relative increase in specific radioactivity of 4-aminobutyrate in relation to that of putrescine.



Scheme 1. *Catabolic pathways of putrescine* (1,4-*diaminobutane*)

The slower decrease in the specific radioactivity of glutamate in the liver of the treated animals in comparison with the controls (Table 3) supports this view.

Pargyline and marsilid, two well-known inhibitors of monoamine oxidase, greatly diminished the expiration of $^{14}CO_2$ (Fig. 1) without influencing significantly the urinary excretion of the [^{14}C]putrescine metabolites (Fig. 3). This shows that they are involved in the mitochondrial pathways. One would think first of the inhibition of acetylputrescine deamination (Scheme 1) and of the significance of the monoacetylputrescine pathway of putrescine catabolism (Seiler & Al-Therib, 1974*a*), not only in brain, but in viscera as well. Indeed, there is evidence for this possibility: excretion of monoacetylputrescine by humans (Perry & Schroeder, 1963); increased amounts of radioactivity in the urinary monoacetylputrescine of hydrazine-treated animals, together with the appearance of a compound that is probably *N*-acetyl-4-aminobutyrate (Fig. 3); detection of the putrescine-acetylating enzyme in brain and other organs, including liver (Seiler & Al-Therib, 1974*b*); and occurrence of monoacetylputrescine in different organs (Seiler *et al.*, 1973). On the other hand, that part of the putrescine-degrading capacity of the organism that can be inhibited by pargyline or marsilid can also be inhibited by aminoguanidine or by hydrazine.

It was believed until recently that pargyline does

not affect diamine oxidase to a significant extent (Zirkle & Kaiser, 1964). However, it has been reported that different inhibitors of monoamine oxidase, including pargyline and marsilid, are capable of considerable inhibition of human placental diamine oxidase (Crabbe & Bardsley, 1974). Among the visceral organs of mammals diamine oxidase activity seems to be highest in the intestines (Zeller *et al.*, 1939). Presumably this organ is therefore of major importance in putrescine catabolism. The lack of specificity of the monoamine oxidase inhibitors towards intestinal diamine oxidase might explain the observed effects; however, until a more detailed knowledge of the extent of the catabolism of putrescine *in vivo* in the different organs is available, and until detailed studies of the effects of monoamine oxidase inhibitors on diamine oxidases of different organs are available, the effects of monoamine oxidase inhibitors on putrescine catabolism cannot be completely understood.

Administration of large doses of 4-aminobutyrate inhibits putrescine catabolism, putrescine excretion, or stimulates putrescine biosynthesis, as is indicated by the considerable increase in putrescine concentrations in brain and especially in liver (Table 1). As neither stimulation of ornithine decarboxylase nor inhibition of diamine oxidase has been reported, the effects of 4-aminobutyrate cannot be attributed to its effects on these enzymes and may be due to its general effects on circulation and respiration (Baxter, 1970).

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