MRC Brain Metabolism Unit,
Thomas Clouston Clinic,
153 Morningside Drive,
Edinburgh EH10 5LG,
Scotland

HAROLD W. READING*
TURGAY ISBIR†

REFERENCES

1. N. J. Birch, *Lancet* **i**, 1384 (1975).
2. J. E. Hesketh, N. Kinloch and H. W. Reading, *J. Neurochem.* **29**, 883 (1977).
3. J. E. Hesketh, J. B. Loudon and H. W. Reading, *J. Neurochem.* **29**, 883 (1977).
4. M. Scott and H. W. Reading, *Biochem. Soc. Trans.* **6**, 642 (1978).
5. J. E. Hesketh, *J. Neurochem.* **28**, 597 (1977).
6. D. Ford, in *Anatomy of the Central Nervous System, in Review* (Ed. D. Ford), p. 149. Elsevier Scientific, Amsterdam (1975).
7. D. F. Cole, in *Biochemistry of the Eye* (Ed. C. N. Graymore), p. 105, Academic Press, New York.
8. K. Thomsen and O. V. Oleson, *Int. Pharmacopsychiat.* **9**, 118 (1974).
9. T. Malmfors, *Acta physiol. scand* **64**, Suppl. 248, 1 (1965).
10. A. J. Dewar and H. W. Reading, *Exp. Neurol.* **40**, 216 (1973).
11. A. Atkinson, A. D. Gatenby and A. G. Lowe, *Biochim. biophys. Acta.* **320**, 195 (1973).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
13. J. E. Hesketh, *Biochem. Soc. Trans.* **4**, 328 (1976).
14. V. Srinivasan, S. Parvath-Devi and A. Venkoba-Rao, in *Lithium in Medical Practice* (Eds. F. N. Johnson and S. Johnson), p. 199. MTP Press, Lancaster (1978).
15. B. Shopsin and S. Gershon, in *Lithium—its role in Psychiatric Research* (Eds. S. Gershon and B. Shopsin), p. 107. Plenum Press, New York (1973).

* To whom correspondence should be addressed.
† Present address: University of Cukurova, Medical Faculty, Biochemistry Department, Adana, Turkey.

Biochemical Pharmacology, Vol. 28, pp. 3474–3476.

0006-2952/79/1201-3474 $02.00/0

Exhalation of mercury—further evidence for an oxidation–reduction cycle in mammalian tissues

(*Received* 25 *January* 1979; *accepted* 24 *May* 1979)

According to the original observations of Nielsen-Kudsk [1,2], the hydrogen peroxide pathway appears to be responsible for the oxidation of inhaled mercury vapor (Hg^0) to divalent inorganic mercury (Hg^{2+}) in mammalian tissues [3,4]. The exhalation of volatile mercury from rats injected with mercuric chloride was the first evidence that Hg^{2+} might be reduced to Hg^0 [5].

The ability of ethanol to depress retention of inhaled vapor [1] and to increase exhalation after exposure of animals to mercury vapor [6] might be accounted for on the basis that ethanol is a substrate in the peroxidatic action of catalase [7], and thus could compete for catalase with mercury. The increase in exhalation produced by ethanol in mice treated with mercuric chloride might be explained by inhibition of the oxidation of Hg^0 that has been produced by reduction of Hg^{2+} [8]. The exhaled mercury was identified as Hg^0.

However, ethanol is known to have a wide variety of metabolic effects. For example, it might directly effect reduction of Hg^{2+} by changing tissue NAD/NADH ratios. In order to test more specifically the role of catalase in the oxidation–reduction of Hg^{2+}, we examined the effects on exhalation of mercury of a well known inhibitor of catalase, 3-amino-1,2,4-triazole (AT). In addition, tests were made on a strain of mice having a genetic variant differing from the normal strain solely in catalase activity [9]. The tissue catalase activities in these animals have been published elsewhere [4].

An initial colony of Cs^B mice (acatalasemic) [9] and Cs^A mice (the corresponding wild type) were provided by Dr. Feinstein, Argonne National Laboratory, Argonne, Il. CBA/J mice were purchased from Jackson Laboratories, ME. Mice treated or not treated with AT were exposed to radioactive metallic mercury vapor in the chamber described previously [10]. AT was recrystallized from a commercial product according to the method described by Tephly *et al.* [11]. AT was injected, i.p. at a dose of 1 g/g body weight, 30 min prior to exposure or immediately after exposure. Exhalation rates were measured by collecting exhaled mercury in an absorbent* held in a tube attached to the outlet of a 500 ml plastic bottle containing the exposed mice. Air was passed through the bottle continuously at a flow rate of 4 liters/min. Radioactivity of the absorbent was counted once an hour by a Packard model 3002 Tri-Carb scintillation spectrometer. During the counting, the collection of air samples was interrupted for about 2 min.

The cumulative amount of exhaled mercury increased continously during the period of observation in all groups of animals (Fig. 1). To assess the contribution of volatilization of mercury from the fur and external surfaces of the mice, volatile mercury was recorded from dead animals (curve F). Volatile mercury from dead animals was always lower than from living animals, confirming previous observations that the main source of volatile mercury was exhalation [5].

In animals exposed to mercury vapor (upper figure), those having reduced catalase activities exhaled more mercury (curves A, B and C) than animals with normal catalase activity. Aminotriazole given 30 min before or after a single i.p. dose of mercuric chloride (lower figure) increased the amount of exhaled mercury. An important difference from the animals exposed to mercury vapor is the 1 to 1.5 hr time lag occurring before the effect of aminotriazole was observed. Another large difference is the much lower amount of mercury exhaled in the mercuric chloride-treated animals.

The increased exhalation cannot be explained by changes in tissue distribution produced by aminotriazole or acatalasemia. Lung deposition is actually reduced in vapor-exposed acatalasemic mice or normal mice pretreated with aminotriazole. Furthermore, aminotriazole given after vapor, or given before or after $HgCl_2$, produced no significant changes in tissue distribution, as evidenced by measurement of mercury in lung, blood, brain, liver, kid-

* Hopcalite (Hopkins & Williams, Ltd., Birmingham, England).

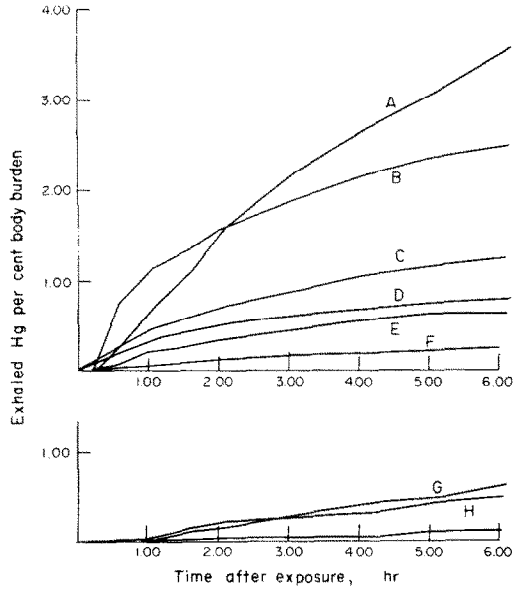

Fig. 1. Cumulative amount of volatile mercury collected from mice following either exposure to mercury vapor (upper graph) or a single dose of $HgCl_2$ (lower graph). Exposure to mercury vapor was for 26 min at concentrations of 0.18 to 0.38 mg Hg/m^3. The $HgCl_2$ was given i.p. in 0.1 ml saline at 10 μg Hg/kg body weight. Collection of volatile mercury commenced immediately after dosing. Volatile mercury was collected in a filter consisting of 10 g of Hopcalite. When the filter was removed for counting, there was an interruption in the collection of mercury for a period of about 2 min. Each curve is labeled by letters having the following significance: (A) CBA/J mice injected i.p. with AT (1 g/kg in 5 ml saline/kg) immediately after exposure to mercury vapor; (B) CBA/J mice injected i.p. with AT (1 g/kg in 5 ml saline/kg) 30 min prior to exposure to mercury vapor; (C) acatalasemic (strain Cs^B) mice; (D) normal (strain Cs^A) mice; (E) CBA/J mice injected with saline (5 ml/kg); (F) CBA/J mice killed by cervical dislocation immediately after exposure to mercury vapor; (G) CBA/J mice injected i.p. with AT (1 g/kg in 5 ml saline/kg) 30 min after a dose of $HgCl_2$; (H) CBA/J mice injected i.p. with AT (1 g/kg in 5 ml saline/kg) 30 min prior to $HgCl_2$; and (I) CBA/J mice injected i.p. with saline (5 ml/kg) 30 min prior to a dosing with $HgCl_2$.

neys, and remaining carcass, in animals killed in the first hour or at the end of the 6 hr observation period.

Our findings of exhaled mercury from mercuric chloride-treated animals confirms previous conclusions that Hg^{2+} may be reduced to Hg^0 in body tissues [5]. Furthermore, the fact that aminotriazole increased exhalation implicates the hydrogen peroxide–catalase pathway in the reoxidation of Hg^0 derived from Hg^{2+}. The conclusion agrees with the suggestion of Dunn *et al.* [8] that ethanol enhances exhalation from mercuric chloride-treated animals by the same mechanism.

The difference in the rates of exhalation in the vapor-exposed versus the mercuric chloride groups may be accounted for by the difference in the quantities of available Hg^0. Of the vapor-exposed groups, residual inhaled Hg^0 presents in tissue should contribute to exhalation [12]. Thus, the exhalation curves level off as this reservoir is depleted. Aminotriazole and acatalasemia lead to decreased rates of removal of this reservoir and consequently greater cumulative exhalation.

Our findings, along with those of others [2,3,4,8,13–15], indicate that the hydrogen peroxide–catalase pathway is responsible not only for the oxidation of inhaled Hg^0 but also for Hg^0 produced in tissues from reduction of Hg^{2+}. Thus, there is mounting evidence for cyclic oxidation and reduction of inorganic mercury in tissues. This may have important pharmacologic and toxicologic implications. Hg^0 is believed to be the form of mercury that most rapidly crosses the blood–brain [16] and placental barriers [17]. Its concentration in plasma and tissues will be determined by the balance of oxidation and reduction reactions. These reactions may be affected by differences in genetic status (e.g. acatalasemia), as well as commonly occurring chemicals (ethanol and aminotriazole; the latter is used as an herbicide).

In summary, the time course of the exhalation of mercury vapor was studied after the exposure of acatalasemic mice or AT-treated mice to metallic mercury vapor. In both cases, the accelerated exhalation of mercury was observed over periods of several hours compared with the corresponding control in catalase activity. AT caused an increased exhalation of mercury even after the dosing of mercuric chloride. These observations indicate that mercury is in a dynamic equilibrium between metallic and mercuric mercury in the body.

Acknowledgements—We are grateful to Mrs. Judi Allen, M.S., for excellent technical assistance. This work was supported in part by a Center grant (ES–10247) from the National Institute of Environmental Health Sciences and on work performed under contract with the U.S. Department of Energy at the University of Rochester, Department of Radiation and Biophysics, and has been assigned Report No. UR–3490–1548.

Environmental Health Sciences Center,
Department of Radiation Biology and Biophysics,
University of Rochester School of Medicine,
Rochester, NY 14642, U.S.A.

YASUO SUGATA*†
THOMAS W. CLARKSON

REFERENCES

1. F. Nielsen-Kudsk, *Acta pharmac. tox.* **23**, 263 (1965).
2. F. Nielsen-Kudsk, in *Mercury, Mercurials and Mercaptans* (Eds. M. W. Miller and T. W. Clarkson), p. 355. Charles C. Thomas, Springfield, (1973).
3. L. Magos, Y. Sugata and T. W. Clarkson, *Toxic. appl. Pharmac.* **28**, 367 (1974).
4. S. Halbach and T. W. Clarkson, *Biochim. biophys. Acta* **523**, 522 (1978).
5. T. W. Clarkson and A. Rothstein, *Hlth Phys.* **10**, 115 (1964).
6. L. Magos, T. W. Clarkson and M. R. Greenwood, *Toxic. appl. Pharmac.* **26**, 180 (1973).
7. D. Keilin and E. F. Hartree, *Biochem. J.* **39**, 293 (1945).
8. J. D. Dunn, T. W. Clarkson and L. Magos, *Br. J. ind. Med.* **35**, 241 (1978).
9. R. N. Feinstein, J. B. Howard, J. T. Braun and J. E. Seaholm, *Genetics, Princeton* **53**, 923 (1966).
10. Y. Sugata, T. W. Clarkson and L. Magos, *Am. ind. Hyg. Ass. J.* **37**, 499 (1976).
11. T. R. Tephly, R. E. Parks, Jr. and G. J. Mannering, *J. Pharmac. exp. Ther.* **131**, 147 (1961).
12. L. Magos, *Envir. Res.* **1**, 323 (1967).

* Present address: Department of Ophthalmology Komagome Hospital, 3–18–22 Hankomagome, Tokyo 113, Japan.

† Reprint requests should be addressed to: Dr. Yasuo Sugata, Department of Ophthalmology, Komagome Hospital, 3–18–22 Hankomagome, Tokyo 113, Japan.

13. F. Nielsen-Kudsk, *Acta pharmac. tox.* **27**, 161 (1969).
14. Y. Sugata, S. Halbach, J. Allen and T. W. Clarkson, *J. envir. Sci. Hlth* **C13** (2), 97 (1979).
15. L. Magos, S. Halbach and T. W. Clarkson, *Biochem. Pharmac.* **27**, 1373 (1978).
16. M. Berlin, J. Fazackerly and G. Nordberg, *Archs envir. Hlth* **18**, 719 (1969).
17. T. W. Clarkson, L. Magos and M. R. Greenwood, *Biol. Neonate* **21**, 239 (1972).

Biochemical Pharmacology, Vol. 28, pp. 3476–3482.

0006-2952/79/1201-3476 $02.00/0

Comparison of hepatic microsomal enzyme induction by methadone, phenobarbital and 3-methylcholanthrene in the mouse*

(*Received* 14 *October* 1978; *accepted* 8 *May* 1979)

In earlier reports, we described an elevation in a variety of hepatic microsomal enzyme activities following oral administation of methadone to the mouse [1–3]. Maximal induction was observed after 6 days of treatment (50 mg/kg/day) for a number of mouse strains [2,3]. Although this type of induction shares some properties with phenobarbital induction, such as an elevation of both type I and type II enzyme activities, increased hepatic microsomal protein, and hepatomegaly [4], it is important to compare methadone to two classic inducers of hepatic microsomal activity: phenobarbital and 3-MC†, which differ from each other in their mechanisms of enzyme induction [4]. Dose–response curves were generated for a family of microsomal parameters and the similarities and differences for the three agents in this study were compared.

Materials and methods

Animals. Male ICR mice (Harlan Industries, Cumberland, IN), with an initial weight of 25–30 g, were housed in clear plastic cages (five to six cage) on San-I-Cel bedding (Paxton Processing Co., White House Station, NJ). The bedding was changed every 2 days to avoid ammonia accumulation, a phenomenon which inhibits microsomal enzyme activity [5]. The mice received Purina Lab Chow (Ralston-Purina Co., St. Louis, MO) and water *ad lib.* The animal quarters were maintained on a 12-hr light/dark cycle at an ambient temperature of 21 ± 1°.

Chemicals and dosing. dl-Methadone hydrochloride was obtained from Mallinckrodt Chemical Works, St. Louis, MO. Sodium phenobarbital and sodium pentobarbital were purchased from Abbott Laboratories, North Chicago, IL; 3-MC was obtained from the Sigma Chemical Co., St. Louis, MO. Methadone was dissolved in water for oral administration, whereas sodium phenobarbital and sodium pentobarbital were dissolved in saline for intraperitoneal administration. 3-MC was administered intraperitoneally dissolved in warm corn oil (Mazola Corn Oil, Best Foods, Englewood Cliffs, NJ). All doses were calculated on the basis of the formula weight of the respective chemical. The volumes of solutions administered via the oral and intraperitoneal routes were 0.02 and 0.01 ml/g body wt, respectively. In the control groups, the appropriate vehicle was substituted for drug administration. The animals were dosed between 2:00 p.m. and 3:00 p.m. daily. Phenobarbital, methadone and 3-MC were given daily for 3, 6 and 5 days, respectively. Phenobarbital and methadone were administered at doses of 3.13, 6.25, 12.5, 25, 35 and 50 mg/kg/day. 3-MC-treated mice received doses of 0.02, 0.20, 2.0, 20 and 200 mg/kg/day.

Tissue preparation and assays. Mice were killed 24 hr after the last dose of chemical or vehicle by cervical dislocation followed by decapitation and exsanguination. The gall bladder was excised, and the liver removed, weighed and immediately homogenized in 3 vol of ice-cold 0.05 M Tris–HCl–0.15 M KCl buffer, pH 7.4. The 12,000 *g* supernatant fraction was used for all enzyme assays as described previously [2]. The procedure and cofactors for each assay were listed by Fouts [6]. Twenty μmoles methadone hydrochloride or 20 μmoles aminopyrine, and 10 μmoles aniline hydrochloride were used as substrates for the *N*-demethylase and hydroxylase assays respectively. Reactions were carried out for 30 min during which time product information in each assay occurred at a constant rate for induced and non-induced animals. Formaldehyde generation in the *N*-demethlase assays was determined according to the method of Nash [7], and PAP formation in the hydroxylase assay was monitored as outlined by Mazel [8]. The microsomal fraction was obtained by centrifuging 0.5-ml aliquots of the 12,000 *g* supernatant fraction for 1 hr at 105,000 *g*. Protein was quantitated according to the method of Lowry *et al.* [9], utilizing bovine serum albumin (Fraction V; Nutritional Biochemical Corp., Cleveland, OH) as the protein standard.

Pentobarbital sleeping times. Animals were challenged 24 hr after the last dose of drug or vehicle with sodium pentobarbital (70 mg/kg) injected intraperitoneally. The time that elapsed between the injection and the loss of righting reflex was noted in addition to the duration of hypnosis (sleeping time), which terminated upon regaining of the righting reflex.

Expression of microsomal parameters. The activities of the three *in vitro* microsomal enzyme systems which were examined are expressed two different ways in the Results. The first expression describes enzyme activity per kg of body weight. This is referred to as relative enzyme activity. The second expression, specific enzyme activity, more conventionally is calculated with respect to enzyme activity per mg of liver microsomal protein per kg of body weight. The rationale for using both expressions has been described previously [10] utilizing two basic concepts.

Statistical analysis. All calculations, statistics and correlations were performed with a DEC-10 computer utilizing SPSS programs [11]. These included calculation of Student's '*t*'-values as well as correlation coefficients (*r*). Dif-

* An initial report of this work was made at the sixtieth Annual Meeting of the Federation of American Societies for Experimental Biology in Anaheim, CA, April 11–16, 1976 (Abstract No. 1450). This work was supported by NIDA Grant DA 01331–02, and in part by NIH Training Grant GM 07099–02, and the University of Mississippi Research Institute of Pharmaceutical Sciences, University, MS.

† Abbreviations: 3-MC, 3-methylcholanthrene; Tris, Tris (hydroxymethyl) aminomethane; NADP, nicotine adeninedinuleotide phosphate; PAP *p*-aminophenol; and *r* correlation coefficient.