- Kermack, W. O. & Matheson, N. A. (1957). *Biochem. J.* **65**, 48.
- Knox, W. E. (1960). In *The Enzymes*, vol. 2, p. 253. Ed. by Boyer, P. D., Lardy, H. & Myrback, K. New York: Academic Press Inc.
- Moulds, L. de V. & Riley, H. L. (1938). *J. chem. Soc.* p. 621.
- Petrovicki, H. (1939). *Biochem. Z.* **303**, 186.
- Platt, M. E. & Schroeder, E. F. (1934). *J. biol. Chem.* **104**, 281.
- Racker, E. (1951). *J. biol. Chem.* **190**, 685.
- Schubert, M. P. (1935). *J. biol. Chem.* **111**, 671.
- Segal, H. L. (1959). In *The Enzymes*, vol. 1, p. 1. Ed. by Boyer, P. D., Lardy, H. & Myrback, K. New York: Academic Press Inc.
- Segal, H. L., Kachman, J. F. & Boyer, P. D. (1952). *Enzymologia*, **15**, 187.
- Strittmatter, P. & Ball, E. G. (1955). *J. biol. Chem.* **213**, 445.
- Waley, S. G. (1957). *Biochem. J.* **67**, 172.
- Waley, S. G. (1958). *Biochem. J.* **68**, 189.
- Wieland, T., Pfeleiderer, G. & Lau, H. H. (1956). *Biochem. Z.* **327**, 393.
- Yamazoe, S. (1936). *J. Biochem., Tokyo*, **23**, 319.

Biochem. J. (1961) **79**, 482

Studies in Detoxication

86. THE METABOLISM OF ¹⁴C-LABELLED ETHYLENE GLYCOL*

By P. K. GESSNER, D. V. PARKE AND R. T. WILLIAMS

Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2

(Received 18 October 1960)

Ethylene glycol (ethane-1:2-diol) and its derivatives are regarded as relatively toxic substances to human beings, and the toxicology has been summarized by Browning (1953). This compound appears to be more toxic to man than to laboratory animals, for while oral LD₅₀ doses vary from about 2 ml./kg. for cats to 13 ml./kg. for mice (Laug, Calvery, Morris & Woodard, 1939; Lehmann & Flury, 1943; Oettingen, 1943; Spector, 1956*a*), the minimum lethal dose for man is about 1.6 g./kg. Fatal poisoning from ethylene glycol in man is usually the result of the accidental drinking of anti-freeze fluid as a substitute for alcoholic beverages (e.g. Pons & Custer, 1946; Harger & Forney, 1959). The cause of death from ethylene glycol has been attributed to lesions of the central nervous system (Pons & Custer, 1946); renal damage due to deposition of calcium oxalate crystals in the renal tubules is usually not severe enough to cause death, although metabolic conversion into oxalate has often been postulated as the reason for the toxic effects. Most studies on the fate of the glycol in animals have been concerned with oxalate formation and excretion (see Oettingen 1943).

The only well established metabolite of ethylene glycol in man and animals is oxalic acid, which, however, accounts for less than 2% of the dose (see Oettingen, 1943). Glycolaldehyde, glyoxal, glycollic acid and glyoxylic acid are possible metabolites. Glycollic acid has been claimed to be a

metabolite in rabbits (Mayer, 1903). Glucuronic acid conjugation of ethylene glycol has not been found (Fellows, Luduena & Hanzlik, 1947; Gessner, Parke & Williams, 1960). Unchanged ethylene glycol, however, is excreted by dogs (Nakazawa, 1950) and humans (Hjelt, Tamminen, Fortelius, Raekallio & Alha, 1958; Harger & Forney, 1959).

This paper shows that the major end product of ethylene glycol metabolism is respiratory carbon dioxide. The main metabolite excreted in urine is unchanged ethylene glycol; oxalic acid is a minor metabolite, the amounts of which depend upon the dose and the species of animal. Intermediates in the formation of carbon dioxide are glycolaldehyde and glyoxylic acid, and these were detected in liver-slice experiments.

MATERIALS AND METHODS

[¹⁴C₂]Ethane-1:2-diol. [¹⁴C₂]Ethylene (The Radiochemical Centre, Amersham, Bucks) was converted into ethanediol according to Milas & Sussman (1937). The [¹⁴C₂]ethylene gas (26 mg., 0.5 μc) was contained in an ampoule which was broken (with a piece of iron and a magnet) in a closed flask containing 1 ml. of a 5.8% (w/v) solution of hydrogen peroxide and 0.07 ml. of a 0.5% (w/v) solution of osmium tetroxide, both in anhydrous *tert*-butyl alcohol. The flask was connected with an arrangement for adding, in the absence of air, non-radioactive ethylene. The reaction mixture was kept at room temperature for 72 hr. and then brought into contact with an excess of non-radioactive ethylene and kept until the appearance of black colloidal osmium oxide indicated total decomposition of the

* Part 85: Parke (1961).

hydrogen peroxide (48 hr.). Shaking of the reaction vessel was avoided since it tended to reduce the yield. Carrier ethanediol (1 g.) was added to the reaction mixture, which was then distilled in a Craig microdistillation apparatus, equivalent to eight theoretical plates at a reflux ratio of 1:5. The yield of ethanediol, b.p. $197^\circ/760$ mm., was 0.97 g. and the recovery of ^{14}C was 43% ($223\mu\text{C/g.}$). The purity of the radioactive glycol was determined by isotope dilution with pure ethanediol (b.p. 198° ; n_D^{20} , 1.4310) and counted as the bisphenylurethane, m.p. and mixed m.p. 158° (see below). The purity was $98 \pm 2\%$.

Animals and dosing. Chinchilla rabbits, albino rats, guinea pigs and cats were used. The ethanediol dissolved in water was administered either by mouth or by subcutaneous injection.

Determination of radioactivity. The radioactivity of the urine, faeces, tissues and expired air were determined as described by Parke (1956).

Determination of metabolites

Ethane 1:2-diol. Carrier ethanediol (400 mg.) was added to the radioactive urine (10 ml.), which was then continuously extracted with ether for 12 hr. The extract was dried with anhydrous Na_2SO_4 and evaporated. The residue was treated with phenyl isocyanate (0.8 g.) and the mixture heated under reflux on a water bath for 0.5 hr. Excess of phenyl isocyanate was allowed to evaporate on the water bath. The resulting crude bisphenylurethane of ethanediol was extracted with light petroleum (b.p. $100\text{--}120^\circ$) to free it from any carbanilide, and then recrystallized to constant activity from ethanol. The bisphenylurethane had m.p. and mixed m.p. 158° .

Oxalic acid. This was determined according to Parke & Williams (1953).

Hippuric acid. To the urine (10 ml.) was added hippuric acid (500 mg.) in a little 2N-NaOH. The urine was acidified with 2N- H_2SO_4 or conc. HCl and the precipitated hippuric acid collected by centrifuging or by filtration. Dissolution in alkali and precipitation with acid were repeated twice. The hippuric acid (m.p. 187°) was recrystallized from water to constant activity and then converted into its *p*-bromophenacyl ester (m.p. and mixed m.p. 151°), which was recrystallized from ethanol.

Glycolaldehyde and glyoxal. Both these aldehydes give the same osazone. Glyoxal (260 mg. of a 30% solution) was added to the urine (10 ml.). This was immediately followed with one drop of saturated aq. Na_2SO_3 to prevent resin formation from the aldehyde. The urine was then treated with 0.5 ml. of a solution of phenylhydrazine (1 ml. of phenylhydrazine in 1 ml. of acetic acid diluted with 5 ml. of water) and the phenylosazone separated immediately. The osazone was recrystallized from ethanol until the activity disappeared (m.p. and mixed m.p. 179°).

Glycollic acid. The acid (600 mg.) was added to urine (10 ml.), followed by 2N- CuSO_4 (5 ml.), and the solution adjusted to pH 7 with 2N- NH_3 solution. After the solution had been kept at 0° for 6 hr., the copper glycollate was collected and suspended in water, and the copper removed with H_2S . The solution was then evaporated to dryness and the residue (110 mg.) was heated with aniline (0.5 g.) at 130° . The product, glycollic acid anilide (m.p. and mixed m.p. 97°), was recrystallized from water until the activity disappeared.

Glyoxylic acid. The acid (200 mg.) was added to urine

(5 ml.) and the mixture treated with a solution of 2:4-dinitrophenylhydrazine (1 g. of 2:4-dinitrophenylhydrazine in 3 ml. of conc. H_2SO_4 made up to 30 ml. with aq. 80% ethanol) until no further precipitation occurred. The glyoxylic acid 2:4-dinitrophenylhydrazone was recrystallized from aq. ethanol until the activity disappeared (m.p. and mixed m.p. 190° ; cf. Ratner, Nocito & Green, 1944).

Urea. Urea (1 g.) was dissolved in urine (about 20 ml.) and conc. HNO_3 (5 ml.) was added. The urea nitrate was filtered off and then dissolved in water (20 ml.). The solution was treated with charcoal and filtered, and 5 ml. of conc. HNO_3 was added to precipitate urea nitrate. This purification was repeated. The salt was then dissolved in 5 ml. of water, and the solution was neutralized with Na_2CO_3 and evaporated to dryness. The urea was extracted with ethanol and then the extract was evaporated. The residue of urea was treated with two equivalents of a 7% (w/v) solution of xanthidol in acetic acid and the mixture refluxed for 0.5 hr. The bisxanthyl derivative of urea (m.p. and mixed m.p. 274°) was recrystallized from aqueous dioxan to constant activity.

Isotope-dilution analyses were also carried out for uric acid, carbonate, pyruvic acid, acetaldehyde, acetic acid and acetone in urine, but none of these compounds were found as metabolites of ethanediol.

Tissue-slice experiments. Rat-liver slices (2 g.) were prepared as described by Umbreit, Burris & Stauffer (1949) and Elliott (1955). The slices were incubated at 37° for 2 hr. in 25 ml. of buffered Krebs-Ringer phosphate solution, pH 7.4 (see Umbreit *et al.* 1949), which had been made 0.01M with respect to the substrates. Incubations were carried out with [$^{14}\text{C}_2$]ethanediol alone, with [$^{14}\text{C}_2$]ethanediol + glycolaldehyde and with [$^{14}\text{C}_2$]ethanediol + glyoxylic acid. At the end of 2 hr. the Ringer solution was filtered from the slices. The CO_2 in the solution was precipitated and counted as BaCO_3 . Glycolaldehyde was estimated by adding 0.2 g. of carrier aldehyde and isolating it as the phenylosazone or the 2:4-dinitrophenylosazone (m.p. and mixed m.p. 310° , from acetone); since glyoxal forms the same osazones this procedure included any glyoxal present. Glyoxylic acid was estimated by adding 70–80 mg. of carrier, which was isolated as the 2:4-dinitrophenylhydrazone.

RESULTS

Oxidation to carbon dioxide. Table 1 shows the distribution of ^{14}C in the expired air and urine of rabbits receiving [$^{14}\text{C}_2$]ethanediol by injection or by mouth. In doses of 0.1–2.0 g./kg. some 20–30% of the radioactivity is excreted in the urine. With the smaller doses of 0.124 g./kg., a large proportion of the ^{14}C is eliminated in the expired air as carbon dioxide and in one animal the amount was 60% of the dose in 3 days (see Fig. 1). Table 1 suggests that with the smaller doses of ethanediol about a quarter is eliminated, mainly unchanged, in the urine, and the main bulk of the rest is oxidized to carbon dioxide. This may be true also for doses up to 1–2 g./kg. Above 2 g./kg. ethanediol is toxic and large amounts are excreted in the urine, presumably unchanged. Table 2 gives similar observations on

rats; it suggests that as the dose rises to about 1 g./kg. a greater proportion of the dose is eliminated in the urine and a smaller proportion is oxidized to carbon dioxide.

Metabolites in urine. Table 3 shows the results of isotope-dilution experiments on the urine of rabbits receiving small doses of [$^{14}\text{C}_2$]ethanediol. The main excretory product is the unchanged diol, which accounts for most of the radioactivity of the urine. Gessner *et al.* (1960) showed that ethanediol does not form a conjugated glucuronic acid. The only other labelled compound found in the urine in appreciable amounts (about 1% of the dose) was urea. Traces (0.1% or less) of oxalic acid were found, but not glycolaldehyde, glyoxal, glycollic acid or glyoxylic acid. No evidence was obtained for the excretion of any other radioactive metabolite.

Formation of oxalic acid. Table 3 shows that with small doses of ethanediol very little is converted into and excreted as oxalate by the rabbit. The formation of oxalate was therefore investigated by the isotope-dilution technique with larger doses and with other species of animals. Table 4 shows that there are species differences in the amount in urine of oxalic acid formed from ethanediol. Cats form much more oxalic acid than do the other species examined and do not survive doses of 1 g. of ethanediol/kg. In rabbits and guinea pigs the oxalate excretion hardly reached 0.5% of the dose at any level tried. Rats seem to be intermediate between cats and the other two species. Doses of 1 g. of ethanediol/kg. were not fatal to rats, rabbits and guinea pigs.

Fig. 1. Excretion of the total radioactivity in the urine (○) and $^{14}\text{CO}_2$ in expired air (●) by a rabbit which had received 0.124 g. of [$^{14}\text{C}_2$]ethanediol/kg. orally.

Table 1. Elimination of ^{14}C by rabbits receiving [$^{14}\text{C}_2$]ethanediol

Dose (g./kg.)	Time after dosing (days)	% of dose of ^{14}C in	
		Urine	Expired air as CO_2
0.10*	2	21	—
0.124	1	21	42
0.124†	3	23	60
0.25*	2	22	—
0.25‡	11	17	—
0.25	11	21	—
0.50*	2	25	—
1.00*	2	26	—
1.50*	2	28	—
2.00	2	26	—
2.50*§	2	46	—
5.00	1	56	—

* These doses were given by subcutaneous injection; the others were oral.

† The animal receiving this dose eliminated 1% of the dose in the faeces and its tissues contained 11%. The total amount accounted for was thus 95% of the dose.

‡ The animal receiving this dose eliminated 1% of the ^{14}C in the faeces.

§ This dose produced symptoms of poisoning.

|| The animal receiving this dose died 36 hr. later.

Table 2. Fate of the ^{14}C in rats receiving [$^{14}\text{C}_2$]ethanediol by subcutaneous injection

The dose of ^{14}C was 1–4 μC . The actual amount of ethanediol oxidized to respiratory CO_2 is given in parentheses.

Dose of glycol (g./kg.)	Wt. of rat (g.)	% of dose of ^{14}C after 24 hr. in		
		Expired air as CO_2	Urine	Body tissues
0.06	115	—	21	18
0.10	160	—	21	13
0.10	300	23 (7 mg.)	35	—
0.60	250	19 (29 mg.)	55	—
1.00	230	14 (39 mg.)	58	—
5.00	320	6* (96 mg.)	32*	—
7.5	250	3.8* (71 mg.)	—†	—
10.0	225	2.4* (54 mg.)	—†	—

* In 12 hr. after dosing; the doses in these cases were fatal.

† No urine obtained.

Table 3. *Metabolites of ethanediol in rabbit urine*

[14C₂]Ethanediol was given orally.

Expt. no.	1	2	3
Wt. of rabbit (kg.)	4.5	3.5	2.9
Dose of ethanediol (mg./kg.)	25	25	125
Dose of 14C (μc)	17.1	14.1	80
Metabolites sought	Percentage of dose in 24 hr.		
Ethanediol	15.1	6.0	10.3
Oxalic acid	0.08	0.11	0.01
Urea	1.4	1.5	0.65
Hippuric acid	0	0	0
Total metabolites	16.6	7.6	11.0
14C excreted in 24 hr.	18.2	9.5	18.0
(14C excreted in 48 hr.	19.4	10.0	22.2)

Table 4. *Urinary excretion of labelled oxalic acid by animals dosed with [14C₂]ethanediol*

The figures are the percentages of the dose of 14C excreted in the urine in 48 hr.

Dose (g./kg.)	Cat		Rat		Rabbit		Guinea pig	
	Total 14C in urine	14C in oxalate	Total 14C in urine	14C in oxalate	Total 14C in urine	14C in oxalate	Total 14C in urine	14C in oxalate
0.10	78	0.72	21	0	21	0.07	26	0
0.25	59	3.65	41	0.97	22	0.05	24	0.06
0.50	43	2.00	45	0.57	25	0.15	27	0.01
1.00*	44†	0.87†	49	1.08	26	0.27	37	0.69
1.50	—	—	—	—	28	0.25	—	—
2.50†	—	—	53	0.98	46	0.45	—	—
5.00†	—	—	60†	0.48†	56†	0.37†	—	—
7.5†	—	—	79†	0.83†	—	—	—	—

* This dose is fatal to cats within 48 hr. † 24 hr. urine. ‡ These doses were fatal in 24–48 hr.

Table 5. *Conversion of [14C₂]ethanediol into labelled hippuric acid in the rat*

The rats were injected subcutaneously with [14C₂]ethanediol (2 m-moles/kg.) and sodium benzoate (2 m-moles/kg.) simultaneously and the urine, collected for 24 hr., examined for hippuric acid by isotopic dilution.

Expt. no.	1	2	3
Wt. of rat (g.)	150	150	170
Dose of ethanediol (mg.)	15.3	15.7	22.0
Dose of 14C (μc)	2.5	2.6	4.92
Dose of sodium benzoate (mg.)	43	43	50
Radioactivity of hippuric acid excreted in 24 hr. (% of dose)	9.6	8.4	7.0

Conversion of ethanediol into glycine. Weinhouse & Friedmann (1951) have shown that glyoxylic acid is converted into glycine, oxalate and carbon dioxide in the rat. Glycollic acid is also converted into glycine and carbon dioxide, but it is not readily converted into oxalate. If glyoxalate and glycollate are intermediates in the metabolism of ethanediol, then the simultaneous administration of benzoic acid with [14C₂]ethanediol to rats should yield radioactive hippuric acid. Table 5 shows the results of three such experiments in which about 8% of the radioactivity of the administered ethanediol was incorporated into glycine.

Formation of glycolaldehyde and glyoxylic acid from ethanediol. Tables 1 and 2 suggest that a

major portion of ethanediol is oxidized to carbon dioxide in the rabbit and rat. It is known that oxalic acid is not oxidized to carbon dioxide appreciably by animal tissues (Weinhouse & Friedmann, 1951; Curtin & King, 1955; Brubacher, Just, Bodur & Bernard, 1956). It is therefore unlikely that ethanediol is converted into carbon dioxide via oxalic acid. It is more probable that it is oxidized via intermediates such as glycolaldehyde, glycollic acid and glyoxylic acid. However, none of these intermediates were found in the urine. [14C₂]Ethanediol was incubated with rat-liver slices alone and in the presence of glycolaldehyde and glyoxylic acid. The results given in Table 6 show that, when [14C₂]ethanediol is

Table 6. *Conversion of ethanediol into carbon dioxide, glycolaldehyde and glyoxylic acid in rat-liver slices*

[$^{14}\text{C}_2$]Ethanediol (0.01 M) was incubated with 3 g. (fresh wt.) of rat-liver slices suspended in 25 ml. of Krebs-Ringer phosphate soln. for 2 hr. at 37° in an atmosphere of O_2 . Glycolaldehyde and glyoxylic acid were added in final concn. of 0.01 M as trapping agents, and isotope dilutions for these substances were determined by procedures described in the text. The values given are in microatoms of labelled substrate carbon incorporated into the metabolite/g. of dry tissue/hr. (see Nakada, Friedmann & Weinhouse, 1955).

Substrates				Products								
				Carbon dioxide			Glycolaldehyde*			Glyoxylic acid		
Expt.	...	...	...	1	2	3	1	2	3	1	2	3
[$^{14}\text{C}_2$]Ethanediol alone				6.4	8.7	7.3	7.0	10.8	9.4	0.3	0.4	0.1
[$^{14}\text{C}_2$]Ethanediol + glycolaldehyde				1.1	1.6	1.2	3.7	2.6	4.4	—	—	—
[$^{14}\text{C}_2$]Ethanediol + glyoxylic acid				1.0	1.4	1.0	—	—	—	2.0	0.9	0.6

* Includes glyoxal.

incubated with liver slices, labelled carbon dioxide and labelled glycolaldehyde and/or glyoxal are formed. Labelled glyoxylic acid is just detectable. When [$^{14}\text{C}_2$]ethanediol is incubated in the presence of unlabelled glycolaldehyde or glyoxylic acid as trapping agent there is a marked reduction in the ^{14}C of the respiratory carbon dioxide with both substances and a significant increase in the labelling of glyoxylic acid. On the basis of the criteria suggested by Nakada, Friedmann & Weinhouse (1955), these experiments indicate that both glycolaldehyde and glyoxylic acid are on the pathway of oxidation of ethanediol to carbon dioxide in the rat-liver slice.

DISCUSSION

The present work shows that, in the rat and rabbit, two main excretory products of ethylene glycol are carbon dioxide eliminated in the expired air and unchanged ethylene glycol eliminated in the urine. Table 1 shows that 17–28 % of the ^{14}C administered to rabbits is excreted in the urine, when the dose of the glycol is 0.1–2.0 g./kg. The results in Table 3 suggest that most of the urinary radioactivity is due to unchanged ethylene glycol, for, apart from labelled urea and traces of oxalic acid, no other labelled compound could be identified in the urine. At low doses (0.12 g./kg.) most of the administered [$^{14}\text{C}_2$]ethanediol appeared as carbon dioxide. One rabbit which excreted 23 % of the dose of ^{14}C in the urine and 60 % in the expired air as carbon dioxide in 3 days (Table 1) also had 11 % of the ^{14}C left in its tissues and 1 % in the faeces, so that 95 % of the administered ^{14}C was accounted for. From Fig. 1 it can be seen that half the dose of ethylene glycol was oxidized to and eliminated as carbon dioxide in 18 hr. after dosing. Table 1 also shows that at doses of 2.5 and 5 g./kg. nearly one-half of the administered radioactivity is eliminated in the urine. This suggests that at the

higher doses more of the glycol is being eliminated unchanged. Similar results were obtained for rats (Table 2). These findings support the view that the toxicity of ethylene glycol may be partly due to the unchanged glycol (cf. Pons & Custer, 1946).

It can be assumed (see Table 3) that the major compound in the urine is the unchanged glycol. In the guinea pig, little or no oxalate is excreted until the dose reaches 1 g./kg. (Table 4). The minimal fatal dose of the glycol by mouth for guinea pigs has been given as 7.4 g./kg. (Laug *et al.* 1939) and 6 g./kg. (Smyth, Seaton & Fischer, 1941). In the rabbit, the oxalate output is also small and even at 5 g./kg. the oxalate output in 24 hr. is no more than 0.37 % of the dose, although 56 % of the dose had been eliminated in the urine. The oral lethal dose of ethylene glycol for rabbits is about 9 g./kg. (Lehmann & Flury, 1943). The glycol is, therefore, not very toxic to rabbits and guinea pigs, and these species do not produce large amounts of urinary oxalate. This would suggest that, at least for rabbits, toxicity is partly due to the unchanged glycol. Cats seem to produce very much more oxalate than rabbits; further, cats eliminate a large proportion of the administered ^{14}C in the urine (see Table 4). We have found that 1 g./kg. of the glycol was fatal to cats but had no effect on rats, rabbits and guinea pigs. Spector (1956a) recorded that the subcutaneous lethal dose of ethylene glycol for cats is 2 g./kg. Thus in cats the toxicity of the glycol could be due to the unchanged glycol and to oxalate formation. Rats excrete appreciably more oxalate in urine than guinea pigs and rabbits and further they seem to excrete more of the administered ^{14}C in the urine. The oral minimum lethal dose of the glycol for rats has been given as 5.5 g./kg. (Laug *et al.* 1939) and 7.7 g./kg. (Smyth *et al.* 1941). Oettingen (1943) recorded that the glycol is consistently more toxic to rats than to rabbits. From a comparison of non-

lethal doses (see Table 4) it appears that ethylene glycol is more toxic to the species which excrete the most oxalate and the most unchanged glycol in the urine, and is least toxic to the species which are more able to oxidize it to carbon dioxide.

The distribution of ^{14}C in some rat tissues is given in Table 7. The brain was examined because Pons & Custer (1946) found some oxalate crystals in the brains of humans fatally poisoned by ethylene glycol; apart from the kidney no other tissues contained oxalate crystals. The concentration of ^{14}C in the brain of rats after 4 days was less than in most tissues (Table 7). The concentration of ^{14}C was much greater in liver, kidney and bone. It seems likely that the ^{14}C in bone is present as oxalate, for Curtin & King (1955) have shown that, in rats killed 10 days after an intraperitoneal injection of [^{14}C]oxalate, the bones contained more $^{14}\text{C/g.}$ than did any other tissue.

Oxidation of ethylene glycol to carbon dioxide. The metabolites of ethylene glycol have been shown to be oxalic acid and carbon dioxide in the whole animal (Tables 1-4) and glycolaldehyde and glyoxylic acid in the rat-liver slice (Table 6). Since oxalic acid is not readily oxidized to carbon dioxide *in vivo* (e.g. Weinhouse, 1955), then the glycol must be converted into carbon dioxide via an intermediate lying between it and oxalic acid. The major pathway for the oxidation of glycolaldehyde in animal tissues as suggested by the work of Weinhouse & Friedmann (1952), Nakada *et al.* (1955) and Friedmann, Levin & Weinhouse (1956) (see also Weinhouse, 1955) is: glycolaldehyde $\rightarrow$ glycollic acid $\rightarrow$ glyoxylic acid $\rightarrow$ carbon dioxide + formic acid $\rightarrow$ carbon dioxide. On this basis glyoxylic acid would be the precursor of carbon dioxide. Glyoxylic acid is known to be converted into carbon dioxide, glycine and oxalate in the whole rat and in liver preparations (Weinhouse, 1955). This work shows that ethylene glycol is also converted into carbon dioxide (Table 1), glycine (Table 5) and oxalate (Table 4). Glyoxylic acid is

therefore the probable precursor of both the carbon dioxide and oxalate formed from the glycol. However, Weinhouse (1955) showed that the extent to which glyoxylic acid is converted into oxalate in rat-liver homogenates depends on its concentration, for at relatively low concentrations glyoxylate is oxidized entirely to carbon dioxide, but at relatively high concentrations it is partly oxidized to oxalate. The increase in oxalate excretion with increasing dose of ethylene glycol observed in the rabbit (see Table 4) could therefore be explained by the formation of increasing concentrations of glyoxylate in the tissues. The difference in oxalate excretion between cats and rabbits (see Table 4) is more difficult to explain, for cats also excrete considerably more of the radioactivity in the urine than do rabbits. This suggests that cats do not oxidize the glycol to the various intermediates given above as efficiently as rabbits, but at the same time cats appear to oxidize glyoxylic acid to oxalic acid more readily than rabbits. Glycolaldehyde, glycollic acid and glyoxylic acid were not found in the urine of rabbits dosed with ethylene glycol but, since they are probable intermediates in the oxidation of the glycol, their participation in the toxic effects should be considered. According to Pohl (1923*a*), 4 g. of oxalic acid by mouth (i.e. about 60 mg./kg.) can be fatal to man and about 100 mg./kg. injected subcutaneously can be fatal to rabbits and cats. The subcutaneous lethal dose of glycolaldehyde for rabbits is 4 g./kg. (Mayer, 1903; Spector, 1956*b*). The intravenous lethal dose of glycollic acid for cats has been given as 1 g./kg. (Merck Index, 1960): according to Riker & Gold (1942) 0.1 g. of sodium glycollate/kg. rarely causes toxic effects in cats and dogs, 0.25 g./kg. is toxic but rarely fatal, whereas 0.5 g./kg. may prove fatal. In herbivores, chronic daily feeding of 300 mg. of sodium glycollate/kg. is tolerated without damage and higher doses up to 1 g./kg. can be tolerated for short periods (Scholz, 1956). According to Pohl (1923*b*)

Table 7. *Distribution of ^{14}C in certain tissues of rats 4 days after dosing with [$^{14}\text{C}_2$]ethanediol*

Animal	Dose of glycol (g./kg.)	% of dose of ^{14}C in				Sp. activity/g. of tissue (relative to liver = 1)			
		Liver	Kidney	Brain	Bone	Liver	Kidney	Brain	Bone
Rabbit	0.10	1.4	0.2	0.01	1.7	1	0.7	0.1	0.3
Rat	0.06	2.2	0.5	0.04	8.2	1	0.9	0.1	1.2
	0.10	2.8	0.3	0.03	5.3	1	0.4	0.04	0.7
	1.00	1.3	0.25	0.11	9.6	1	0.5	0.4	2.0
	2.50	0.8	0.2	—	4.2	1	0.8	—	1.5
	5.00	0.5	0.1	0.02	3.7	1	1.1	0.3	0.3
	7.5	0.4	0.2	0.02	2.9	1	1.3	0.2	1.7
	10.0*	1.3	1.4	0.10	4.0	1	1.8	0.3	0.7
					Mean for rats		0.97	0.22	1.16

* The animal receiving this dose died on the fourth day.

glyoxylic acid is much less toxic than oxalic acid. None of these intermediates appear to be as toxic as oxalic acid, but they all seem to be more acutely toxic than the glycol.

The probable fate of ethylene glycol in the body is shown in Scheme 1.

SUMMARY

1. [$^{14}\text{C}_2$]Ethane-1:2-diol has been prepared from [$^{14}\text{C}_2$]ethylene and its fate in the animal body investigated.

2. In the rabbit, at dose levels of 0.1–2.0 g./kg. about 20 % of the dose of radioactivity is excreted in the urine in 2 days, mainly as unchanged ethanediol, with smaller amounts as urea and oxalic acid; at higher doses (2.5 and 5.0 g./kg.), the radioactivity in the urine increases to about 50 %. At a dose level of 0.1 g./kg. about 40 % of the glycol is eliminated as $^{14}\text{CO}_2$ in the expired air in 1 day, and 60 % in 3 days. The remainder of the activity is mostly located in the tissues.

3. In the rat, the radioactivity excreted in the urine in 24 hr. increases from 21 % at a dose level of 0.1 g./kg. to 58 % at 1.0 g./kg., and the $^{14}\text{CO}_2$ in the expired air decreases from 23 % in 24 hr. at 0.1 g./kg. to 2.4 % in 12 hr. at 10 g./kg.

4. Rabbit and guinea pig excrete only traces (about 0.05 % of dose) of oxalate in the urine at dose levels of ethanediol of less than 1 g./kg., which increases (about 0.5 % of dose) at doses of 1–5 g./kg. Larger amounts of oxalate are excreted by rats (0.5–1.1 % of dose) and by cats (0.7–3.7 % of dose). At dose levels of 0.1–1 g. of [$^{14}\text{C}_2$]ethanediol/kg., the total radioactivity excreted in the urine by cats (43–78 %) is greater than that excreted by rats (21–49 %), rabbits (21–26 %) or guinea pigs (26–37 %), but both rats and rabbits show considerable increases at higher doses.

5. The concentration of radioactivity in the tissue of rats was greatest in liver, kidney and bone.

6. It has been shown in rat-liver slices that

glycolaldehyde and glyoxylic acid are intermediates in the oxidation of [$^{14}\text{C}_2$]ethanediol to $^{14}\text{CO}_2$.

7. Further evidence that glyoxylic acid is a metabolite of ethanediol is shown by the excretion of [^{14}C]hippuric acid by rats injected with [$^{14}\text{C}_2$]ethanediol and benzoic acid simultaneously.

8. The relative toxicity of ethanediol in different species, probable metabolic pathways and the toxicities of the metabolic intermediates are discussed.

This work was supported by a grant from The Distillers Co. Ltd.

REFERENCES

- Browning, E. (1953). *Toxicity of Industrial Organic Solvents*, p. 340. London: H.M.S.O.
- Brubacher, G., Just, M., Bodur, M. & Bernhard, K. (1956). *Hoppe-Seyl. Z.* **304**, 173.
- Curtin, C. O'H. & King, C. G. (1955). *J. biol. Chem.* **216**, 539.
- Elliott, K. C. A. (1955). In *Methods in Enzymology*, vol. 1, p. 3. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Fellows, J. K., Luduena, F. P. & Hanzlik, P. J. (1947). *J. Pharmacol.* **89**, 211.
- Friedmann, B., Levin, H. W. & Weinhouse, S. (1956). *J. biol. Chem.* **221**, 665.
- Gessner, P. K., Parke, D. V. & Williams, R. T. (1960). *Biochem. J.* **74**, 1.
- Harger, R. N. & Forney, R. B. (1959). *J. forensic Sci.* **4**, 136.
- Hjelt, E., Tamminen, V., Fortelius, P., Raekallio, J. & Alha, A. (1958). *Dtsch. Z. ges. gerichtl. Med.* **46**, 730.
- Laug, E. P., Calvery, H. O., Morris, H. J. & Woodard, G. (1939). *J. industr. Hyg.* **21**, 173.
- Lehmann, K. B. & Flury, F. (1943). *Toxicology and Hygiene of Industrial Solvents*, p. 257. Baltimore: The Williams and Wilkins Co.
- Mayer, P. (1903). *Hoppe-Seyl. Z.* **38**, 135.
- Merck Index (1960). *The Merck Index of Chemicals and Drugs*, 7th ed., p. 491. Rahway, N.J., U.S.A.: Merck and Co. Inc.

- Milas, N. A. & Sussman, S. (1937). *J. Amer. chem. Soc.* **59**, 2345.
- Nakada, H. I., Friedmann, B. & Weinhouse, S. (1955). *J. biol. Chem.* **216**, 583.
- Nakazawa, Y. (1950). *Folia pharm. jap.* **46**, 6.
- Oettingen, W. F. von. (1943). *Publ. Hlth Bull., Wash.*, no. 281. p. 166.
- Parke, D. V. (1956). *Biochem. J.* **62**, 339.
- Parke, D. V. (1961). *Biochem. J.* **78**, 262.
- Parke, D. V. & Williams, R. T. (1953). *Biochem. J.* **54**, 231.
- Pohl, J. (1923*a*). *Handb. exp. Pharm.* **1**, 860.
- Pohl, J. (1923*b*). *Handb. exp. Pharm.* **1**, 867.
- Pons, C. A. & Custer, R. P. (1946). *Amer. J. med. Sci.* **211**, 544.
- Ratner, S., Nocito, V. & Green, D. E. (1944). *J. biol. Chem.* **152**, 119.
- Riker, W. F. & Gold, H. (1942). *J. Amer. pharm. Ass. (sci. ed.)* **31**, 306.
- Scholz, J. (1956). *Arzneim. Forsch.* **6**, 227.
- Smyth, H. F., jun., Seaton, J. & Fischer, L. (1941). *J. industr. Hyg.* **23**, 259.
- Spector, W. S. (1956*a*). *Handbook of Toxicology*, vol. 1, p. 134. Philadelphia and London: W. B. Saunders Co. Ltd.
- Spector, W. S. (1956*b*). *Handbook of Toxicology*, vol. 1, p. 152. Philadelphia and London: W. B. Saunders Co. Ltd.
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1949). *Manometric Techniques and Tissue Metabolism*, p. 110. Minneapolis: Burgess Publishing Co.
- Weinhouse, S. (1955). In *Amino Acid Metabolism*, p. 637. Ed. by McElroy, W. D. & Glass, H. B. Baltimore: The Johns Hopkins Press.
- Weinhouse, S. & Friedmann, B. (1951). *J. biol. Chem.* **191**, 707.
- Weinhouse, S. & Friedmann, B. (1952). *J. biol. Chem.* **197**, 733.

Biochem. J. (1961) **79**, 489

Induction and Repression of β -Galactosidase in Non-growing *Escherichia coli*

By J. MANDELSTAM

National Institute for Medical Research, Mill Hill, London, N.W. 7

(Received 18 November 1960)

It has been known since the earliest studies of enzyme induction that glucose will often prevent the induced synthesis of enzymes concerned with the metabolism of other carbohydrates. The glucose effect and similar phenomena have been summarized in a number of reviews (Monod & Cohn, 1952; Mandelstam, 1956; Pollock, 1959). It appears to be an instance of a more general type of reaction, now termed repression (Vogel, 1957), in which the rate of synthesis of an enzyme is specifically decreased by a repressor, which is generally, but not necessarily, a normal metabolite of the cells.

The inhibitory effect of glucose upon the induced synthesis of β -galactosidase in growing cultures of *Escherichia coli* has been examined by Cohn & Horibata (1959). The present experiments show that, in non-growing cells (i.e. in the absence of net protein synthesis), the repressor action of glucose is not specific, and that the synthesis of β -galactosidase is effectively inhibited by all substances tested which the cells can utilize as sources of carbon and energy.

MATERIALS AND METHODS

Organisms. The following strains of *E. coli* were used: ML 30 and K₁₂ (wild type); ML 328c (Lac⁺) (requiring

leucine and able to form β -galactosidase); 160-37 (requiring arginine); 26-26 (requiring lysine); N.C.T.C. 8009 (requiring methionine); 15⁻ (requiring thymine); W 3301 (requiring uracil and biotin). All experiments were done at 35°. The wild-type strains were grown with shaking in a synthetic medium (pH 7.2) containing phosphate and inorganic salts (Mandelstam, 1958*a*) and glucose or another carbon source (1%, w/v). All organic acids were neutralized to pH 7.0 with NaOH; concentrations given in the text refer to the acid and not to its salt. For the auxotrophic mutants, the medium was supplemented with the appropriate growth factor as follows: DL-leucine (300 μ g./ml.), L-arginine-HCl (100 μ g./ml.), L-lysine-HCl (150 μ g./ml.), L-methionine (100 μ g./ml.), thymine (10 μ g./ml.), uracil (100 μ g./ml.), biotin (0.05 μ g./ml.).

Induction of β -galactosidase. The cells were harvested when the culture contained about 0.8 mg. dry wt. of bacteria/ml., and were washed with phosphate buffer (having the same composition and pH as the phosphate in the growth medium). In the earlier experiments the bacteria were first starved of their specific requirement or, with the wild-type strains, of ammonium salts (see below). In later experiments this was omitted, and the cells, after washing, were suspended in the same phosphate buffer (at 0.2–0.4 mg. dry wt./ml.) with methyl β -D-thiogalactoside (MTG; 0.1 mg./ml.) as inducer. MTG was synthesized by the method of Helferich & Türk (1956).

Incorporation of [¹⁴C]leucine. Washed bacteria were incubated in buffer as already described, with the addition of DL-[¹⁴C]leucine (200 μ g./ml.; specific activity about 30