Metabolism of β-Mercaptoethylamine

2. IN THE DOG

W. G. VERLY* AND G. KOCH
Department of General Pathology, University of Liege, Belgium

(*Received* 29 *June* 1954)

β-Mercaptoethylamine forms a part of coenzyme A but is not known to occur naturally in the free state, for instance as a hydrolysis product from coenzyme A. Since β-mercaptoethylamine affords good protection against irradiation by X-rays (Bacq *et al.* 1953), it seemed worthwhile to look for sulphur compounds which can be derived metabolically from β-mercaptoethylamine.

The persistance of ^{35}S in the body of the mouse after intraperitoneal injection of 3 mg. of [^{35}S]β-mercaptoethylamine has been studied (Verly, Bacq, Rayet & Urbain, 1954), and it has been shown that 1 hr. or more after the injection, only part of the ^{35}S still present in the organism is in β-mercaptoethylamine (cysteamine)–β:β′-diaminodiethyldisulphide (cystamine) molecules (Verly, Gregoire, Rayet & Urbain, 1954). It was concluded that the injected cysteamine was partly excreted unchanged in the urine and partly metabolized in the tissues, the metabolites being eventually excreted in the urine.

In this work, some labelled molecules that appear in the urine after injection of [^{35}S]β-mercaptoethylamine to a dog, have been identified and their amount measured.

EXPERIMENTAL

The synthesis of the [^{35}S]β-mercaptoethylamine hydrochloride has been previously described. The solution injected into the dog contained 85–88% of cysteamine and 12–15% of cystamine (Verly, Gregoire, Rayet & Urbain, 1954).

The total sulphur and the total sulphates were determined by the methods of Fiske (1921).

Isolation of taurine and of cysteamine–cystamine

To 25 ml. of the diluted urine sample, accurately weighed amounts (about 200 mg.) of taurine and cystamine dihydrochloride, about 40 mg. of Na_2SO_4 and 30 ml. of conc. HCl were added. The solution was allowed to boil 1 hr. After cooling, an excess of I_2 was added, followed by $BaCl_2$ to precipitate the sulphates. The solution was filtered and evaporated under reduced pressure.

The residue was taken up in water, the solution filtered and passed through a column of 25 ml. of Amberlite IR-120(H); the resin was washed with 200 ml. of water; the filtrate and washings (Fraction *A*) contained the taurine (Carsten, 1952). The substances adsorbed on the sulphonated resin were removed with 500 ml. of 3N-HCl; this eluate (Fraction *B*) contained the cystamine; it was worked up as previously described (Verly, Gregoire, Rayet & Urbain, 1954).

Fraction *A* was evaporated to dryness under reduced pressure. The residue was taken up in 2–3 ml. of water and the solution filtered; crystals appeared on addition of 25 ml. of ethanol to the filtrate. After standing 2 hr. in the ice box, the mother liquors were removed and the crystals washed with ethanol.

The taurine was twice recrystallized from a water–ethanol mixture (except samples 1*a*, 2*a* and 2*b* which were recrystallized three times); the crystals were dried at 78° *in vacuo*. The nitrogen content of the samples was determined by Kjeldahl's method; the results are recorded in Table 1. The last recrystallization did not alter the specific activity of the taurine samples.

The isolated cystamine dihydrochloride samples were twice recrystallized from an ethanol–water–conc. HCl (12:1:1, by vol.) and ether mixture. The m.p.'s of these samples are recorded in Table 1. The last crystallization did not change the specific activity of the cystamine dihydrochloride.

Determinations of radioactivity

A portion of the [^{35}S]cysteamine hydrochloride solution was oxidized with Benedict's reagent (Fiske, 1921). The crystalline taurine and cystamine dihydrochloride samples were oxidized with HNO_3 in sealed tubes (Carius) (Niederl, Baum, McCoy & Kuck, 1940). In every instance, benzidine sulphate precipitates were prepared. Radioactivity measurements were performed on these precipitates using the Tracerlab windowless flow counter in the Geiger region. Results were corrected for background, self absorption and radioactive decay; they are expressed in counts/min./total sample.

RESULTS

A 7 kg. male dog, anaesthetized with α-chloralose was injected, into the femoral vein with 153 mg. [^{35}S]β-mercaptoethylamine hydrochloride (104 mg. of the base) containing a radioactivity of $10{\cdot}75 \times 10^7$ counts/min. The ureters were catheterized and urine collected for 8 hr.; the receiver was changed every 2 hr.

Each 2 hr. specimen was filtered, then diluted to 100 ml. from which samples were used for determinations of total ^{35}S, free and esterified

* Associé du Fonds National Belge de la Recherche Scientifique.

Table 1. *Properties of the isolated taurine and cystamine dihydrochloride*

For details see text.

Sample		0–2 hr. (1)	2–4 hr. (2)	4–6 hr. (3)	6–8 hr. (4)
Taurine N content (%)	*a*	11·25	11·1	11·1	11·1
(theoretical value (11·19%)	*b*	—	11·1	10·9	11·0
Cystamine, 2HCl,	*a*	217–218·5	—	219–220	—
m.p.'s ° (corr.)	*b*	218–220	219–220	—	218–220

Table 2. *Radioactivities of materials isolated from dog urine after injection of* [^{35}S]*cysteamine*

Sample	Total radioactivity (counts/min. × 10^{-5}) 0–2 hr.	2–4 hr.	4–6 hr.	6–8 hr.
Total ^{35}S	72·0	42·8	33·6	18·8
Total sulphates	18·5	20·9	17·7	11·7
Taurine	0·6	2·2	2·7	2·2
Cystamine, 2HCl	33·0	7·1	4·2	1·5

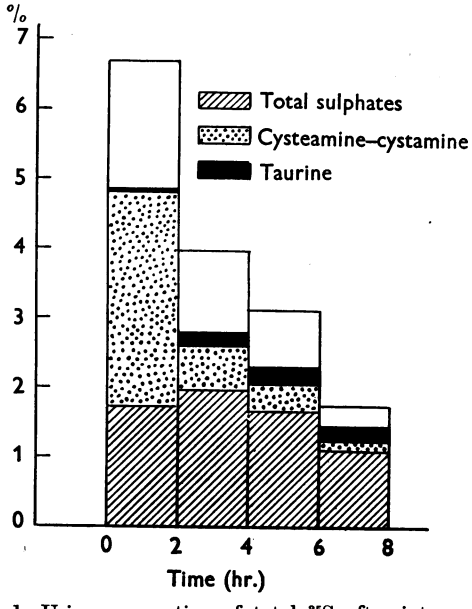

Fig. 1. Urinary excretion of total ^{35}S after intravenous injection of 104 mg. labelled cysteamine into a 7 kg. dog; radioactivity distribution between sulphates, taurine, cysteamine–cystamine and unidentified metabolites as percentages of the injected dose.

[^{35}S]sulphates, [^{35}S]taurine and [^{35}S]cysteamine–cystamine. Nearly all determinations were done in duplicate; the mean results are recorded in Table 2; Fig. 1 gives these results expressed as percentages of the injected dose. Similar results were obtained with another dog.

DISCUSSION

To calculate the activities of urine taurine and cysteamine–cystamine, the amounts of inactive taurine and cystamine added as carriers were assumed to be large compared with the amounts of labelled taurine and cysteamine–cystamine present in the very small volume (fourth of a 2-hour specimen) of urine used for analysis.

In 8 hr., 16% of the injected ^{35}S were excreted in the urine, 4% as cysteamine–cystamine. Since radioactive cysteamine–cystamine was mainly excreted during the first 2 hr. and practically none was present in the last urine sample collected, it may be concluded that from the 104 mg. of cysteamine injected to this 7 kg. dog, only 4–5% were excreted unchanged in the urine.

Fischer & Goutier Pirotte (1954) injected 150 and 190 mg. of cysteamine in the marginal vein of the ears of two rabbits and detected polarographically in the urine substances that discharged at the same potentials as cysteamine and cystamine. Expressed in cysteamine equivalents the amount of these substances excreted in the urine represented 30–35% of the injected dose.

An important fraction of the urinary ^{35}S is present as sulphate; there is also some excretion of labelled taurine. Eldjarn (1954) also reported the formation of taurine and sulphate from cystamine in the rat, rabbit and man.

It is clear from Table 2 and Fig. 1 that cysteamine–cystamine, taurine and sulphates do not account for the total ^{35}S excreted in the urine; there are thus other labelled metabolites still unidentified.

SUMMARY

1. A 7 kg. male dog was injected intravenously with 104 mg. of [^{35}S]cysteamine.

2. After 8 hr., 16% of the ^{35}S had been excreted in the urine. The greater part of it was present as sulphate, cysteamine–cystamine and taurine.

The expenses of this work have been defrayed by grants from the Institut Interuniversitaire des Sciences Nucléaires and the Conseil Supérieur de la Sécurité Civile.

The authors wish to thank Dr P. Fischer for injecting the radioactive compound into the dog and collecting the urine, and G. Espreux and A. Tremsky for their careful assistance.

REFERENCES

Bacq, Z. M., Dechamps, G., Fischer, P., Herve, A., LeBihan, H., Lecomte, J., Pirotte, M. & Rayet, P. (1953). *Science*, **117**, 633.

Carsten, M. (1952). *J. Amer. chem. Soc.* **74**, 5954.

Eldjarn, L. (1954). *J. biol. Chem.* **206**, 483.

Fischer P. & Goutier Pirotte, M. (1954). *Arch. int. Physiol.* **62**, 76.

Fiske, C. H. (1921). *J. biol. Chem.* **47**, 59.

Niederl, J. B., Baum, H., McCoy, J. S. & Kuck, J. A. (1940). *Industr. Engng Chem. (Anal.)*, **12**, 428.

Verly, W. G., Bacq, Z. M., Rayet, P. & Urbain, M. F. (1954). *Biochim. biophys. Acta*, **13**, 233.

Verly, W. G., Gregoire, S., Rayet, P. & Urbain, M. F. (1954). *Biochem. J.* **58**, 660.

Sulphate Formation from Dimethylthetin in Rat Liver

By G. A. MAW

Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E. 1

(*Received* 9 *July* 1954)

Dimethylthetin chloride, i.e.

$$\{(CH_3)_2.S^+.CH_2.CO_2H\}\ Cl^-,$$

has been established as a biological methyl donor on the basis of its ability to enhance the growth rates of rats fed on a methyl-free diet supplemented with homocystine (du Vigneaud, Moyer & Chandler, 1948; Maw & du Vigneaud, 1948) and its ability to promote methionine synthesis from homocysteine in the presence of liver preparations (Dubnoff & Borsook, 1948). Transfer of one methyl group from the thetin to homocysteine or homocystine takes place and the compound is believed to be converted into methylthioacetic acid. The methyl group of methylthioacetic acid, however, does not appear to be labile, since the acid does not have the growth-promoting properties of a methyl donor, and since the administration of trideuteriomethylthioacetic acid to rats does not result in the appearance of deuteriomethyl-labelled choline or creatine in the tissues (Maw & du Vigneaud, 1948). Furthermore, the acid is unable to methylate homocysteine *in vitro* (Dubnoff & Borsook, 1948).

In the intact rat the sulphur of dimethylthetin chloride, administered orally or by injection, has been shown to be converted to a considerable extent into urinary inorganic sulphate (Maw, 1953). This oxidation is considered to be connected with the methyl group lability, since ethylmethylthetin chloride and dimethyl-β-propiothetin chloride, i.e.

$$\{(CH_3)_2.S^+.CH_2.CH_2.CO_2H\}\ Cl^-,$$

which are also methyl donors, are likewise oxidized to sulphate, whereas diethylthetin chloride and trimethylsulphonium chloride, i.e. $\{(CH_3)_3.S^+\}\ Cl^-$, which are not methyl donors, are not so oxidized.

The present communication describes a series of *in vitro* experiments in which the formation of inorganic sulphate by rat-tissue preparations incubated with dimethylthetin chloride and some related compounds has been followed. It has been found that in contrast to its behaviour in the whole animal, dimethylthetin chloride alone is not oxidized to sulphate by liver or kidney slices, but that a mixture of the thetin and homocysteine gives rise to a higher output of sulphate in liver-slice preparations than does homocysteine alone. This implies that the oxidation of the thetin is dependent on its preliminary demethylation, as suggested by earlier animal experiments (Maw, 1953). Methylthioacetic acid, the most likely demethylation product, is readily oxidized by liver slices and to a lesser extent by kidney slices. Conversion of this compound into sulphate in the presence of fortified liver suspensions and acetone-dried liver powders has not so far been obtained. Its oxidation by liver slices has been compared with that of a number of related compounds, and also with that of cysteine, *S*-methylcysteine and methionine.

MATERIALS AND METHODS

Preparation of compounds

Dimethylthetin chloride and the sodium salts of mercaptoacetic (thioglycollic), methylthioacetic (*S*-methylthioglycollic) and ethylthioacetic (*S*-ethylthioglycollic) acids were prepared as described in a previous paper (Maw, 1953). Dithiodiglycollic acid, i.e. $(—S.CH_2.CO_2H)_2$, was prepared by the method of Westerman & Rose (1927) and converted into the neutral sodium salt. Sodium *S*-phenylthioacetate (*S*-phenylthioglycollate) was made from thiophenol and ethyl chloroacetate by a method similar to that used for the two alkylthioacetates. *S*-Methyl-L-cysteine was prepared by the reduction and subsequent methylation of L-cystine in liquid ammonia (du Vigneaud, Loring & Craft, 1934). L-Cysteine hydrochloride, DL-homocysteine, L-methionine and adenosine 5′-phosphate (AMP) were obtained from Roche Products Ltd., diphosphopyridine nucleotide (DPN) and cytochrome *c* from L. Light and Co. Ltd.