of the product is D-lactate, which cannot be oxidised by lactic dehydrogenase. A more likely fate for methylglyoxal is oxidation to pyruvate.[14] This reaction is catalysed by ketoaldehyde oxidase, which preferentially uses NADP as a coenzyme, with a slightly lower affinity for NAD, which is more abundant in the cell than NADP. Fig. 2 shows that triose phosphate metabolism via methylglyoxal makes the same demands on the cell's pyridine nucleotides as the "normal" glycolytic pathway.

Since ketoaldehyde oxidase is inhibited by cyanide, the extremely efficient non-enzymic dismutation of methylglyoxal should take precedence in the livers of rats fed cyanide. As Fig. 2 shows the metabolism of triose phosphates to L-lactate in this way results in the oxidation of one mole of $NADH_2$ per mole of triose phosphate, and this accounts for the increase in $NAD/NADH_2$ ratio observed in the cytoplasm of liver cells of rats administered cyanide.

This scheme also explains the observed decrease in the sum of the concentrations of lactate and pyruvate in cyanide-fed rats. Assuming that the normal cell lactate level is the balance of the synthesis of lactate and its removal, then the fact that the cyanide pathway converts 50 per cent of the methylglyoxal formed to "dead-end" condensation products should result in decreased lactate formation and a lower cell lactate plus pyruvate concentration.

Department of Biochemistry,
University of Sydney,
Sydney, N.S.W., Australia

R. C. BAXTER
W. J. HENSLEY

REFERENCES

1. E. R. STADTMAN, *Adv. Enzym.* **28**, 41 (1966).
2. J. R. WILLIAMSON, M. S. OLSON and E. T. BROWNING, *Biochem. J.* **104**, 45P (1967).
3. T. BÜCHER and M. KLINGENBERG, *Angew. Chem.* **70**, 552 (1958).
4. D. H. WILLIAMSON, P. LUND and H. A. KREBS, *Biochem. J.* **103**, 514 (1967).
5. M. E. SMITH and H. W. NEWMAN, *J. biol. Chem.* **234**, 1544 (1959).
6. E. KIRSTEN, C. GEREZ and R. KIRSTEN, *Biochem. Z.* **337**, 312 (1963).
7. E. BERNT and H. U. BERGMEYER, in *Methods of Enzymatic Analysis* (Ed. H. U. BERGMEYER), p. 384. Academic Press, New York (1963).
8. H. U. BERGMEYER and E. BERNT, in *Methods of Enzymatic Analysis* (Ed. H. U. BERGMEYER) p. 324. Academic Press, New York (1963).
9. H. J. HOHORST, F. H. KREUTZ and T. BÜCHER, *Biochem Z.* **332**, 18 (1959).
10. N. O. KAPLAN and M. M. CIOTTI, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN), vol. 3, p. 253. Academic Press, New York (1957).
11. D. PETTE and G. RUGE, *Biochem. biophys. Res. Commun.* **13**, 150 (1963).
12. C. V. SMYTHE, *Biochem Z.* **257**, 371 (1933).
13. H. A. KREBS and P. LUND, *Biochem. J.* **98**, 210 (1966).
14. C. MONDER, *Biochim. biophys. Acta* **99**, 573 (1965).

Biochemical Pharmacology, Vol. 18, pp. 236–238. Pergamon Press 1969. Printed in Great Britain

Metabolic fate of zoxazolamine in tumor bearing rats

A MARKED reduction in the rate of Pentobarbital and *d*-Amphetamine metabolism in tumor bearing rats and mice even at a very early stage of tumor growth, has been already reported.[1,2] Further support for the hypothesis that an impairment of drug metabolizing microsomal enzymes occurs in tumor bearing animals has been obtained with zoxazolamine, which is transformed by such enzymes in the presence of oxygen and NADPH.[3–5] Male, Sprague–Dawley rats of the average weight of 150 ± 10 g, received s.c. the following transplantable tumors: Walker 256 carcinosarcoma,

Flexner-Jobling carcinoma, T_8 uterine epithelioma of Guèrin and Sarcoma 45. Usually sixteen days after the transplantation, animals were used for both *in vivo* and *in vitro* experiments. Pharmacological activity of zoxazolamine was estimated by measuring the duration of paralysis by means of a rotating bar (9 cm dia.; 2 rpm).

Table 1 indicates that rats transplanted with tumor show a paralysis longer than normal rats after intravenous administration of zoxazolamine (kindly obtained by McNeil Labs. Inc., Washington,

TABLE 1. ZOXAZOLAMINE PARALYSIS (50 mg/kg i.v.)

Group	Body weight (g ± S.E.)	Tumor weight (g ± S.E.)	Duration of paralysis (min ± S.E.)	% of increase	P
Normal	240 ± 6 (30)*	—	161 ± 4·7	—	
Walker	188 ± 12 (13)*	23 ± 2·7	*328 ± 13·8	103	< 0·001
Flexner–Jobling	207 ± 9 (9)*	23 ± 2·5	300 ± 8·0	86	< 0·001
Sarcoma 45	210 ± 10 (6)*	11 ± 1·3	209 ± 10·0	29	< 0·001
T_8 Guèrin	162 ± 9 (6)*	32 ± 4·8	515 ± 32·0	219	< 0·001

* Four animals died during the paralysis. Tumors were implanted 16 days before the experiment.

U.S.A.) (50 mg/kg). The difference ranged between a maximum (219 per cent increase in respect to control rats) in the case of T_8 Guèrin tumor and a minimum (29%) in the case of Sarcoma 45. The increased effect of zoxazolamine seems to be correlated with the weight of the tumor. Zoxazolamine levels in plasma, brain and liver following intravenous injection of 50 mg/kg, were estimated according

TABLE 2. TISSUES LEVELS OF ZOXAZOLAMINE (50 mg/kg i.v.) IN NORMAL AND WALKER 256 CARCINOSARCOMA BEARING RATS

No. of rats	Group	Body weight (g ± S.E.)	Tumor weight (g ± S.E.)	Time after treatment (min)	Zoxazolamine μg/g or ml ± S.E. brain	liver	plasma
7	Normal	220 ± 10	—	60	50·4 ± 3·2	46·1 ± 1·3	11·6 ± 0·6
7	Normal	243 ± 7	—	240	18·2 ± 1·8	23·3 ± 2·1	6·2 ± 0·6
7	Normal	213 ± 6	—	360	3·8 ± 0·3	7.3 ± 1·0	3·3 ± 0·7
7	Walker	144 ± 12	17·9 ± 2	60	53·9 ± 2·5	47·8 ± 2·3	11·5 ± 0·6
7	Walker	147 ± 14	17·8 ± 3	240	24·9 ± 4·3	30·7 ± 1·3*	8·3 ± 0·8
7	Walker	185 ± 11	17·2 ± 5	360	12·5 ± 0·8†	13·7 ± 1·8†	6·5 ± 1·0†

* $0{\cdot}01 < P < 0{\cdot}05$.

† $P < 0{\cdot}01$.

Tumors were transplanted 16 days before.

to the spectrophotometric method described by Juchau *et al.*[6] A statistically significant delay in the disappearance of the drug was observed for rats bearing a 16 day old Walker tumor, in respect to normal animals (see Table 2).

The half-life of zoxazolamine in plasma was 5 hrs in Walker bearing rats as opposed to 3 hr in normal animals. Further support for an impairment of zoxazolamine biotransformation has been obtained by *in vitro* experiments. The 9000 *g* liver supernatant fraction obtained from Walker tumor bearing and normal rats was incubated with zoxazolamine according to Juchau *et al.*[6] Protein determinations were performed according to the method of Lowry[7] and no differences in protein content were observed in the supernatant liver fraction between normal and tumor bearing rats. Table 3 shows that, beginning at the 11th day after tumor transplantation, there is a considerable reduction in the amount of zoxazolamine metabolized by the liver of tumor bearing animals in respect to normal rats.

TABLE 3. ZOXAZOLAMINE METABOLISM IN RAT LIVER (9000 *g* FRACTION)

No. of rats	Group	Time after tumor transplantation days	Body weight (g ± S.E.)	Tumor weight (g ± S.E.)	μg Zoxazolamine metabolized/100 mg/60 min ± S.E.	μg Zoxazolamine metabolized/mg protein/60 min ± S.E.
5	Normal	—	198 ± 6	—	11·7 ± 2·8	0·59 ± 0·13
5	Walker	4	152 ± 8	—	8·4 ± 2·0	0·45 ± 0·10
5	Normal	—	191 ± 6	—	10·8 ± 1·6	0·53 ± 0·08
5	Walker	11	176 ± 6	3·3 ± 0·5	4·4 ± 0·9*	0·23 ± 0·06*
5	Normal	—	236 ± 13	—	13·5 ± 1·9	0·66 ± 0·07
5	Walker	16	182 ± 15	15·5 ± 3·2	2·7 ± 0·5†	0·14 ± 0·03†
5	Normal	—	284 ± 18	—	12·0 ± 2·2	0·66 ± 0·13
5	Walker	22	182 ± 29	34·7 ± 5·0	3·0 ± 0·7†	0·18 ± 0·04†

* $0{\cdot}01 < P < 0{\cdot}05$.
† $P < 0{\cdot}01$.

These results agree with previous results obtained with Pentobarbital and *d*-Amphetamine.[1,2] Further studies to elucidate the possible mechanisms involved and their specificity are in progress.

Istituto di Ricerche Farmacologiche "Mario Negri" G. FRANCHI
Via Eritrea, 62, 20157 *Milano, Italy* R. ROSSO

Acknowledgements—The valuable technical help of Mr. M. Marchesini is gratefully acknowledged. This work was supported by the contract DHEW/PHS/NIH PH-43-67-83.

REFERENCES

1. R. ROSSO, E. DOLFINI and M. G. DONELLI, *Eur. J. Cancer* **4**, 133 (1968).
2. R. ROSSO, DOLFINI and G. FRANCHI, *Biochem. Pharmac.* **17**, 633 (1968).
3. B. B. BRODIE, J. AXELROD, J. R. KOOPER, L. GAUDETTE, B. N. LADU, C. MITOMA and S. UDENFRIEND, *Science* **121**, 603 (1955).
4. A. H. CONNEY, N. TROUSOF and J. J. BURNS, *J. Pharmac. exp. Ther.* **128**, 333 (1960).
5. C. MITOMA, H. S. POSNER, H. C. REITZ and S. UDENFRIEND, *Archs. Biochem. Biophys.* **61**, 431 (1950).
6. M. R. JUCHAU, R. L. CRAM, G. L. PLAA and J. R. FOUTS, *Biochem. Pharmac.* **14**, 473 (1965).
7. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. Biol. Chem.* **193**, 265 (1951).

Biochemical Pharmacology, Vol. 18, pp. 238–243. Pergamon Press. 1969. Printed in Great Britain

The separation and detection of metabolites of guanethidine

(*Received* 24 *June* 1968; *accepted* 15 *August* 1968)

THE ANTIHYPERTENSIVE drug guanethidine has been widely used in investigations of the adrenergic neurone. Although several studies[1–3] suggest that the drug is metabolised in animals, until recently little was known about its metabolites.

Furst[4] showed that in rats, radioactivity labelled guanethidine was extensively converted into what appeared to be a single polar metabolite and that oxygen-dependent metabolism of the drug took place in rat and rabbit liver microsomal preparations.[5]