REFERENCES

- Conway, E. J. (1950). *Micro-diffusion Analysis and Volumetric Error*. London: Crosby Lockwood.
- Conway, E. J. & O'Malley, E. (1942). *Biochem. J.* **36**, 655.
- Dische, Z. & Borenfreund, E. (1950). *J. biol. Chem.* **184**, 517.
- Elson, L. A. & Morgan, W. T. J. (1933). *Biochem. J.* **27**, 1824.
- Folkes, B. F., Grant, R. A. & Jones, J. K. N. (1950). *J. chem. Soc.* p. 2136.
- Horowitz, H. N., Ikawa, M. & Fling, M. (1950). *Arch. Biochem.* **25**, 226.
- Immers, J. & Vasseur, E. (1950). *Nature, Lond.*, **165**, 898.
- Johnston, J. P., Ogston, A. G. & Stanier, J. E. (1951). *Analyst*, **76**, 88.
- Long, C. (1951). Personal communication.
- Lutwak-Mann, C. (1941). *Biochem. J.* **35**, 610.
- McCrea, J. F. (1951). *Biochem. J.* **48**, xlix.
- Markham, R. (1942). *Biochem. J.* **36**, 790.
- Morgan, W. T. J. (1936). *Biochem. J.* **30**, 909.
- Ogston, A. G. & Stanier, J. E. (1950). *Biochem. J.* **46**, 364.
- Pucher, G. W., Vickery, H. B. & Leavenworth, C. S. (1935). *Industr. Engng Chem. (Anal. ed.)*, **7**, 152.
- Schloss, B. (1951). *Anal. Chem.* **23**, 1321.
- Sideris, C. P., Young, H. Y. & Krauss, B. H. (1938). *J. biol. Chem.* **126**, 233.
- Tracey, M. V. (1951). *Biochem. J.* **49**, xx.
- Vanselow, A. P. (1940). *Industr. Engng Chem. (Anal. ed.)*, **12**, 516.

The Metabolism of Methyl Carbamate

By E. BOYLAND AND D. PAPADOPOULOS*
The Chester Beatty Research Institute, London, S.W. 3

(Received 3 March 1952)

Urethane or ethyl carbamate, which is carcinogenic (Nettleship & Henshaw, 1943), mutagenic (Vogt, 1948) and used in the treatment of leukaemia (Paterson, Ap Thomas, Haddow & Watkinson, 1946), is considered to be a radiomimetic agent. It is destroyed relatively slowly in the bodies of normal rats and mice and even more slowly by tumour-bearing animals (Boyland & Rhoden, 1949; Skipper *et al.* 1951).

Methyl carbamate is not a radiomimetic agent, being neither carcinogenic nor mutagenic (Larsen, 1947). The rate of destruction of methyl carbamate by rats has been followed by estimation of the concentration of the compound in the tissues at different times after injection.

EXPERIMENTAL

Estimation of methyl carbamate. Methyl carbamate was determined by the method used for urethane by Boyland & Rhoden (1949). The sample containing less than 0.40 mg. methyl carbamate in 2 ml. 2N-KOH was placed in the outer

ring of a Conway unit (Conway, 1947), the central compartment of which contained 1 ml. of 0.025N-K₂Cr₂O₇ in 10N-H₂SO₄. The unit, along with a blank unit in which m-K₂CO₃ replaced the 2N-KOH solution, was heated to 37° for 16 hr. At the end of that time, KI was added to the K₂Cr₂O₇, and the liberated I₂ titrated with 0.1N-Na₂S₂O₃ from a Conway burette. The difference between the titration figures of the KOH and K₂CO₃ units was proportional to the methyl carbamate present. As in the estimation of ethyl carbamate, the reaction was stoichiometric and reproducible under constant conditions (as shown in Table 1). All determinations were carried out in duplicate, and duplicate sets of blank units with reagents alone were set up with each set of estimations. One ml. 0.1N-Na₂S₂O₃ is equivalent to 1.87 mg. methyl carbamate.

Table 2. *Excretion of methyl carbamate in urine of rats dosed with 50 mg./100 g. body weight*

Weight of rat (g.)	Methyl carbamate (mg.) excreted in urine		Percentage of dose excreted in 24 hr.
	During first 4 hr. after injection	Between 4 and 24 hr. after injection	
150	2.3	1.4	4.9
200	2.2	7.6	9.8
190	1.8	7.0	9.2
160	1.4	2.7	5.1

Urinary excretion of methyl carbamate by rats

Adult rats weighing between 100 and 200 g. were dosed by intraperitoneal injection with 50 mg./100 g. body weight of methyl carbamate. The animals were kept in metabolism cages and the urine collected for estimation of the excreted carbamate. The cages were washed 4 and 24 hr. after injection and the washings added to the collected urine.

Table 1. *Estimation of known quantities of methyl carbamate*

Methyl carbamate taken (mg.)	0.1N-Na ₂ S ₂ O ₃ used (ml.)	Titre as % of theory
0.05	0.0262	96
0.10	0.0547	103
0.20	0.1188	96
0.30	0.1551	98
0.40	0.2232	103

* Present address: The Greek Anti-Cancer Institute, Athens.

The amounts of methyl carbamate excreted shown in Table 2 suggest that the drug is not concentrated in the kidney as the proportion of the dose of carbamate excreted in 24 hr. is of the same order as the proportion of urine excreted in 24 hr. to the total body water.

carbamate was then estimated in samples of the extracts.

The theoretical figures for the expected concentrations are based on the following assumptions: (1) the methyl carbamate is uniformly distributed in the body water (as indicated by the similar con-

Table 3. *The concentration of methyl carbamate in tissues of normal rats at different times after intraperitoneal injection of the drug*

Dose (mg./100 g. body wt.)	Time after injection (hr.)	Concentration of methyl carbamate found (mg./100 g.)			Theoretical expected concentration* (mg./100 g.)
		Blood	Lungs	Liver	
50	0	—	—	—	60
50	1	54	56	57	—
50	4	51	44	44	48
50	24	26	24	26	30
50	48	16	14	14	15
50	72	8	13	10	7
50	96	7	5	4	4
50	120	0	0	0	2
100	0	—	—	—	120
100	1	125	106	100	—
100	4	132	86	99	95
100	24	61	47	47	60
100	48	27	18	20	30
100	96	17	19	16	7
100	120	20	23	21	—
100	144	0	0	2	—

* Assuming that the concentration in the body falls by 50% in 24 hr.

Table 4. *The concentration of methyl carbamate in tissues of rats with Walker carcinomata at different times after intraperitoneal injection of the drug*

Dose (mg./100 g. body wt.)	Time after injection (hr.)	Concentration of methyl carbamate found (mg./100 g.)			Theoretical expected concentration* (mg./100 g.)
		Blood	Lungs	Liver	
50	0	—	—	—	57
50	4	67	61	70	—
50	24	50	43	40	38
50	48	20	12	23	28
50	72	—	—	—	19
50	96	—	—	—	14
50	120	16	10	4	—
50	144	—	—	—	7
100	0	—	—	—	114
100	4	138	77	108	—
100	24	56	40	53	85
100	48	67	48	55	57
100	96	31	27	28	28
100	120	27	10	24	21

* Assuming that the concentration in the body falls by 50% in 48 hr.

Distribution of methyl carbamate

Adult rats, with grafted Walker carcinomata, and normal rats were given doses of 50 and 100 mg./100 g. body weight of methyl carbamate by intraperitoneal injection and killed at different intervals after dosing. A sample of blood was collected and extracted with 2 volumes of 10% trichloroacetic acid. The lungs and a sample of liver were similarly extracted with the trichloroacetic acid. Methyl

concentrations in blood and the tissues examined); (2) the body water forms two-thirds of the body weight of normal rats (Moulton, 1923) and 70% in tumour-bearing rats (an estimate based on scattered data in the literature); (3) the tissues examined contain 80% water; and (4) the rate of elimination of methyl carbamate at any time is proportional to the concentration in the body at that time.

The data on the distribution of methyl carbamate in rat tissues (Tables 3 and 4) show that the com-

pound is fairly quickly distributed in the body, but that the concentration in the tissues examined falls very slowly. It appears that the rate of elimination of methyl carbamate is such that the time required for the concentration to fall to half the maximum value is 24 hr. for normal rats and almost twice this time for tumour-bearing rats.

DISCUSSION

The data on elimination of ethyl carbamate (Boylard & Rhoden, 1949; Skipper *et al.* 1951) indicated that the time for 50 % elimination was of the order of 12 hr. for normal rats or mice and 24 hr. for tumour-bearing animals. The present data indicate that methyl carbamate, like ethyl carbamate or ethanol, is not concentrated by the kidney, so that elimination must be mainly by metabolism. Methyl carbamate appears to be metabolized at about half the rate of ethyl carbamate.

The relationship of metabolism of the methyl and ethyl carbamates to their biological action is difficult to understand. If the radiomimetic action were due to hydrolysis of the carbamate, then the slower metabolism of methyl carbamate should be compensated for by increase of the dose. This, however, is not the case.

If the carcinogenic action of urethane were due to slow liberation of ethanol oxidized to acetaldehyde within the tissues then methanol would be expected to be as effective, as formaldehyde has been found to be mutagenic (Rapoport, 1946).

The discovery that ethyl carbamate is a much more effective inhibitor of transmethylation reactions than is methyl carbamate (McKinney, 1950) suggests that such inhibition may be concerned with the radiomimetic effects produced by urethane.

SUMMARY

1. Methyl carbamate, like urethane, does not appear to be concentrated by the kidney. Methyl carbamate is eliminated from the body by metabolism at about half the rate at which urethane is eliminated.

2. Tumour-bearing rats eliminate methyl carbamate only about half as fast as normal rats.

We should like to thank Mr R. W. White for his help with these experiments. This investigation has been supported by grants to The Royal Cancer Hospital and Chester Beatty Research Institute from The British Empire Cancer Campaign, The Jane Coffin Childs Memorial Fund for Medical Research, The Anna Fuller Fund, and The National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

REFERENCES

- Boylard, E. & Rhoden, E. (1949). *Biochem. J.* **44**, 528.
 Conway, E. J. (1947). *Microdiffusion Analysis and Volumetric Error*, revised ed. London: Crosby Lockwood.
 Larsen, C. D. (1947). *J. nat. Cancer Inst.* **8**, 99.
 McKinney, G. R. (1950). *J. Pharmacol.* **100**, 45.
 Moulton, C. R. (1923). *J. biol. Chem.* **57**, 79.
 Nettleship, A. & Henshaw, P. S. (1943). *J. nat. Cancer Inst.* **4**, 309.
 Paterson, E., Ap Thomas, I., Haddow, A. & Watkinson, J. M. (1946). *Lancet*, **1**, 677.
 Rapoport, I. A. (1946). *C.R. Acad. Sci., U.R.S.S.*, **54**, 65.
 Skipper, H. E., Bennett, L. L., Bryan, C. E., White, L., Newton, M. A. & Simpson, L. (1951). *Cancer Res.* **11**, 46.
 Vogt, M. (1948). *Experientia*, **4**, 68.

Sorbitol Antiketogenesis and the Dehydrogenation and Oxidation of Fatty Acids

By R. L. BLAKLEY*

*Department of Biochemistry, Medical School, University of Otago,
 Dunedin, New Zealand*

(Received 8 January 1952)

Sorbitol is remarkable for its ability to decrease the spontaneous ketogenesis of liver slices from fasted rats, being more efficient in this respect than related hexoses and most other compounds (Edson, 1936b; Blakley, 1951). During the investigation of this effect a dehydrogenase was found in rat liver which converts D-sorbitol to D-fructose in the presence

* Present address: National Institute for Medical Research, Mill Hill, London, N.W. 7.

of coenzyme I (CoI). The enzyme also converts L-iditol to sorbose, and iditol is as effective as sorbitol in diminishing spontaneous ketogenesis (Blakley, 1951).

A simple explanation of sorbitol antiketogenesis suggested by Edson (1936b) is that it results from competition between sorbitol and fatty acids for oxidative enzymes and coenzymes in the liver. When the course of sorbitol metabolism is taken