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The physiological disposition of ^{14}C-methsuximide in the rat

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Summary

1. ^{14}C-Methsuximide (N-methyl-^{14}C-2-methyl-2-phenylsuccinimide) was rapidly absorbed from the small intestine of the rat ($t_{\frac{1}{2}}$ 17·4 min).

2. The drug was rapidly and fairly evenly distributed throughout the body with peak blood and tissue levels occurring 1 h after oral administration. At all times, adrenals, body fat, kidneys and liver had higher levels of ^{14}C-methsuximide than other tissues and the drug freely traversed the blood-brain barrier. However, radioactivity disappeared rapidly from most tissues after the initial phase of the distribution.

3. During 24 h, 26% of the orally administered radioactivity was recovered in urine and 29% appeared in expired air as $^{14}CO_2$. The excretion of $^{14}CO_2$ indicated N-demethylation of ^{14}C-methsuximide to 2-methyl-2-phenylsuccinimide. 2·7% of an administered dose of methsuximide was excreted unchanged in 24 h urine and 2·7% appeared as 2-methyl-2-phenylsuccinimide. 2-Methyl-2-phenylsuccinimide was also detected as a urinary metabolite of methsuximide in man.

4. 2-Methyl-2-phenylsuccinimide possesses anticonvulsant activity and it is suggested that this metabolite contributes to the overall anticonvulsant activity and toxicity of methsuximide.

5. Rat urine also contained a radioactive substance with similar chromatographic properties to one of the products of alkaline hydrolysis of methsuximide. This compound may arise from the spontaneous decomposition of the parent drug *in vivo*.

Introduction

Methsuximide (N,2-dimethyl-2-phenylsuccinimide) is effective against both chemically- and electrically-induced convulsions and has been used for several years in the treatment of petit mal epilepsy. Although its pharmacological and clinical efficacy has been extensively studied (Zimmerman, 1953, 1956; Gibbs & Stamp, 1958; Dow, Macfarlane & Stevens, 1958; Carter & Maley, 1957; Chen, Weston & Bratton, 1963), there is no information available on the fate of the drug in animals and man apart from a recent preliminary study (Nicholls & Orton, 1971).

For absorption, distribution, excretion and metabolism studies, methsuximide was prepared with carbon-14 in the N-methyl position. By analogy with the metabolic fate of the closely related antiepileptic drug phensuximide, it is probable that methsuximide is mainly N-demethylated to 2-methyl-2-phenylsuccinimide

and/or hydrolyzed in the succinimide ring to yield two possible structural isomers, N,2-dimethyl-2-phenyl and N,3-dimethyl-3-phenyl succinamic acids (Fig. 1).

Methods

Synthesis of ^{14}C-methsuximide

N-Methylation of 2-methyl-2-phenylsuccinimide with ^{14}C-methyl iodide was carried out by the procedure of El-Zanfally, Khalifa & Abou-Zeid (1968). 2-Methyl-2-phenylsuccinimide, 1·5 mmol, was mixed with equimolar amounts of anhydrous potassium carbonate and ^{14}C-methyl iodide in a flask fitted with a reflux condenser and was heated gently for 2 h to a state of fusion. The crude product was extracted into ether and purified by crystallization from 20% ethanol. Thin layer chromatography showed that the compound obtained was identical with non-labelled reference substance and that all the radioactivity present ran as a single entity. The specific activity of the ^{14}C-methsuximide was 0·2 mCi/mmol (1 μCi/mg). For administration to rats, a known amount of the ^{14}C-labelled methsuximide (ranging between 10 to 20% of the final dose) was mixed with non-radioactive methsuximide.

Animals and administration of drug

Male CFHB-Wistar rats (Carworth Europe, Huntingdon, England) weighing 70–120 g were used. All animals were fasted for 18 h prior to experimentation but were allowed free access to water. It was decided to administer ^{14}C-methsuximide at a dosage known to be effective against both leptazol- and electrically-induced convulsions. A dose of 100 mg/kg was therefore chosen (Chen *et al.*, 1963; Miller & Long, 1951).

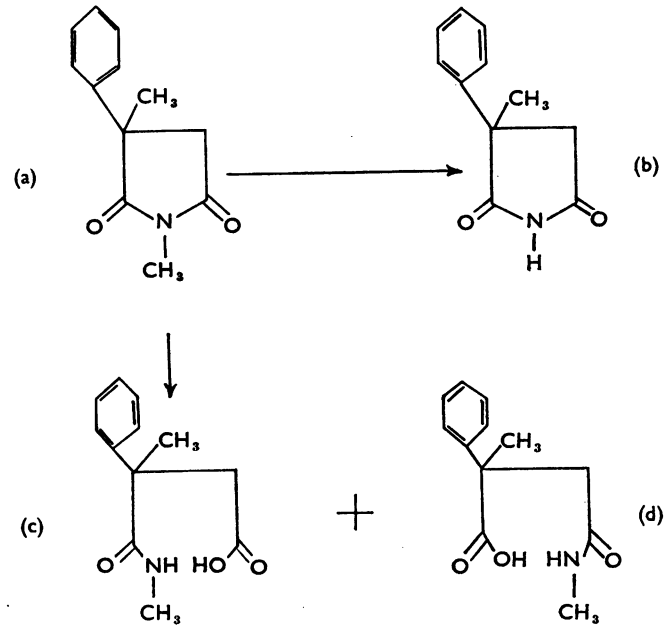

FIG. 1. Scheme of the possible metabolism of methsuximide (a). (b), 2-methyl-2-phenyl succinimide; (c), N,2-dimethyl-2-phenyl succinamic acid; (d), N,3-dimethyl-3-phenyl succinamic acid.

Radioactivity measurement

Radioactivity was measured by liquid scintillation counting with a Tracerlab Coru/Matic 100 Dual Channel Scintillation Spectrometer. The counting solution consisted of 4 g/litre 2,5-dephenyloxazole (PPO), 0·1 g/litre 1,4-bis-5-phenyloxazol-2-yl benzene (POPOP) and 20 ml/litre formic acid in toluene:2-ethoxyethanol (7:3 v/v) (Hall & Cocking, 1965).

The scintillation mixture for the counting of $^{14}CO_2$ consisted of 5·5 g PPO/litre toluene:2-ethoxyethanol mixture (2:1 v/v) (Jeffray & Alvares, 1961). Each sample was counted in triplicate. Quench correction was made by internal standardization with ^{14}C-hexadecane (1·06 μCi/g; Radiochemical Centre, Amersham).

Absorption

The absorption of ^{14}C-methsuximide was studied essentially by the method of Doluisio, Billups, Dittert, Sugita & Swintosky (1969) which utilizes *in situ* segments of the gastrointestinal tract of anaesthetized rats. Groups of 2 male rats (100–120 g) were anaesthetized with pentobarbitone sodium (30 mg/kg, i.p.). The proximal and distal ends of either the small intestine or the stomach were then cannulated. 10 ml of pH 6·0 Sørenson's phosphate buffer containing 0·2 μCi ^{14}C-methsuximide and 5 ml of pH 3·0 citrate–phosphate buffer containing 0·15 μCi ^{14}C-methsuximide were then placed in the small intestine and stomach respectively. Aliquots (0·1 ml) of the intraluminal solutions from the small intestine and the stomach were removed at 10 and 15 min intervals respectively and assayed for radioactivity. The pattern of disappearance from the small intestine during the initial ten minutes was determined by sampling at two minute intervals.

The disappearance of radioactivity from the entire gastrointestinal tract was determined during the studies on the distribution of ^{14}C-methsuximide following oral administration to unanaesthetized rats. In these experiments, the radioactivity of the gastrointestinal tract and its contents was measured and expressed as a percentage of the dose administered.

Tissue distribution studies

Male rats (70–95 g) received ^{14}C-methsuximide (0·15 μCi/mg in a dose of 100 mg/kg) by stomach tube. Two animals were examined several times up to six hours. Animals were decapitated and blood samples collected in heparinized test tubes. Various tissues were rapidly removed, rinsed in saline, blotted and weighed. Radioactivity of blood and tissues was measured according to the method of Petroff, Patt & Nair (1965). Tissues were treated individually with 2 N methanolic potassium hydroxide solution (1 ml/50 mg wet tissue), placed in a pre-heated water bath (70° C) and agitated continuously for 120 min to ensure dissolution. Whole blood was digested with methanolic KOH (1 ml/ml blood) and then decolourized with 100 volume hydrogen peroxide solution (3 drops/0·2 ml blood digest). Peroxide did not liberate any volatile ^{14}C-material from the digested blood samples. Aliquots (0·2 ml) of the blood and tissue digests were counted in scintillation mixture (10 ml).

Excretion of radioactivity after administration of ^{14}C-methsuximide

Groups of 2 male rats (70–95 g) received 100 mg/kg ^{14}C-methsuximide (15 μCi/kg) either orally or intraperitoneally. Each rat was immediately placed in a

separate metabolism cage fitted for the simultaneous collection of urine, faeces and expired carbon dioxide. Carbon dioxide was collected in a solution of ethanolamine in 2-ethoxyethanol (1:2 v/v) (Jeffray & Alvares, 1961). Aliquots (3 ml) of the absorbing fluid were counted in 15 ml of scintillation mixture. Aliquots (0·2 ml) of urine and the methanolic KOH digest of faeces were counted in 10 ml of scintillation mixture.

Biliary excretion of ^{14}C-methsuximide (0·3 μCi/rat) was studied in male rats (100–120 g) anaesthetized with pentobarbitone sodium (30 mg/kg, i.p.). The common bile duct was cannulated and bile was collected during 3 h, at 15 min intervals, after intravenous administration and during 6 h, at 90 min intervals, after oral administration. Aliquots (0·2 ml) of the bile were counted in 10 ml of the scintillation mixture.

Metabolic fate of ^{14}C-methsuximide

Urine from rats given ^{14}C-labelled or unlabelled methsuximide (100 mg/kg, orally) was collected for 24 h and investigated by thin layer, paper and gas-liquid chromatography.

(a) Thin-layer and paper chromatography

Ascending paper (Whatman No. 1) and silica gel thin layer (Eastman Chromagram Sheet 6060) chromatography was carried out in the following solvent systems; *n*-butanol:glacial acetic acid:water, 4:1:2 v/v/v (acidic system); chloroform: methanol, 1:1 v/v (neutral system); *n*-butanol saturated with 3% v/v ammonia (basic system). For investigation of conjugates, urine (1 volume) was autoclaved with 3 N hydrochloric acid (3 volumes) at 100° C and 0·85 kg/cm^2 for 60 min (Yeh & Woods, 1969) or incubated for 3 hours at 37° C with β-glucuronidase solution (0·5 volume 10,000 units/ml, 0·1 M acetate buffer pH 5·0; Akagi, Aoki & Uematsu, 1966).

Acid and enzyme hydrolyzed urine and untreated urine were freeze-dried. The residue was then dissolved in methanol and aliquots (20 μl) were spotted on to chromatograms. After development of chromatograms, ^{14}C-methsuximide and its metabolites were visualized under ultraviolet light (254 nm) and with location reagents (0·5 w/v ninhydrin in *n*-butanol, 1% w/v iodine in carbon tetrachloride). Autoradiography (Ilford G X-ray film) and 2π scanning (Tracerlab Radiochromatogram Scanner) were employed to locate radioactive areas on chromatograms of 24 h urine.

(b) Gas-liquid chromatographic analysis

Diluted rat urine samples (2 ml) were adjusted to pH 2·0 by the addition of 6 N hydrochloric acid. The urine was extracted by shaking for 2 h with chloroform (10 ml). The chloroform was separated off with phase separation paper (Whatman IPS) and then removed by distillation *in vacuo*. The residue was taken up into 100 μl chloroform containing glutethimide as internal standard (retention time 4·5 min). Samples (0·5 μl) were then analysed on a Pye Panchromatic gas chromatograph, equipped with a hydrogen flame ionization detector. The column was 150 × 0·4 cm with a packing of 2·5% w/w SE 30 on silanized, 80–120 mesh, acid washed Celite. The column temperature was 190° C and nitrogen flow rate was 50 ml/minute.

Anticonvulsant activity of methsuximide and its probable metabolites

The time-course of the anticonvulsant activity of methsuximide, 2-methyl-2-phenylsuccinimide and the alkaline hydrolysis product of methsuximide (hydrolyzed methsuximide) was studied following oral administration (100 mg/kg) to rats. At various time intervals, convulsions were induced by the continuous infusion (0·5 ml/min), via a tail vein, of leptazol solution (10 mg/ml saline) (Fingl & McQuarrie, 1960). The minimum amount of leptazol (mg/kg) that induced tonic convulsions was recorded.

In a separate group of animals the levels of methsuximide and 2-methyl-2-phenylsuccinimide in blood was determined at various times after administration of methsuximide (100 mg/kg, orally). Blood was obtained by cardiac puncture and methsuximide and 2-methyl-2-phenylsuccinimide were extracted from plasma according to the method of Huisman (1966) with a chloroform:methyl acetate mixture (6:4 v/v). The residue obtained after removal of the solvents was finally taken up into 100 μl extracting mixture containing glutethimide as internal standard and analysed by gas-liquid chromatography.

Results

Absorption

Figure 2 shows the disappearance of ^{14}C-methsuximide from the stomach and small intestine of the rat. Each line is plotted from the mean of results from two rats. After approximately 10 to 15 min, the disappearance of the drug followed

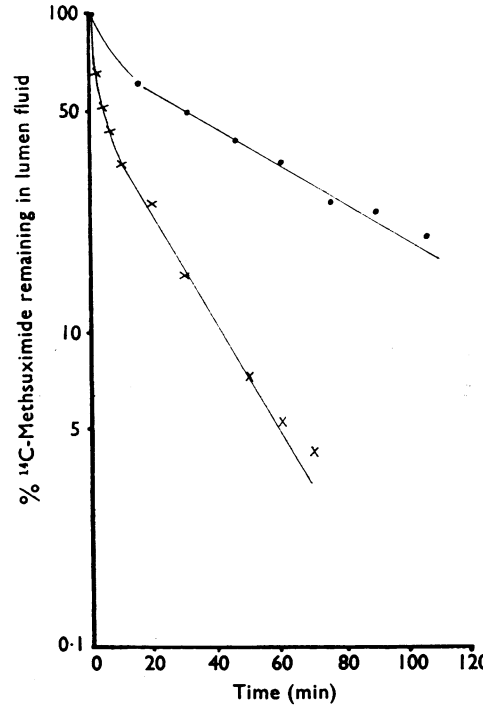

FIG. 2. Disappearance of ^{14}C-methsuximide from cannulated stomach (●) and small intestine (×) of anaesthetized male rats. At zero time, the intestine contained 0·2 μCi in 0·2 mg ^{14}C-methsuximide dissolved in 10 ml Sørenson's phosphate buffer pH 6·0. Separate animals were used for experiments with the stomach and the small intestine. Each line is the mean of results from two rats.

apparent first-order kinetics. When the half-life for disappearance was calculated from the straight ' tails ' of these semilog plots, it was found that ^{14}C-methsuximide disappeared more rapidly from the small intestine than from the stomach ($t_{\frac{1}{2}}$ 17·4 and 52 min respectively). After oral administration of ^{14}C-methsuximide, radioactivity disappeared steadily from the entire gastrointestinal tract until at 6 h only 12·7% of the radioactivity administered remained (Table 1).

TABLE 1. *Distribution of ^{14}C-methsuximide (0·15 μCi/mg), expressed as μg methsuximide/g, in tissues of the male rat after oral administration of 100 mg/kg*

	Time (min) after administration					
	15	30	60	120	180	360
Kidney	40·69	48·89	60·24	33·72	104·44	73·36
Spleen	3·5	29·42	39·80	21·25	9·11	7·16
Heart	3·18	28·72	43·78	13·83	6·95	5·03
Lung	6·37	31·56	40·55	21·65	9·52	10·13
Adrenal gland	8·98	79·16	117·00	41·70	29·34	18·71
Salivary gland	3·97	32·19	41·48	16·31	7·70	7·66
Testis	2·27	23·73	33·86	19·13	8·21	5·02
Gastrointestinal tract+contents*	92·22	64·5	45·3	—	23·1	12·7

*Expressed as % of the dose of methsuximide administered. The values at each time are the means from two rats.

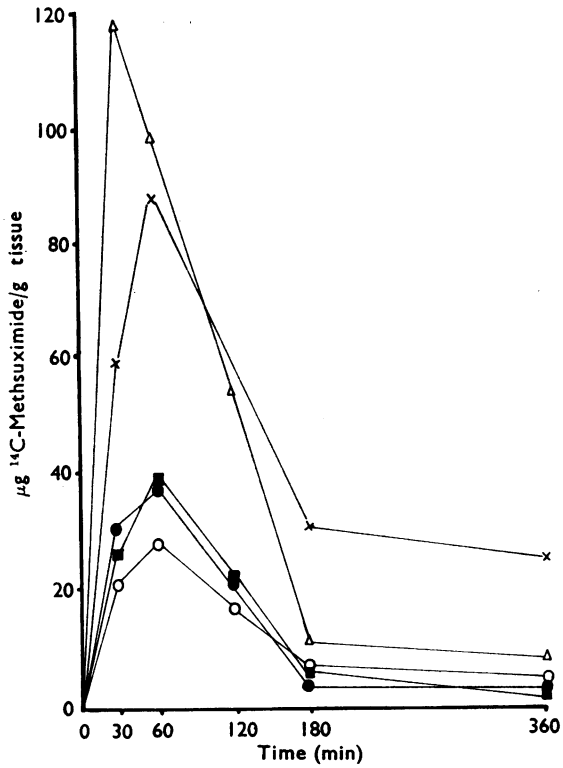

FIG. 3. Distribution of ^{14}C-methsuximide (0·15 μCi/mg) in various tissues of the male rat following oral administration (100 mg/kg). Each result is the mean from two rats. △——△, Body fat ; ×——×, liver; ●——●, brain ; ■——■, skeletal muscle ; ○——○, blood (μg/ml).

Distribution

The levels of radioactivity in most of the tissues of the rat, at periods of time between 15 and 360 min after oral administration of ^{14}C-methsuximide, are presented in Table 1. The values at each time interval represent the mean of results from tissues of 2 rats. The individual values did not differ by more than 10% from each other. The pattern of distribution of ^{14}C-methsuximide in the remaining tissues (fat, liver, brain, skeletal muscle and blood) is presented graphically in Fig. 3. These latter tissues showed distribution profiles which were representative of the other tissues.

There was rapid distribution of ^{14}C-methsuximide throughout all tissues examined. Peak concentrations in the blood and tissues were observed after 60 min and in the case of fat after 30 minutes. Brain, spleen, heart, testes, lungs, salivary glands, and skeletal muscle showed fairly similar levels of ^{14}C-methsuximide at each time interval studied. The compound quickly traversed the blood-brain barrier but was efficiently cleared from the brain. High levels of ^{14}C-methsuximide were rapidly attained in the liver, adrenal glands, fat and kidneys. The concentration of ^{14}C-methsuximide in fat reflects the lipophilic character of the compound.

Between 60 and 180 min after dosing, levels of radioactivity in the blood and the majority of the tissues fell rapidly. High levels, however, persisted in the kidneys, liver and adrenals up to 360 minutes.

Assuming that all radioactivity represents ^{14}C-methsuximide the half-life of methsuximide in blood is approximately 120 minutes.

Excretion

The excretion of radioactivity in the urine and the expired air (as $^{14}CO_2$) up to 9 h after oral and intraperitoneal administration of ^{14}C-methsuximide is presented in Fig. 4 where the radioactivity excreted is expressed as a percentage of the radioactivity administered. The appearance of $^{14}CO_2$ in expired air indicates that the carbon-14 labelled methyl group is removed from the drug in the body.

After oral administration, radioactivity slowly appeared in urine (2% in 3 h) and expired CO_2 (6% in 3 h). The maximum phase of excretion of radioactivity was observed between 3 and 6 h and the fall off after this plateau was gradual. During 24 h, 26 and 29% of the orally administered radioactivity appeared in the urine and expired CO_2 respectively. After intraperitoneal administration, however, radioactivity appeared more quickly in urine (12% in 3 h) and expired CO_2 (20% in 3 h). Excretion into the urine then decreased gradually with time while the excretion of $^{14}CO_2$ fell off rapidly. The excretion of radioactivity in urine and expired CO_2 during 24 h was 34 and 29% respectively.

During 72 h, 4% of the administered radioactivity was excreted in the faeces after intraperitoneal injection and 12% after oral dosing. Part of the radioactivity in the faeces following oral administration may represent unabsorbed drug. Excretion of radioactivity in the bile was demonstrated after both i.v. (12% in 3 h) and oral (8·5% in 3 h) administration of ^{14}C-methsuximide. This excretion may also contribute to the radioactivity of the faeces.

After 72 h, the total recovery of radioactivity in urine expired CO_2 and faeces was 69 and 71% of that administered for the oral and intraperitoneal routes respectively.

Chromatographic studies

Two radioactive spots were observed on the thin layer silica gel chromatograms of 24 h urine developed in the acidic solvent system (Rf 0·95 and 0·44) and the neutral solvent system (Rf 0·80 and 0·69). Chromatograms developed in the basic solvent system showed only a single radioactive spot (Rf 0·46). The major and faster moving spot in the acidic and neutral solvent systems corresponded to the mobility of ^{14}C-methsuximide (either alone or added to urine), and of unlabelled methsuximide. No additional radioactive spots were observed after either acid-heat or enzyme treatment of urine. However, a reversal in the intensities of the two radioactive spots was observed after acid-heat treatment of urine followed by thin layer chromatography in the acidic solvent system. A decrease in the ratio (major:minor) of the two radioactive spots was also observed after storage of a methanolic extract of 24 h rat urine at 4° C for several weeks. In this connexion it should be noted that chromatograms of a methanolic solution of ^{14}C-methsuximide stored at 20° C for 14 days indicated, in addition to methsuximide, the presence of a minor, slow moving, ninhydrin-sensitive radioactive spot (Rf 0·43 in the acidic system). These observations suggest that the second (minor) radioactive spot seen in some chromatograms of urine samples may be a simple breakdown product of

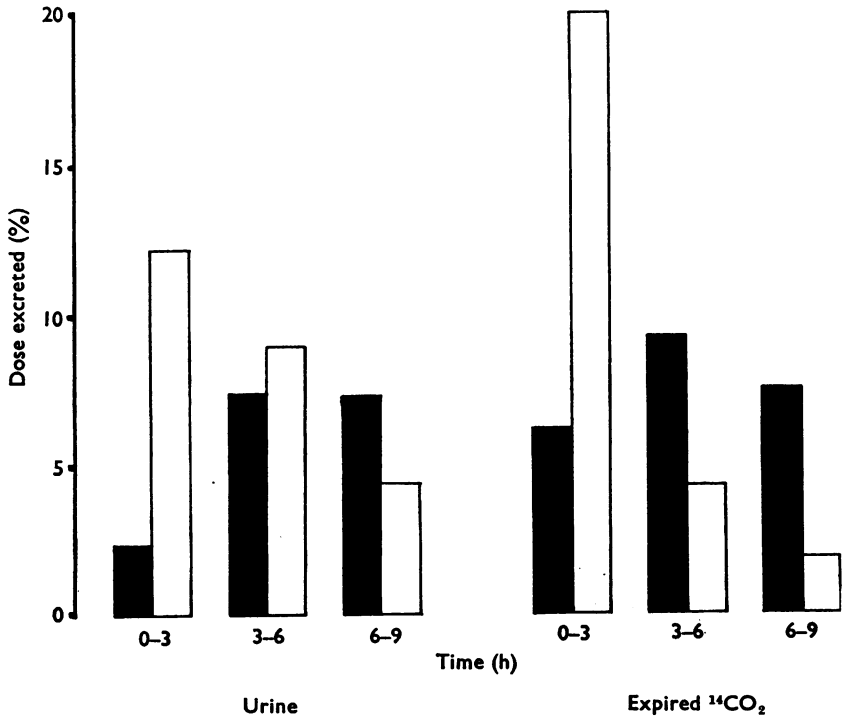

FIG. 4. Excretion of radioactivity in expired $^{14}CO_2$ and urine of rats receiving ^{14}C-methsuximide (100 mg/kg), orally (solid columns) and intraperitoneally (open columns). The results are the mean from two rats and are expressed as a percentage of the dose administered.

methsuximide such as the succinamic acid derivative. In fact, thin layer chromatograms of recently-collected 6 h urine developed in the acidic solvent system indicated a single radioactive spot with a mobility similar to ^{14}C-methsuximide.

Hydrolysis of the succinimide ring of methsuximide can give rise to two possible structural isomers, N,2-dimethyl-2-phenyl and N,3-dimethyl-3-phenyl succinamic acids (Fig. 1). To achieve this, methsuximide (1 g) was refluxed with 2 N NaOH (20 ml) for 2 hours. On cooling, the solution was adjusted to pH 2·0 with 2 N HCl. The colourless flocculent precipitate was recrystallized from *n*-butanol: chloroform mixture (1:1 v/v) m.p. 147–149° C. This hydrolyzed methsuximide was identified as an acid-amide by qualitative spot-tests, infra-red spectra and elemental analysis, found: C, 66·06; H, 6·67; N, 6·28%; $C_{12}H_{15}O_3N$ requires: C, 65·16; H, 6·79; N, 6·33%. ν_{max} (K Br) 3340 (N-H of secondary amide), 1720 (C=0 of acid), 1640 cm^{-1} (C=0 of amide). Thin layer and paper chromatography of the product in the acidic solvent system indicated the presence of two spots (Rf 0·88 and 0·43). The faster moving spot gave a more intense purple colouration than the slower spot after reaction with ninhydrin. It was observed that the Rf value of the slower moving spot corresponded to the Rf of the minor radioactive spot detected chromatographically in 24 h urine of rats receiving ^{14}C-methsuximide.

Gas–liquid chromatography provided both qualitative and quantitative information on the metabolic fate of methsuximide. Peaks corresponding to unchanged methsuximide (retention time 2·06 min) and 2-methyl-2-phenylsuccinimide (retention time 2·6 min) were demonstrated in chloroform extracts of 24 h urine (pH 2·0) collected from rats receiving both labelled and unlabelled methsuximide. A third peak with an identical retention time (1·31 min) to that of hydrolyzed methsuximide was also detected in 24 h rat urine (pH 2·0).

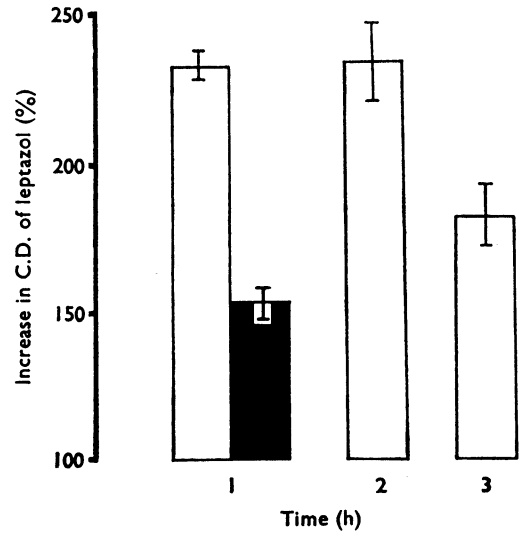

FIG. 5. Time-course of anticonvulsant activity after oral administration of methsuximide (100 mg/kg, open columns) and 2-methyl-2-phenyl succinimide (100 mg/kg, solid column) in male rats. Anticonvulsant activity was measured against leptazol-induced convulsions and is expressed as percentage increase in minimum convulsive dose of leptazol (CD) in succinimide-treated rats over the CD control animals. Each result is the mean (±S.E.) from five rats. The CD of leptazol in control animals was 41·6±3·7 mg/kg.

Quantitative analysis of 24 h rat urine (pH 2·0) by gas–liquid chromatography determined that 2·70 ± 0·13% of a dose (100 mg/kg) of methsuximide was excreted unchanged and 2·73 ± 0·21% was excreted as 2-methyl-2-phenylsuccinimide.

The 24 h urine of human volunteers who had received a single oral dose of methsuximide (300 mg) was extracted and gas chromatographed as described for rat urine. The presence of unchanged methsuximide and 2-methyl-2-phenylsuccinimide was established. It was not possible to demonstrate unequivocally the presence of hydrolyzed methsuximide in human urine.

Anticonvulsant activity of methsuximide and its probable metabolites

The anticonvulsant activity of methsuximide and 2-methyl-2-phenylsuccinimide is shown in Fig. 5. Anticonvulsant activity was expressed as the percentage increase over the control animals of the threshold dose of leptazol needed to induce convulsions and histograms represent the mean results from 5 rats. The hydrolyzed methsuximide did not possess anticonvulsant activity even at a dose of 500 mg/kg. Anticonvulsant activity (196 ± 13% of controls) was demonstrated 30 min after oral administration of methsuximide. A peak of anticonvulsant activity was observed between the 1 and 3 h time periods. The plasma levels of methsuximide determined by the gas–liquid chromatographic technique at 1 and 3 h were 26·8 ± 0·9 and 18·6 ± 0·5 μg/ml respectively. Significant anticonvulsant activity (182 ± 19%) was demonstrated up to 6 h although the plasma level of methsuximide at this time was only 2·0 ± 0·5 μg/ml. 2-Methyl-2-phenylsuccinimide possessed significant anticonvulsant activity (153 ± 9%) when tested 1 h after administration. Small concentrations of this metabolite (<1 μg/ml plasma) could be detected in rats 6 h after the oral administration of methsuximide (100 mg/kg).

Discussion

The rapid appearance of radioactivity in the tissues and blood of rats, after oral administration of ^{14}C-methsuximide, reflects the rapid absorption of the drug primarily from the small intestine. The rate of disappearance of ^{14}C-methsuximide from the small intestine was 4% per minute.

The onset of anticonvulsant activity paralleled the rise in the levels of ^{14}C-methsuximide in the blood and brain. Peak anticonvulsant activity and peak levels of ^{14}C-methsuximide in blood and brain were observed after 60 minutes.

The blood level of methsuximide after 60 min, determined by liquid-scintillation counting and gas–liquid chromatography, was 27·7 and 26·8 μg/ml respectively. The similar value determined by the two techniques suggests that little or no metabolism of ^{14}C-methsuximide has occurred within this time and that the anticonvulsant activity at this time is due almost entirely to the drug.

The appearance of $^{14}CO_2$ in expired air indicated N-demethylation of ^{14}C-methsuximide and this was confirmed by the detection of 2-methyl-2-phenylsuccinimide in 24 h rat urine. 2-Methyl-2-phenylsuccinimide was also identified in urine after oral administration of methsuximide to man. As an anticonvulsant, 2-methyl-2-phenylsuccinimide was shown to be about half as potent as methsuximide when tested 1 h after administration to rats. Previous work by Chen, Portman, Ensor & Bratton (1951) has demonstrated significant anti-leptazol activity up to 6 h after oral administration of 2-methyl-2-phenylsuccinimide (125 mg/kg) to rats.

The formation of active metabolites by *N*-demethylation occurs with the potent anticonvulsants paramethadione, mephenytoin, mephobarbitone and trimethadione. When these methylated drugs are administered chronically there is, in every instance, evidence indicative that the product of demethylation accumulates in the body (Butler & Waddell, 1958). The question then arises as to what extent the demethylated products may be responsible for the therapeutic and toxic effects seen after administration of the methylated drugs. In the case of trimethadione, the drug is converted entirely to dimethadione (Butler & Waddell, 1954; Butler, Waddell & Poole, 1965). Dimethadione is not metabolized further (Butler, 1953; Waddell & Butler, 1957), is very slowly excreted (Waddell & Butler, 1957; Jensen, 1962) and has similar anticonvulsant and toxic properties to trimethadione (Taylor, Davin & Richards, 1956; Chamberlaine, Waddell & Butler, 1965). Recently, Withrow, Stout, Barton, Beacham & Woodbury (1968) have shown that dimethadione can account for most of the anticonvulsant activity of trimethadione. The existence of a similar situation with regard to methsuximide and its N-demethylated metabolite, at the later time periods after oral administration of methsuximide is highly probable. However, the present work did not eliminate the possibility that 2-methyl-2-phenylsuccinimide is further metabolized to compounds possessing anticonvulsant activity. From the $^{14}CO_2$ excretion data, it is estimated that approximately 15% of the *N*-demethylated metabolite is formed during the first 6 hours. This may represent the production of sufficient 2-methyl-2-phenylsuccinimide to account for the anticonvulsant activity up to 6 h following oral administration of methsuximide, as the work with both ^{14}C-labelled and unlabelled methsuximide indicated that the levels of methsuximide in blood and brain at this time were low.

2-Methyl-2-phenylsuccinimide has a pK of 8·52 (determined by u.v. spectrophotometry) and is very lipophilic, which suggests that the compound will persist in blood and tissues. This was further illustrated by the small percentage excretion of the demethylated metabolite into 24 h rat urine (2·7% of an oral dose of methsuximide) although from the $^{14}CO_2$ excretion data about 29% would have been expected. From the results of a small clinical trial (Zimmerman, 1953) 2-methyl-2-phenylsuccinimide also appears to have a similar spectrum of toxic effects to methsuximide. Although a 56% reduction in the number of seizures per week was reported, a figure comparable to that of methsuximide, 20% of the patients receiving this demethylated compound exhibited toxic signs such as nausea, vomiting and drowsiness.

Recently, methsuximide has been implicated in the precipitation of acute intermittent porphyria in an epileptic patient (Birchfield & Cowger, 1966). By means of a chick embryo liver screening technique, 2-methyl-2-phenylsuccinimide has been shown to be approximately eight times less porphyrinogenic than methsuximide whilst hydrolyzed methsuximide possessed no such activity (Orton, unpublished observations).

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