J. Pharm. Pharmac., 1967, **19**, 1–9 Received August 18, 1966

The metabolism of ethionamide and its sulphoxide

J. P. JOHNSTON, P. O. KANE,* AND M. R. KIBBY†

The fate of orally administered ethionamide (2-ethyl-4-thiocarbamoylpyridine) and ethionamide sulphoxide in man, rats, mice and dogs has been examined. Though spontaneous interconversion between the two antituberculosis drugs does not take place, there was extensive interconversion *in vivo*, both appearing in the blood within 15 min of dosing irrespective of which compound was administered. Species differences were evident in both the rate of metabolism and in the ethionamide: ethionamide sulphoxide ratio. Apart from this interconversion, ten probable urinary metabolites were detected. These consisted of six fluorescent and four non-fluorescent metabolites, three of the latter being identified as 2-ethylisonicotinamide, 2-ethylisonicotinic acid and inorganic sulphate. A scheme is proposed to cover part of the common metabolic route of the two drugs.

ETHIONAMIDE (2-ethyl-4-thiocarbamoylpyridine or 2-ethylisothionicotinamide) is an active antituberculosis drug (Rist, Grumbach, Libermann, Moyeux, Cals & Clavel, 1958; Brouet, Marche, Rist, Chevallier & LeMeur, 1959). Recently the sulphoxide of ethionamide has been shown to behave similarly (Kreis, B., unpublished).

The structure of ethionamide shows similarities to other antituberculosis compounds, notably the thiosemicarbazones and isonicotinic hydrazide. Unlike these drugs the metabolism of thioamides has not been investigated although the behaviour of the $-CSNH_2$ group in thioureas and thiosemicarbazones is to some extent understood (Arita, 1956; Shibata, 1958; Scheline, Smith & Williams, 1961).

Thiorea and thiosemicarbazide (released from thiosemicarbazones) appear to be stable. Mono-*N*-substituted thioureas are highly toxic to rats (Dieke, Allen & Richter, 1947), probably due to release of hydrogen sulphide (Smith & Williams, 1961), whereas *NN'*-diarylthioureas are less so. On the basis of toxicity thioamides would be labile, being converted into the corresponding amide with the associated production of hydrogen sulphide.

Preliminary clinical trials of ethionamide indicated a very low urinary recovery of unchanged drug; blood concentrations, although not high, indicated that appreciable absorption of the compound had occurred (Hughes, Smith & Kane, 1962). Since then other workers have demonstrated extensive metabolism of ethionamide in man. Bieder & Mazeau (1962) have detected 2-ethylisonicotinamide and unchanged ethionamide in human urine by chromatography; they have also detected and obtained data on fluorescent metabolites. Kane (1962) demonstrated convincingly that a product found by polarography in the serum of patients receiving ethionamide was ethionamide sulphoxide; ethionamide was also detected. Bieder, Brunel & Mazeau (1963) have further shown the conversion of ethionamide sulphoxide into ethionamide in man. Yamamoto (1962) claimed to have identified a fluorescent product found by chromatography

From the Research Laboratories, May & Baker Ltd., Dagenham, Essex.

Present address: * Mond Division (Research Department), Imperial Chemical Industries Ltd., P.O. Box 8, Heath Laboratory, Runcorn, Cheshire. † Ministry of Defence, Porton Down, Nr. Salisbury, Wiltshire.

as 1-methyl-2-ethyl-4-thiocarbamoyl-6-pyridone, and has recently described the isolation and identification of the amide and acid from the urine of rabbits receiving ethionamide only. This is the first time that the biochemical conversion of $-CSNH_2$ into $-C\overset{O}{\overset{\uparrow}{S}}NH_2$ has been described, but the chemical conversion has been demonstrated (Walter & Curts, 1960).

We have attempted to extend and integrate existing knowledge of the metabolism of ethionamide and its sulphoxide in man and experimental animals.

Experimental

MATERIALS

Ethionamide was used as a finely milled yellow powder m.p. 165°. Ethionamide sulphoxide was supplied by Rhône-Poulenc as a bright yellow finely crystalline solid m.p. 141–142°. The remaining reference compounds were synthesized in the Research Laboratories of May & Baker Ltd. These were 2-ethyl-isonicotinamide–white prisms m.p. 131–132°; 2-ethyl-isonicotinic acid–white solid m.p. 237–239°; 2-ethyl-4-pyridoyl-glycine–white prisms m.p. 186–187°. (Found C, 57·5; H, 5·8; N, 13·7; $C_{10}H_{13}N_2O_3$ requires C, 57·7; H, 5·8; N, 13·5%); 2-ethyl-4-carboxy-6-pyridone–off-white solid m.p. 308° (decomp.); 2-ethyl-4-carboxamide-6-pyridone–white solid m.p. 303–304° (decomp.). (Found, C, 58·1; H, 6·55; N, 16·6; $C_8H_{10}N_2O_2$ requires C, 57·8; H, 6·05; N, 16·85%). The 1-methyl derivatives of these last two pyridones were obtained only partially purified.

DOSAGE AND SAMPLE COLLECTION

Ethionamide and ethionamide sulphoxide were given in 0·1N hydrochloric acid to rats, 150–200 g, and mice, 15–25 g, by stomach tube. Dogs received the compounds as aqueous suspensions. A few experiments using 10 mg/kg (the approximate dose in man) showed identical metabolic patterns to those obtained with 100 mg/kg which was used in most experiments. Blood samples were obtained by cardiac puncture from lightly ether-anaesthetized rats and mice. Dogs were bled by inserting a wide-bore hypodermic needle into the cephalic vein of the foreleg. Serum was obtained by collecting the blood in well-oiled syringes and transferring it to a centrifuge tube where it was allowed to clot. Plasma was obtained by collecting the blood into citrated syringes and subsequently spinning off the cellular fraction. With mice, three animals were required to provide sufficient material for one analysis; for other species one animal was used.

Urine was obtained from rats and mice housed in metabolism cages. The animals were allowed access to food for ½ hr each day; water was given *ad libitum*. Dogs were fed normally. Samples of human urine were also obtained from volunteers who took single doses of 0·5–1 g of ethionamide in the form of uncoated 250 mg tablets of Trescatyl.

CHROMATOGRAPHY

Urine was prepared for descending chromatography on Whatman No. 1 paper by concentration to a small volume under reduced pressure at 45°, extraction into ethyl acetate and concentration of the extract. Unless otherwise indicated, the running time was 16 hr. The Dragendorff spray reagent was prepared according to Vagùjfàlvi (1960).

ESTIMATION METHODS

Plasma and serum were analysed polarographically (Kane, 1959). In addition to the waves due to ethionamide and its sulphoxide (Kane, 1962), a third wave with $E_{\frac{1}{2}} = -1{\cdot}15$ V was recorded.

Urinary inorganic sulphate excretion was determined gravimetrically by precipitation of barium sulphate from urine.

Results

EXAMINATION OF BLOOD

The blood concentrations (Table 1) show that both ethionamide and its sulphoxide occur in the blood of mouse, rat and dog irrespective of which is administered. The concentrations of the two compounds parallel each other, except in the rat where detectable sulphoxide concentrations are transient. The peak ethionamide level occurs earliest in the mouse and latest in the dog. A third compound found in these animals forms the major component detectable in the later blood samples.

EXAMINATION IN URINES

Urines were collected and chromatographed after similar dosage with either compound. The metabolites found are listed in Table 2, which also contains data from man after the administration of ethionamide. Eleven different probable metabolites were found by chromatography, ten of which occur in more than one species. These included six unidentified fluorescent compounds. Three of the remainder (M_3, M_9 and M_{10}) have been further investigated. M_{10} has been identified as ethionamide from its Rf, ultraviolet quenching, colour reaction with Dragendorff reagent and polarographic half-wave potential after elution. The Rf values of M_9, using several solvent systems, showed no significant difference from those of 2-ethylisonicotinamide. Polarography of eluates of this spot confirmed this identification by revealing a wave with the characteristic half-wave potential of the amide. The polarographic records further show a second wave characteristic of ethionamide sulphoxide, demonstrating that the spot is a binary mixture of 2-ethylisonicotinamide and ethionamide sulphoxide. (In all the solvent systems so far tested it has proved impossible to separate the sulphoxide from this amide by chromatography). On the basis of its chromatographic behaviour (Table 3), ultraviolet and polarographic properties and colour reactions M_3 was indistinguishable from 2-ethylisonicotinic acid. A fraction obtained by passing the urine of human volunteers, who had taken ethionamide,

TABLE 1. MEAN SERUM OR PLASMA CONCENTRATIONS (μG/ML) OF ETHIONAMIDE, ETHIONAMIDE SULPHOXIDE AND THE THIRD METABOLITE IN THREE SPECIES FOLLOWING ORAL DOSAGE (100 MG/KG) WITH EITHER ETHIONAMIDE OR ITS SULPHOXIDE

Time (hr)		Mouse†						Rat*						Dog†					
		Ethionamide dosed			Sulphoxide dosed			Ethionamide dosed			Sulphoxide dosed			Ethionamide dosed			Sulphoxide dosed		
		S	E	T	S	E	T	S	E	T	S	E	T	S	E	T	S	E	T
0·25	Mean	19·0	41	15·7	26	23	20	2·4	18·4	5·6	7·5	19·1	16·9	5·8	16	3·7	7·9	6·8	8·1
	s.e.m. ±	2·7	6	2·2	3	5	2	1·3	3·2	0·5	4·0	7·3	3·9	0·3	7	2·5	3·0	4·4	3·5
0·5	Mean	25	34	22	25	31	28	0·0	35·0	7·5	0·0	4·9	6·5	9·7	31·6	4·6	10·5	7·7	13·5
	s.e.m. ±	6	8	2	5	5	5	0·0	5	0·0	0·0	0·7	1·4	0·8	12·2	2·7	0·6	2·7	4·4
1·0	Mean	20	29	24	14	19	43	0·0	20	10·7	0·0	13·3	13·2	13·2	40	12·9	7·5	9·1	13·2
	s.e.m. ±	5	9	3	6	4	14	0·0	1	0·6	0·0	1·5	0·6	—	—	—	—	—	—
2·3–3·0	Mean	6·2	5·5	16	0·0	1·0	4·4	0·0	8·1	11·8	0·0	5·3	8·3	13·0	40	23	3·5	4·9	24
	s.e.m. ±	1·1	0·9	3	0·0	0·8	1·3	0·0	1·2	0·7	—	—	—	0·5	4	3	—	—	—
5·0–6·0	Mean	0·6	1·5	2·4	0·0	0·0	0·0	0·0	3·5	8·7	0·0	0·8	5·6	9·4	19	28	0·0	1·0	32
	s.e.m. ±	0·6	1·5	0·8	0·0	0·0	0·0	0·0	0·5	0·7	0·0	0·8	0·4	3·1	6	10	—	—	—

* Plasma, † Serum; S = ethionamide sulphoxide; E = ethionamide, T = "third metabolite" (see text).
As the third metabolite is unidentified, its concentrations cannot exactly be given. Derivatives of ethionamide, however, show little variation in polarographic wave height with concentration; as a rough guide, concentrations were calculated assuming for historical reasons that the metabolite is 2-ethyl-4-carbamoyl-pyridine.

TABLE 2. COMPARATIVE TABLE OF URINARY "METABOLITE SPOTS"

Metabolites	Rf values				Fluorescence ultraviolet	Colour reaction with Dragendorff reagent
	Mouse	Rat	Dog	Man		
M_1	0·16*	0·16*	0·15*	—	blue	—
M_2	—	0·32*	—	—	yellow	—
M_3	0·35	0·35	0·34	0·32	none	+
M_4	0·38*	0·38	0·38*	—	blue	—
M_5	—	0·49*	0·49*	—	blue	—
M_6	0·60*	—	0·64*	0·58	blue	—
M_7	0·64*	0·65*	—	—	quench	—
M_8	0·80	0·80	—	0·82	blue	—
M_9	0·85	0·85	0·84	0·82	none	+
M_{10}	—	—	0·91*	0·92*	quench	+

* Indicates minor "metabolite" or one intermittent in appearance.
Dosage with either ethionamide or ethionamide sulphoxide. In chromatography the solvent system was n-butanol-0·2N ammonia (1:1 by volume); under these circumstances reference compounds have the following Rf values—2-ethylisonicotinic acid, 0·35; ethionamide sulphoxide, 0·82; 2-ethylisonicotinamide, 0·84; ethionamide, 0·89.

TABLE 3. CHROMATOGRAPHIC BEHAVIOUR OF 2-ETHYLISONICOTINIC ACID AND M_3

Solvent systems	Rf values	
	2-ethylisonicotinic acid	M_3
Butanol-ammonia (0·2N) (1:1)	0·32	0·34
Butanol-pyridine-water (140:30:30)	0·39	0·42
Benzene-acetic acid-water* (125:72:3)	0·85	0·85
Methyl ethyl ketone-acetic acid-water* (50:1:49)	0·64	0·64

* Running time only 4 hr.
M_3 is a urinary metabolite detected after the administration of ethionamide or ethionamide sulphoxide (see Table 2).

through an anion exchange column (Amberlite IRA-410, 40–60 mesh, OH form, 2·5 × 15 cm) and eluting with N acetic acid had the same chromatographic properties as M_3. The nature of this isolated fraction of M_3 was established from its infrared spectrum as being 2-ethylisonicotinic acid.

Rats were dosed orally (100 mg/kg) with either 2-ethylisonicotinamide or the acid, and their urine was collected and chromatographed. The amide yielded both amide and acid from the urine, whilst only the acid was detected after its administration. Since nicotinic and isonicotinic acids give rise *in vivo* to glycine conjugates (Cuthbertson, Ireland & Wolff, 1953; Komori & Sendju, 1926), a search for similar conjugates was made in the urine of all the species. In no case was evidence of a foreign glycine conjugate found using Altman's reagent (Gaffney, Schreier, DiFerrante & Altman, 1954) on chromatograms. The chance of this being a false negative result was reduced by finding that chromatograms of the synthetic glycine conjugate of 2-ethylisonicotinic acid gave a strong positive reaction with this reagent.

Though none of the fluorescent metabolites was identified, one, M_8, was isolated from human urine by chromatography and partially purified by ion-exchange methods. It formed pale yellow crystals (m.p. 132–142°). In saturated aqueous solutions of borax it had a polarographic half-wave potential of −1·25 V. An aqueous solution had fluorescence excitation and emission maxima of 355 and 410 mμ. The ultraviolet absorption

spectra varied with pH in a manner characteristic of a 6-pyridone. Four possible structures for this compound were eliminated on chromatographic grounds (Table 4).

TABLE 4. CHROMATOGRAPHIC BEHAVIOUR OF SYNTHETIC PYRIDONES AND OF M_8

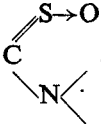
Compounds	Rf
2-Ethyl-4-carboxy-6-pyridone	0·08
2-Ethyl-4-carboxamide-6-pyridone	0·55
1-Methyl-2-ethyl-4-carboxy-6-pyridone	0·05
1-Methyl-2-ethyl-4-carboxamide-6-pyridone	0·36
M_8	0·82

Solvent system was butanol-ammonia 0·2N (1 : 1 by volume). Detection was by fluorescence under 365 mμ light.
M_8 is a urinary metabolite detected after the administration of ethionamide or ethionamide sulphoxide (see Table 2).

Attempts to repeat the synthesis of 1-methyl-2-ethyl-4-thiocarbamoyl-6-pyridone by the method of Yamamoto (1961) have consistently failed. In every instance, the thiocarbamoyl group was also oxidized and the resulting product was 1-methyl-2-ethyl-4-carbamoyl-6-pyridone. This compound showed blue fluorescence, but chromatography demonstrated that it was not one of the urinary metabolites.

Excretion of sulphur. The metabolic conversion of ethionamide and ethionamide sulphoxide to the corresponding amide would involve loss of the sulphur atom. On this reasoning the excretion of inorganic sulphate by rats before and after receiving either of the two antituberculosis drugs (100 mg/kg) was determined. An increase occurred during the first day after administration, equivalent to 80% of the dose. The increase in excretion was statistically indistinguishable whichever of the two drugs was given. There was no indication of increased sulphate excretion extending into the second day after administration.

Discussion

The interconversion between ethionamide and its sulphoxide found in the rat, mouse and dog is similar to that found in man (Bieder & Mazeau, 1962). These seem to be the only instances of such an interconversion between two therapeutically active foreign substances. Other sulphur-containing compounds, chlorpromazine for example, are known to be metabolized to sulphoxides *in vivo*, but this reaction is thought to be irreversible. These other sulphoxides are of the type $C-\overset{\overset{O}{\uparrow}}{S}-C$ whereas thioamide sulphoxides have the structure  . The latter compounds have only recently been discovered (Walter & Curts, 1960).

An analysis of the blood-level figures made at each time on each species shows there is no statistical difference between the two treatments ($P > 0·05$). In view of this, it is not surprising that the pattern of

urinary metabolites is independent of the antituberculosis compound given.

The blood levels also indicate interesting species differences in the metabolism of ethionamide and its sulphoxide, both in rate of metabolism and in the balance between ethionamide and ethionamide sulphoxide. For example, after 5 to 6 hr there are only traces of ethionamide derivatives remaining in the blood stream of the mouse but much greater amounts in both the rat and the dog; the rat demonstrates only transitory amounts of ethionamide sulphoxide, which appears only in the $\frac{1}{4}$ hr sample, whereas the ratio between the blood concentrations of the two drugs is nearer unity in the other two species.

Further examination of the figures in Table 1 shows that the polarographic wave due to the third component tends to increase to a maximum, the time of peak concentration occurring after the ethionamide peak. Although no identification of this wave has been made, it appears to be a metabolite which is formed at a later stage than the interconversion and the parent compounds. Its polarographic half-wave potential coincides with that of 2-ethylisonicotinamide, and this metabolite has been found by us in animal urine; its presence in human urine is reported by Bieder & Mazeau (1962) as well as in our results. However, chromatographic examination of ultrafiltrates of plasma in this laboratory indicates that the third wave is attributable to metabolites other than the simple amide (Law, 1963, unpublished).

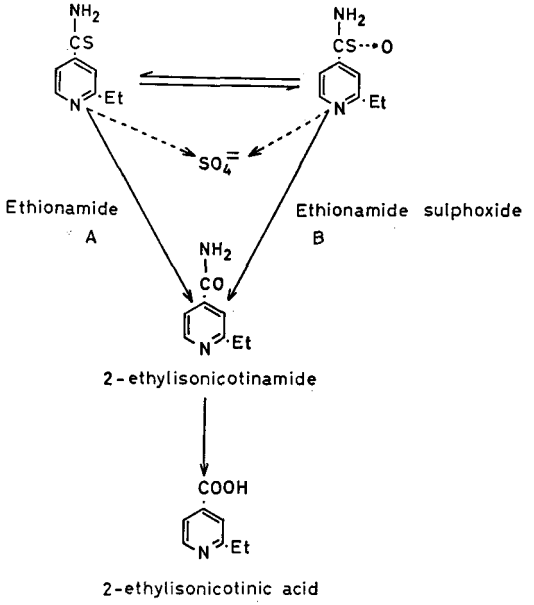
Urinary metabolites detected in the three animal species and man fall into two classes, fluorescent and non-fluorescent. Of the latter, five have been identified. Two of these are the two drugs and the others are 2-ethylisonicotinamide, 2-ethylisonicotinic acid and inorganic sulphate. There was no evidence of the corresponding glycine conjugate of the acid, an unusual contrast with nicotinic and isonicotinic acids (Cuthbertson & others, 1953; Komori & Sendju, 1926). The ready conversion of the amide into the acid, demonstrated by the feeding of 2-ethylisonicotinamide to rats, is however similar to known *in vivo* reactions of other pyridine acids and amides.*

The increased excretion of inorganic sulphate in the urine of dosed rats, provides supporting evidence for the formation of these sulphur-free metabolites.

Although none of the fluorescent metabolites has been identified, there is some evidence that these are pyridones. There is a precedent for this in that one of the metabolites of nicotinamide, 1-methyl-3-carbamoyl-6-pyridone, is fluorescent (Chang & Johnson, 1959). The pyridone claimed to have been found by Yamamoto, 1-methyl-2-ethyl-4-thiocarbamoyl-6-pyridone, could be the metabolite designated M_8 in our nomenclature on the basis of Rf values, but unfortunately we failed to confirm his synthetic route. Bieder & Mazeau describe similar fluorescent metabolites, without further identification other than fluorescence data.

* Since the completion of this work the results of a study of the metabolism of ethionamide in man have been published by Bieder, A. & Mazeau, L. (1964). *Therapie*, **19**, 897–907.

The five metabolites that we have identified are consistent with the following metabolic scheme:



There is nothing to indicate whether route A, route B or both routes are operative *in vivo*. The placing of the amide higher in the metabolic pathway than the acid is justified by the results from the experiment where both compounds were fed to rats. The acid was detected in urine in both instances, but amide was only found after dosing with amide. It is not clear at which point *N*-methylation may occur or the suggested pyridones be formed; by analogy with nicotinamide this would be at the amide stage. The reactions may, however, take place earlier or later in the metabolic pathway.

Acknowledgements. The authors thank Dr. R. Slack and Mr. D. L. Pain for providing synthesized reference compounds, Dr. D. F. Muggleton for advice and facilities over physical chemical methods and specifically Mr. T. L. Threlfall and Mr. B. J. Ward for infrared and fluorimetric examinations. They also gratefully acknowledge the technical assistance of Messrs. R. Buckler, D. I. Edwards and R. G. Mundy.

References

Arita, T. (1956). *J. pharm. Soc. Japan*, **76**, 987–990.
Bieder, A. & Mazeau, L. (1962). *Ann. pharm. fr.*, **20**, 211–216.
Bieder, A., Brunel, P. & Mazeau, L. (1963). *Ibid.*, **21**, 375–387.
Brouet, G., Marche, J., Rist, N., Chevallier, J. & LeMeur, G. (1959). *Am. Rev. Tuberc. pulm. Dis.*, **79**, 6–18.
Chang, M. L. W. & Johnson, B. C. (1959). *J. biol. Chem.*, **234**, 1817–1821.
Cuthbertson, F. W. J., Ireland, D. M. & Wolff, W. (1953). *Biochem. J.*, **55**, 669–671.
Dieke, S. H., Allen, G. S. & Richter, C. P. (1947). *J. Pharmac. exp. Ther.*, **90**, 260–270.

Gaffney, G. W., Schreier, K., DiFerrante, N. & Altman, K. I. (1954). *J. biol. Chem.*, **206**, 695–698.
Hughes, I. E., Smith, H. & Kane, P. O. (1962). *Lancet*, **1**, 616–617.
Kane, P. O. (1959). *Advances in Polarography*, **3**, pp. 1076–1086, London: Pergamon Press.
Kane, P. O. (1962). *Nature, Lond.*, **195**, 495–496.
Komori, Y. & Sendju, Y. (1926). *J. Biochem., Tokyo*, **6**, 163–169.
Rist, N., Grumbach, F., Libermann, D., Moyeux, M., Cals, S. & Clavel, S. (1958). *Rev. Tuberc.*, **22**, 278–283.
Scheline, R. R., Smith, R. L., & Williams, R. T. (1961). *J. mednl pharm. Chem.*, **4**, 109–135.
Shibata, I. (1958). *Jap. J. med. Prog.*, **45**, 455.
Smith, R. L. & Williams, R. T. (1961). *J. mednl pharm. Chem.*, **4**, 137–146.
Vagùjfàlvi, D. (1960). *Planta med.*, **8**, 34–43.
Walter, W. & Curts, J. (1960). *Chem. Ber.*, **93**, 1511–1517.
Yamamoto, M. (1962). *Jap. J. Chest Dis.*, **6**, 1036–1041.