*Acta Medica Okayama*

*Volume* 41, *Issue* 6 1987 *Article* 2

DECEMBER 1987

# Effect of Aminotriazole on Mercury Uptake by the Fetus of Normal and Acatalasemic Mice Exposed to Metallic Mercury

Tadamichi Meguro*

*Okayama University,

# Effect of Aminotriazole on Mercury Uptake by the Fetus of Normal and Acatalasemic Mice Exposed to Metallic Mercury*

Tadamichi Meguro

## Abstract

Pregnant normal (N) and acatalasemic (A) mice treated with aminotriazole (AT) were exposed to metallic mercury. The mercury contents of the fetus and maternal organs were subsequently determined. The fetal and placental mercury contents were the highest in the AT-treated A mice (A-AT), and the contents decreased in the order of AT-treated N mice (N-AT), non-treated N mice (N-C) and non-treated A mice (A-C). Statistically significant differences in the fetal mercury levels were observed between N-C and A-C, A-C and N-AT, and N-AT and A-AT. The ratios of the mercury concentration in the fetus to that in the maternal blood decreased in the order of A-AT, N-AT, A-C and N-C. The differences in the ratio were significant between these groups. Similar results were obtained when the ratios of the maternal liver level to the maternal blood level or the ratios of the placental level to the maternal blood level were compared. The effect of AT on mercury uptake is remarkable in the fetus of both normal and acatalasemic mice exposed to metallic mercury.

**KEYWORDS:** aminotriazole, mercury uptake, fetus, acatalasemic mice, metallic mercury

---

*PMID: 3439479 [PubMed - indexed for MEDLINE]

Acta Med Okayama 41 (6) 243-248 (1987)

# Effect of Aminotriazole on Mercury Uptake by the Fetus of Normal and Acatalasemic Mice Exposed to Metallic Mercury

Tadamichi Meguro

*Department of Public Health, Okayama University Medical School, Okayama 700, Japan*

Pregnant normal (N) and acatalasemic (A) mice treated with aminotriazole (AT) were exposed to metallic mercury. The mercury contents of the fetus and maternal organs were subsequently determined. The fetal and placental mercury contents were the highest in the AT-treated A mice (A-AT), and the contents decreased in the order of AT-treated N mice (N-AT), non-treated N mice (N-C) and non-treated A mice (A-C). Statistically significant differences in the fetal mercury levels were observed between N-C and A-C, A-C and N-AT, and N-AT and A-AT. The ratios of the mercury concentration in the fetus to that in the maternal blood decreased in the order of A-AT, N-AT, A-C and N-C. The differences in the ratio were significant between these groups. Similar results were obtained when the ratios of the maternal liver level to the maternal blood level or the ratios of the placental level to the maternal blood level were compared. The effect of AT on mercury uptake is remarkable in the fetus of both normal and acatalasemic mice exposed to metallic mercury.

*Key words :* aminotriazole, mercury uptake, fetus, acatalasemic mice, metallic mercury

Metallic mercury is rapidly oxidized to mercuric ion when it is exposed to human blood *in vitro* (1-3). It has been reported that the uptake of mercury by erythrocytes of Japanese acatalasemic patients is lower than that by erythrocytes of normal subjects (4-7), indicating that catalase (EC 1.11.1.6) oxidizes metallic mercury into mercuric ions and facilitates the uptake of mercury. The transport of elemental mercury into fetal tissues of rats was reported by Clarkson *et al.* (8). Ogata and Meguro reported the distribution of inhaled mercury vapor in the fetus of normal and acatalasemic mice (9).

Aminotriazole (AT), an inhibitor of catalase (14), inhibits oxidation of mercury vapor. The effect of AT on mercury uptake was previously studied *in vitro* using human erythrocytes in the presence of methylene blue and in whole rats (10).

In this study, the effect of AT on mercury uptake by the fetus of normal and acatalasemic mice was determined in order to elucidate the cause of the difference in the organ distribution of mercury between normal and acatalasemic mice.

## Materials and Methods

*Animals.* Normal and acatalasemic female mice of an inbred strain ($C_3H$/AnlC$_s$), which were in the 17th or 18th day of gestation, were used.

*Aminotriazole injection.* 3-Amino-1*H*-1, 2, 4-triazole (AT) (Kanto Chemical Co., Inc, Tokyo)

dissolved in 0.9% saline was injected intraperitoneally at a dose of 1.6 g/kg 30 min prior to the exposure to metallic mercury.

*Exposure to metallic mercury vapor.* Metallic mercury vapor was generated by adding a stannous chloride solution to an aqueous solution of radioactive mercuric chloride ($^{203}HgCl_2$) (specific activity, 0.52 mCi/mg; Amersham International plc, Buckinghamshire, England) in a dish on the bottom of an exposure chamber. After injection of AT, normal and acatalasemic pregnant mice were placed in the middle of the exposure chamber and exposed to metallic mercury vapor (2.0 mg/m$^3$) for 1 h at room temperature (28°C). Mice receiving no injection were used as controls. Thirty minutes after the mice were removed from the exposure chamber, the mice were anesthesized with diethyl ether, and venous blood was taken from the orbital vein. Maternal organs, fetuses and fetal appendices were removed, washed in 0.9% saline and weighed. The amount of mercury was determined by a Multi-Mode Scaler scintillation counter (TDC-601, Aloka Co., Mitaka, Tokyo). The mercury contents were expressed as ng per g wet weight of tissue.

*Statistical analysis.* Statistical significance was evaluated by one way analysis of variance (ANOVA) (11) and Tukey's method (12).

## Results

*Mercury concentration in the fetus and maternal organ.* Mercury concentrations in the maternal blood, fetus and placenta of pregnant non-treated normal (N-C), non-treated acatalasemic (A-C), AT-treated normal (N-AT) and AT-treated acatalasemic (A-AT) mice exposed to metallic mercury are shown in Table 1. The mercury concentration in the maternal blood was the highest in N-AT and decreased in the order of N-C, A-AT, and A-C. On the other hand, the mercury concentration in the fetus was the highest in A-AT and decreased in the order of N-AT, N-C and A-C. Similar results as in the fetus were obtained in the comparison of placental mercury levels.

The results of one way ANOVA of mercury concentrations in the maternal blood, fetus and placenta of the four groups of mice (N-C, A-C, N-AT and A-AT) are shown in Table 2. Differences between mean tissue concentrations of mercury in these groups were analyzed by Tukey's method (12) (Table 3), and it was shown that the mercury concentration in the maternal blood was significantly different ($p < 0.05$) between N-C and N-AT, A-C and A-AT, N-C and A-C, and N-AT and A-AT. The mercury concentration in the fetus was also significantly different between N-C and N-AT ($p < 0.001$), A-C and A-AT ($p < 0.001$), and N-AT and A-AT ($p < 0.05$). Similar results were obtained in the comparison of mercury concentrations in the placenta.

**Table 1** Mercury concentrations (ng Hg/g wet weight) in the maternal blood, fetus and placenta of normal and acatalasemic pregnant mice exposed to metallic mercury

| | Maternal blood | | Fetus | | Placenta | |
|---|---|---|---|---|---|---|
| | (n)[a] | Mean±USD[b] | (n)[a] | Mean±USD[b] | (n)[a] | Mean±USD[b] |
| N-C[c] | (4) | 24.17±7.15 | (18) | 5.79±2.61 | (18) | 55.29±27.50 |
| A-C | (3) | 8.16±1.52 | (11) | 4.51±0.79 | (11) | 26.23± 5.81 |
| N-AT | (3) | 36.20±1.97 | (13) | 20.61±3.83 | (13) | 85.70±17.58 |
| A-AT | (3) | 18.52±5.57 | (16) | 22.89±1.36 | (16) | 102.41±13.18 |

*a*: Number of mice.
*b*: Unbiased standard deviation.
*c*: N-C, non-treated normal mice; A-C, non-treated acatalasemic mice; N-AT, AT-treated normal mice; A-AT, AT-treated acatalasemic mice.

**Table 2** One way ANOVA[a] of mercury concentrations in normal and acatalasemic pregnant mice exposed to metallic mercury

| Factor | d. f.[b] | Mean square (factor) | d. f.[b] | Mean square (error) | F-value |
|---|---|---|---|---|---|
| Maternal blood | 3 | 411.54 | 9 | 25.3327 | 16.25***[c] |
| Fetus | 3 | 1340.98 | 54 | 6.0374 | 222.11*** |
| Placenta | 3 | 14987.7 | 54 | 361.244 | 41.49*** |

*a*: Analysis of variance.
*b*: Degree of freedom.
*c*: $p < 0.001$.

**Table 3** Analysis by Tukey's method of differences between mean values for the mercury concentrations in maternal blood, fetus and placenta in normal and acatalasemic pregnant mice exposed to metallic mercury

| Groups | Maternal blood | | | Fetus | | | Placenta | | |
|---|---|---|---|---|---|---|---|---|---|
| | A-C | N-AT | A-AT | A-C | N-AT | A-AT | A-C | N-AT | A-AT |
| N-C[a] | ** | * | − | − | *** | *** | *** | ** | *** |
| A-C | | *** | * | | *** | *** | | *** | *** |
| N-AT | | | * | | | * | | | * |

*a*: For the abbreviation of groups, see the legend for Table 1.
−, $p > 0.05$; *, $0.05 > p \geq 0.01$; **, $0.01 > p \geq 0.001$; ***, $0.001 > p$.

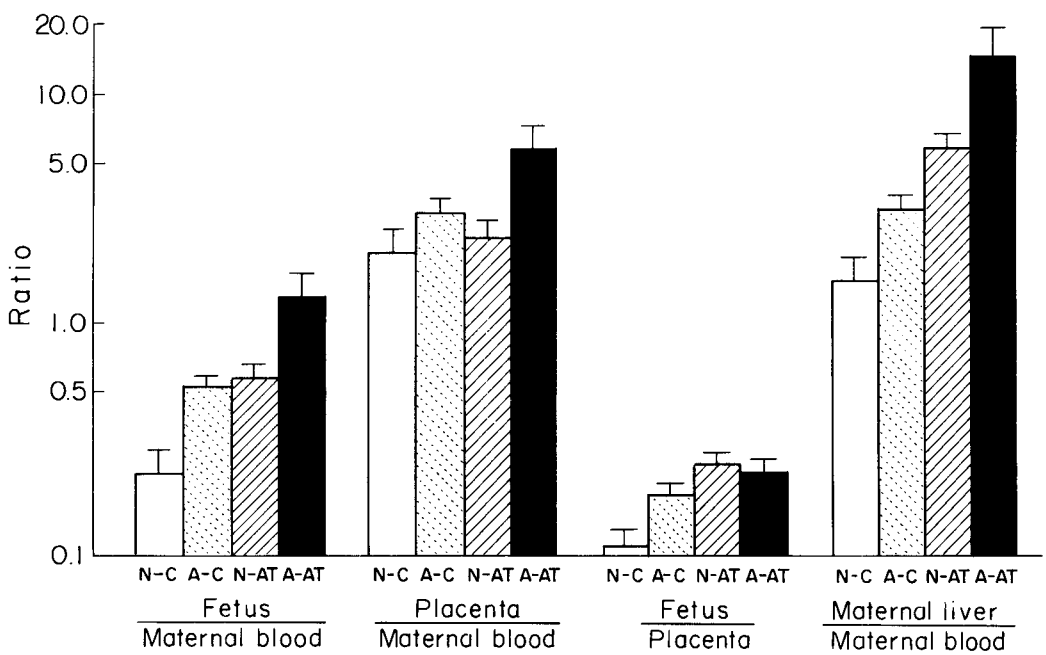


**Fig. 1** Organ/maternal blood ratios and fetus/placenta ratio of mercury concentrations in normal and acatalasemic pregnant mice exposed to metallic mercury. N-C, non-treated normal mice; A-C, non-treated acatalasemic mice; N-AT, AT-treated normal mice; A-AT, AT-treated acatalasemic mice. The bars show mean values, and the lines represent one USD.

**Table 4** One way ANOVA[a] of organ/maternal blood ratios in normal and acatalasemic pregnant mice exposed to metallic mercury

| Factor (Distribution) | d. f.[a] | Mean square (factor) | d. f.[a] | Mean square (error) | F-value |
|---|---|---|---|---|---|
| Maternal liver/maternal blood | 3 | 120.81 | 9 | 5.0285 | 24.03***[b] |
| Placenta/maternal blood | 3 | 47.595 | 54 | 0.7890 | 60.32*** |
| Fetus/placenta | 3 | 0.057066 | 54 | 0.000834 | 68.42*** |
| Fetus/maternal blood | 3 | 3.5451 | 54 | 0.031344 | 113.10*** |

*a*: For abbreviations, see Table 2.
*b*: ***, $p < 0.001$.

**Table 5** Analysis by Tukey's method of differences between mean values for the organ/maternal blood and fetus/placenta ratios of mercury concentrations in normal and acatalasemic pregnant mice exposed to metallic mercury

| | Maternal liver / Blood[a] | | | Placenta / Blood[a] | | | Fetus / Placenta | | | Fetus / Blood[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A-C | N-AT | A-AT | A-C | N-AT | A-AT | A-C | N-AT | A-AT | A-C | N-AT | A-AT |
| N-C | * | * | *** | ** | − | *** | *** | *** | *** | *** | *** | *** |
| A-C | | − | *** | | − | *** | | *** | *** | | − | *** |
| N-AT | | | *** | | | *** | | | − | | | *** |

*a*: Maternal blood.
−, $p > 0.05$; *, $0.05 > p \geq 0.01$; **, $0.01 > p \geq 0.001$; ***, $p < 0.001$.

*The ratios of the mercury concentration in the fetus, placenta and maternal liver to that in the maternal blood, and the mercury concentration in the fetus to that in the placenta (Fig. 1).* The fetus/maternal blood ratio and maternal liver/maternal blood ratio were markedly increased by AT in N and A mice. The placenta/maternal blood ratio was markedly increased by AT in A mice, but the AT-induced increase in the ratio was slight in N mice. The fetus/placenta ratio was markedly increased by AT in N mice, but the increase was rather slight in A mice. The results of one way ANOVA of the data given in Figure 1 are shown in Table 4. There were highly significant differences ($p < 0.001$) in all four ratios (maternal liver/maternal blood, placenta/maternal blood, fetus/placenta and fetus/maternal blood) among the four groups (N-C, A-C, N-AT and A-AT). The results of differences between mean ratios of the four groups of an analysis by Tukey's method are shown in Table 5. The maternal liver/maternal blood ratio was significantly different in all combinations of animal groups, except the combination of A-C versus N-AT. The placenta/maternal blood ratio was significantly different in all combinations of the groups, except the combinations of N-C versus N-AT and A-C versus N-AT. The fetus/placenta ratio was significantly different in all combinations of the groups, except the combination of N-AT versus A-AT. The fetus/maternal blood ratio was significantly different in all combinations of the groups, except the combination of A-C versus N-AT.

## Discussion

The concentration of metallic mercury in the arterial blood has been shown to be

higher in acatalasemic mice exposed to metallic mercury than in normal mice similarly exposed, whereas the concentration of mercuric ion in the blood was lower in acatalasemic mice than in normal mice (13). These results suggest that larger amounts of elemental mercury, which is lipid soluble, pass through the blood-brain, blood-placenta and placenta-fetus barriers in acatalasemic mice than in normal mice. In fact, our previous study (9) indicated that the fetal level of mercury is higher in acatalasemic mice than in normal mice. The concentration of mercury in the liver of acatalasemic mice exposed to metallic mercury has been shown to be significantly higher than that of normal mice (5). Similar results were observed in a comparison of the maternal liver of acatalasemic and normal mice.

As to the inactivation of catalase by aminotriazole, Heim *et al.* (14) observed about a 90% decrease in hepatic and renal catalase activities. Feinstein (15) observed the inhibition of catalase activity by the treatment of whole animals with aminotriazole. They showed the ratio of whole body catalase levels in N-C, A-C, N-AT and A-AT mice to be 100 : 22 : 12 : 1.

Ogata *et al.* (16) have reported the recovery of catalase activity after inhibition by aminotriazole in the liver of acatalasemic mice. Marked differences in the mercury levels in the maternal blood, fetus and fetal appendix were observed between N-C and N-AT and A-C and A-AT.

These findings as a whole suggest that AT increases the concentration of metallic mercury in the maternal blood by inhibiting catalase activity, and that the increased metallic mercury, which is lipid soluble, readily passes through the blood-placenta and placenta-fetus barriers.

Consistent with previous reports (9, 10, 13, 15), the results of the present study show that the mercury contents of organs in mice exposed to metallic mercury vapor vary depending on the catalase activity of the organ, because chemical forms of mercury differ according to the catalase activity.

Investigation of the quantitative relationship between catalase activity and mercury uptake by tissues is under way.

Acknowledgments. I wish to express my sincere thanks to Prof. Masana Ogata for giving me valuable suggestions during this investigation. I am also grateful to Dr. Katashi Kenmochi, Dr. Fumihiko Jitsunari and Dr. Noboru Hirota of the Department of Public Health for technical assistance.

## References

1. Clarkson TW, Gatzy J and Dalton C: Studies on the equilibration of mercury vapor with blood. AEP Report, No. 582, New York, University of Rochester (1961).
2. Kudsk N: Absorption of mercury vapor from the respiratory tract in man. Acta Pharmacol Toxicol (1965) **23**, 250-262.
3. Kudsk N: Factors influencing the *in vitro* uptake of mercury vapor in blood. Acta Pharmacol Toxicol (1969) **27**, 161-172.
4. Ogata M and Ikeda M: *In vitro* mercury uptake by human acatalasemia erythrocytes. Arch Environ Health (1979) **34**, 218-221.
5. Ogata M and Ikeda M: Mercury uptake by acatalasemia mice and their erythrocytes, lungs and liver homogenates. Int Arch Occup Environ Health (1978) **41**, 87-93.
6. Ogata M, Kenmotsu K, Naito T, Meguro T and Hirota N: *In vitro* mercury uptake by hypocatalasemic and acatalasemic mouse hemolysates. Ind Health (1980) **18**, 217-220.
7. Ogata M and Aikoh H: The oxidation of metallic mercury by catalase in relation to acatalasemia. Ind Health (1983) **21**, 219-229.
8. Clarkson TW, Magos L and Greenwood MR: The transport of elemental mercury into fetal tissues. Biol Neonate (1972) **21**, 239-244.
9. Ogata M and Meguro T: Fetal distribution of inhaled mercury vapor in normal and acatalasemic mice. Physiol Chem Phys Med NMR (1986) **18**, 165-170.
10. Magos L, Sugata Y and Clarkson TW: Effects of 3-amino-1, 2, 4-triazole on mercury uptake by *in vitro* human blood samples and by whole rats.

Toxicol and Appl Phar (1974) **28**, 367-373.
11. Snedecor GW and Cochran WG: Statistical methods. The Iowa State University Press, Ames, Iowa, U. S. A. (1982) pp. 97.
12. Fukui S, Yamaoka A, Okada F and Orii M: Primary excercises of stochastics (in Japanese). Sangyo Tosho Publishing Co., Ltd., Tokyo (1965) pp. 311-312.
13. Ogata M, Kenmotsu K, Hirota N, Meguro T and Aikoh H: Mercury uptake *in vivo* by normal and acatalasemic mice exposed to metallic mercury vapor ($^{203}Hg^0$) and injected with metallic mercury or mercuric chloride ($^{203}HgCl_2$). Arch Environ Health (1985) **40**, 151-154.
14. Heim WG, Appleman D and Pyfrom HT: Effects of 3-amino-1, 2, 4-triazole (AT) on catalase and other compounds. Amer J Physiol (1956) **186**, 19-23.
15. Feinstein RN: Acatalasemia in the mouse and other species. Biochem Genetics (1970) **4**, 135-155.
16. Ogata M, Mizugaki J and Takahara S: Recovery of catalase activity after inhibition with aminotriazole in acatalasemia mice. Tohoku J Exp Med (1975) **116**, 39-43.

Received: April 6, 1987
Accepted: August 25, 1987

Correspondence to:
Tadamichi Meguro
Department of Public Health
Okayama University Medical School
2-5-1 Shikatacho
Okayama 700, Japan