Trans. Nat. Acad. Science
& Technol. 1984.6:113-125

ANTIMUTAGENIC EFFECTS OF SOME INORGANIC BIOCHEMICAL SYSTEMS

Clara Y. Lim-Sylianco and Estrella C. Daya
Department of Chemistry, College of Science
University of the Philippines
Diliman, Quezon City

ABSTRACT

Dimethylnitrosamine and benzo(a)pyrene are two carcinogens which induced the formation of micronucleated polychromatic erythrocytes in bone marrow cells of mice. This shows that both carcinogens are mutagenic and clastogenic.

Antimutagenic effects against dimethylnitrosamine and benzo(a)pyrene were studied using biochemical inorganic systems. The assessment of their antimutagenic effects was based on the reduction of micronucleated polychromatic erythrocytes. While the carcinogen was administered intraperitoneally, the inorganic ion was given orally. Except for magnesium and calcium ions, all the others were administered at the same time as the carcinogen. Magnesium and calcium ions were given an hour after the carcinogen.

Calcium was given as calcium chloride, magnesium as $MgCl_2 \cdot 6H_2O$, zinc as zinc chloride, manganese as $MnCl_2 \cdot 4H_2O$ and iron as $FeSO_4 \cdot 7H_2O$. Dolomite, a combination of calcium and magnesium, was also used.

Calcium ions, magnesium ions, manganese ions, zinc ions and ferrous ions all reduced the formation of micronucleated polychromatic erythrocytes induced by dimethylnitrosamine and benzo(a)pyrene. All exhibited antimutagenic and anticlastogenic effects.

The best effect was given by a combination of calcium and magnesium.

Introduction

In the past few years, there has been escalated efforts to uncover substances and physical agents that exhibit genetic toxicity not only to germ cells but also to somatic cells. Greater efforts are encouraged to discover the tendency of some systems to reduce or abolish the genotoxic effects of chemical and physical agents.

Antimutagenic effects of some organic systems have been studied in our laboratory. Antimutagenic effects against aflatoxin B 1, aflatoxin G 1, dimethylnitrosamine, mitomycin C and metronidazole have been studied (1). Vitamin A, E, C, riboflavin and thiamine exhibited antimutagenic effects. Vitamin C was also shown to exhibit antimutagenic effects against alkylating agents, intercalating

Table 1. Formation of Micronucleated Polychromatic Erythrocytes in Bone Marrow Cells as Induced by Dimethylnitrosamine.

Dose of dimethylnitrosamine mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand***
Control*	0.33 ± 0.08
DMN 20	10.89 ± 1.23
15	9.11 ± 0.96
10	5.33 ± 0.34

*Control was untreated
**an average of 15 slides

Table 2. Formation of Micronucleated Polychromatic Erythrocytes in Bone Marrow Cells as Induced by Benzol(a)pyrene.

Dose of Benzo(a)pyrene mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand***
375.00	9.78 ± 1.18
187.50	9.44 ± 1.08
93.75	6.45 ± 0.91
Control*	0.33 ± 0.08

*Control was untreated
**An average of 15 slides

agents, amine derivatives and sodium nitrite (2), and also against two pesticides (3). Vitamin E was also found to be antimutagenic against chloroform (4) and against hexachlorophene (5). Niacin was antimutagenic against metronidazole and benzidine (6).

Among inorganic biochemical systems, only cobalt chloride has been studied (7). It was shown to be antimutagenic against N-methyl-N-nitro-N-nitrosoguanidine. It was also antimutagenic against Trp-p-1, a carcinogen found in charred portions of broiled meat and fish (8). It is therefore of great interest if other inorganic biochemical systems also exhibit antimutagenic effects.

The inorganic systems reported in this study are those which contain calcium ions, magnesium ions, zinc ions, manganese ions and ferrous ions.

Table 3. Effect of Calcium Chloride on Micronuclei Formation of Bone Marrow Cells.

Dose of calcium chloride mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
500	0.84 ± 0.07
400	0.84 ± 0.06
250	0.84 ± 0.05
125	0.84 ± 0.07
Control*	1.00 ± 0.05

*Control was given triple-distilled water

Table 4. Effect of Magnesium Chloride on Micronuclei Formation in Bone Marrow Cells.

Dose of Magnesium chloride mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
450.0	0.16 ± 0.05
300.0	0.40 ± 0.12
225.0	0.50 ± 0.16
112.5	0.50 ± 0.15
Control*	1.00 ± 0.05

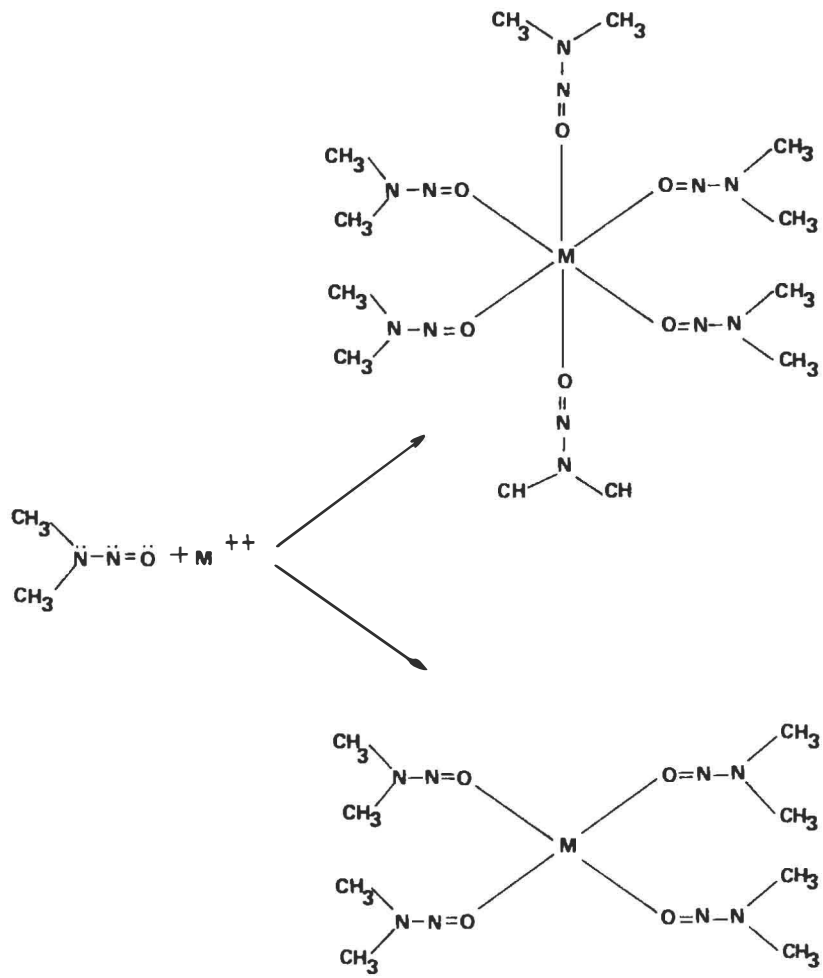
*Control was given triple-distilled water.

Materials and Methods

Dimethylnitrosamine was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin. Benzo(a)pyrene was a gift from Dr. H. Matsushita, Institute of Public Health, Tokyo, Japan. The inorganic salts were purchased from J.T. Baker Chemical Company, N.J. The mice that were used were of the Swiss Webster strain.

Mutagenic and antimutagenic effects were assessed using the micronucleus test of Schmid (9). Mice weighing 20-25 grams were used.

Mutagenic and clastogenic effects of dimethylnitrosamine and benzo(a)pyrene, and the inorganic systems were determined separately.



Formation of DMN-metal ion complex.

For antimutagenic effects, dimethylnitrosamine and benzo(a)pyrene were given intraperitoneally, while the inorganic systems were administered orally at approximately the same time except for calcium and magnesium which were given an hour after the carcinogens. The carcinogens and the inorganic systems were administered twice, 30 hours and 6 hours prior to the preparation of the bone marrow.

Bone marrow of the femur was flushed into a test tube using fetal calf serum. Air dried smears were made from the pellet. These were stained and examined for micronuclei formation in polychromatic erythrocytes.

Table 5. Effect of Manganese Chloride on Micronuclei Formation in Bone Marrow Cells.

Dose of manganese chloride mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
420	1.67 ± 0.08
320	1.67 ± 0.09
210	1.67 ± 0.07
105	1.67 ± 0.08
Control*	

*Control was given triple-distilled water

Table 6. Effect of Ferrous Sulfate on Micronuclei Formation in Bone Marrow Cells.

Dose of ferrous sulfate mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
625.00	1.67 ± 0.21
312.50	1.34 ± 0.09
220.0	1.33 ± 0.08
156.25	1.33 ± 0.09
Control*	1.66 ± 0.12

*Control was given triple-distilled water.

Results and Discussion

Dimethylnitrosamine and benzo(a)pyrene induced the formation of micronucleated polychromatic erythrocytes (Tables 1 and 2). This indicates that these carcinogens affected the DNA of the bone marrow cells. Mitotic bone marrow cells with chromatid breaks or chromatid exchanges suffer from disturbance in the anaphase distribution of their chromatin. Chromosome pieces lag in the anaphase. After telophase, a sizable portion of the displaced chromatin is not included in the nuclei of the daughter cells. Instead, they form single or multiple micronuclei in the cytoplasm of these cells. Thus, both dimethylnitrosamine and benzo(a)pyrene are not only mutagenic but also clastogenic.

Table 7. Effect of Zinc Chloride on Micronuclei Formation In Bone Marrow Cells.

Dose of Zinc Chloride mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
150.0	0.84 ± 0.07
100.0	0.95 ± 0.09
75.0	0.95 ± 0.09
37.5	0.95 ± 0.09
Control*	1.66 ± 0.12

*Control was given triple-distilled water

Table 8. Antimutagenic Effects of Calcium Ions Against Dimethylnitrosamine*

Dose of calcium chloride mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
Dimethylnitrosamine alone	9.11 ± 0.96
Dimethylnitrosamine plus	
Calcium – 500	2.22 ± 0.05
Calcium – 250	2.33 ± 0.07
Calcium – 125	2.89 ± 0.08

*Dimethylnitrosamine – 15 mg/kg

Table 9. Antimutagenic Effects of Calcium Ions Against Benzo(a) Pyrene.

Dose of calcium chloride mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
Benzo(a)pyrene alone*	9.44 ± 1.08
Benzo(a)pyrene plus	
Calcium – 500	2.89 ± 0.08
Calcium – 250	2.89 ± 0.08
Calcium – 125	2.67 ± 0.09

*Benzo(a)pyrene –187.5 mg/kg

Table 10. Antimutagenic Effects of Magnesium Ions Against Dimethylnitrosamine.

Dose of magnesium chloride mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
Dimethylnitrosamine alone*	9.11 ± 0.96
Dimethylnitrosamine plus	
magnesium – 450.0	1.33 ± 0.09
magnesium – 225.0	2.22 ± 0.05
magnesium – 112.5	2.45 ± 0.10

*Dimethylnitrosamine – 15 mg/kg

Table 11. Antimutagenic Effects of Magnesium Ions Against Benzo(a)pyrene.

Dose of magnesium chloride mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
Benzo(a)pyrene alone*	9.44 ± 1.08
Benzo(a)pyrene plus	
magnesium – 450.0	1.22 ± 0.11
magnesium – 225.0	2.11 ± 0.12
magnesium – 112.5	2.33 ± 0.09

*Benzo(a)pyrene – 187.5 mg/kg

Table 12. Antimutagenic Effects of Zinc Ions Against Dimethylnitrosamine.

Dose of Zinc chloride mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
Dimethylnitrosamine alone*	9.11 ± 0.96
Dimethylnitrosamine plus	
Zinc chloride – 150.0	1.33 ± 0.11
Zinc chloride – 75.0	1.44 ± 0.12
Zinc chloride – 37.5	1.67 ± 0.09

*Dimethylnitrosamine – 15 mg/kg

Table 13. Antimutagenic Effects of Zinc Ions Against Benzo(a)pyrene.

Dose of Zinc chloride mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
Benzo(a)pyrene alone*	6.45 ± 0..91
Benzo(a)pyrene plus	
Zinc chloride – 150.0	1.67 ± 0.12
Zinc chloride – 75.0	1.78 ± 0.13
Zinc chloride – 37.5	1.78 ± 0.12

*Benzo(a)pyrene – 93.75 mg/kg

Table 14. Antimutagenic Effects o f Manganese Ions Against Dimethylnitrosamine.

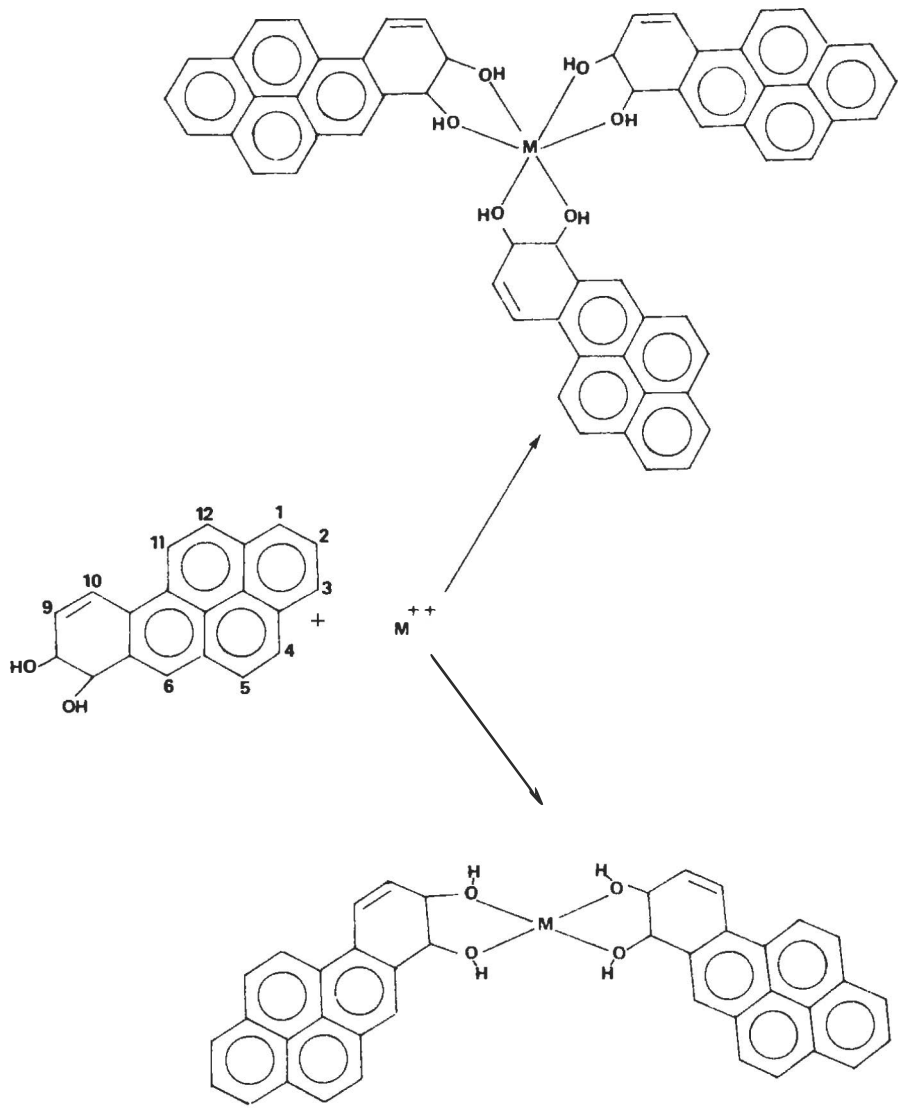
Dose of manganese chloride mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
Dimethylnitrosamine alone	9.11 ± 0.96
Dimethylnitrosamine plus	
Mn chloride – 420	1.67 ± 0.15
Mn chloride – 210	1.78 ± 0.18
Mn chloride – 105	2.11 ± 0.15

*Dimethylnitrosamine – 15 mg/kg

Table 15. Antimutagenic Effects of Manganese Ions Against Benzo(a)pyrene.

Dose of manganese chloride mg/kg	*No of micronucleated polychromatic erythrocytes per thousand*
Benzo(a)pyrene alone*	6.45 ± 0.91
Benzo(a)pyrene plus	
Mn chloride – 420	1.78 ± 0.14
Mn chloride – 210	1.89 ± 0.16
Mn chloride – 105	2.00 ± 0.18

*Benzo(a)pyrene – 93.75 mg/kg



Complex formation between a metal ion and BP-7, 8-diol.

Table 16. Antimutagenic Effects of Ferrous Ions Against Dimethylnitrosamine.

Dose of ferrous chloride mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
Dimethylnitrosamine alone*	9.11 ± 0.96
Dimethylnitrosamine plus	
ferrous chloride – 625.00	1.78 ± 0.12
ferrous chloride – 312.50	2.00 ± 0.15
ferrous chloride – 156.25	2.11 ± 0.16

*Dimethylnitrosamine – 15 mg/kg

Table 17. Antimutagenic Effects of Ferrous Ions Against Benzo(a)pyrene.

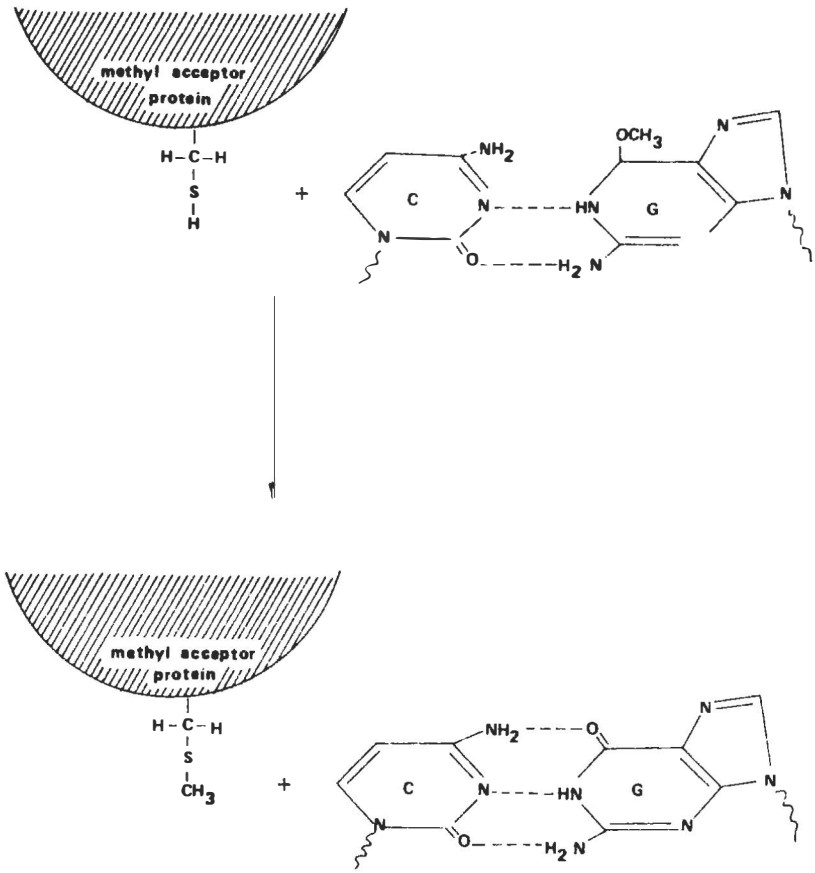
Dose of ferrous chloride mg/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
Benzo(a)pyrene alone*	6.45 ± 0.91
Benzo(a)pyrene plus	
ferrous chloride – 625.00	2.11 ± 0.17
ferrous chloride – 312.50	2.33 ± 0.12
ferrous chloride – 156.25	2.67 ± 0.21

*Benzo(a)pyrene – 93.75 mg/kg

The inorganic biochemical systems used were also studied as regards their effect on DNA of the bone marrow cells. The results are shown in Tables 3 to 7. None of the systems tested induced the formation of micronucleated polychromatic erythrocytes. These systems did not affect the DNA of the bone marrow cells.

Calcium ions caused the reduction of micronucleated polychromatic erythrocytes induced by dimethylnitrosamine and benzo(a)pyrene (Tables 8 and 9). The same observation was made of magnesium ions (Tables 10 and 11), of zinc ions (Tables 12 and 13), of manganese ions (Tables 14 and 15), and of ferrous ions (Tables 16 and 17). A combination of calcium and magnesium gave the best reduction in the formation of micronucleated polychromatic erythrocytes (Tables 18 and 19).

It is very clear that the five metal ions counteract the mutagenic and clastogenic effects of dimethylnitrosamine and benzo(a)pyrene. Each of the metal ions



Demethylation reaction

through their vacant hybrid orbitals can interact with dimethylnitrosamine resulting in the inhibition of its metabolic activation to a mutagen.

Dimethylnitrosamine can readily bind with metal ions through the lone pairs of oxygen. Both calcium and magnesium ions can form hexacoordinated compounds. The transition metal ions, ferrous ions, zinc ions and manganese ions can form 4- and 6- coordinate complexes. Of the tetracoordinated compounds, the tetrahedral configuration is more favored for steric reasons.

Benzo(a)pyrene after metabolism forms diols (10) which can form metal complexes through the lone pairs of oxygen.

It is possible that metal ions can deplete the concentration of cytochrome P-450 in the cells. The enzymes in this system are responsible for the mutagenic activation of dimethylnitrosamine and benzo(a)pyrene (11).

Table 18. Antimutagenic Effects of Dolomite* Against Dimethylnitrosamine.**

Dose of Dolomite tablet/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
Dimethylnitrosamine alone	9.11 ± 0.96
Dimethylnitrosamine plus	
Dolomite – 2 tablets	0.56 ± 0.08
Dolomite – 1 tablet	0.67 ± 0.08
Dolomite – ½ tablet	0.78 ± 0.08

*Dolomite – 1 tablet contains 390 mg calcium and 180 mg magnesium
**Dimethylnitrosamine – 15 mg/kg

Table 19. Antimutagenic Effects of Dolomite Against Benzo(a)pyrene.**

Dose of Dolomite tablet/kg	*No. of micronucleated polychromatic erythrocytes per thousand*
Benzo(a)pyrene alone	6.45 ± 0.91
Benzo(a)pyrene plus	
Dolomite – 2 tablets	0.89 ± 0.05
Dolomite – 1 tablet	1.11 ± 0.08
Dolomite – ½ tablet	1.11 ± 0.08

*Dolomite – 1 tablet contains 390 mg calcium and 180 mg magnesium
**Benzo(a)pyrene – 93.75 mg/kg

Co^{++} has been shown to increase the rate of heme oxidation which led to reduction in the activity of cytochrome P-450 (12). This effect was also observed with Fe^{++}, Zn^{++}, Mn^{++}, and other metal ions (13).

Thus, the antimutagenic effect of the metal ions in this study could be a consequence of reduced activity of cytrochrome P-450. Divalent metal ions have been shown not only to reduce the activity of cytochrome P-450 but also to enhance the activity of glutathione epoxide transferase (14). This enzyme opens up epoxides and therefore reduces their alkylating ability. Extent of alkylation of DNA by benzo(a)pyrene epoxides can thus be reduced in the presence of metal ions.

Another possibility is the activation of the adaptive response repair system by the metal ions. This repair system has been shown to remove methyl groups from 06 guanine (15). Methylated 06 guanine is formed in the presence of dimethylnitrosamine. Therefore, if DNA is alkylated by dimethylnitrosamine, the adaptive

response repair mechanism can transform the altered base to its original form. This is illustrated on page 123.

Conclusion

Dimethylnitrosamine and benzo(a)pyrene are mutacarcinogens since they induce the formation of micronucleated polychromatic erythrocytes in bone marrow cells.

Their mutagenicity, however, was reduced when calcium ions, magnesium ions, ferrous ions, zinc ions, and manganese ions. Calcium and magnesium ions were administered an hour after the mutagen while the other ions were given at the same time as the mutagen.

A combination of calcium and magnesium gave the best anti-mutagenic effects.

Literature Cited

1. Sylianco, C.Y. Lim, J. San Agustin, I. Panizares, S.M. Karunungan and A.P. Jocano, 1981. Antimutagenic Effects of Some Vitamins on Bone Marrow erythrocytes of Mice Treated with Various Mutagens, *Asian J. of Pharmaceutical Sciences* 3:1925.
2. Sylianco, C.Y. Lim and L.C. Brion, June. 1981. Antimutagenic Effects of L-Ascorbic Acid, *NRCP Research Bulletin* **36**(2): 208-219.
3. Sylianco, C.Y. Lim, I. Panizares, E. Español, C. Lui, 1978. Mutagenicity Potential of Some Pesticides, *NRCP Research Bulletin*, **33**:91-100.
4. San Agustin, J. and Sylianco, C.Y. Lim, 1978. Mutagenic and Clastogenic Effects of Chloroform, *Bull. Phil. Biochem. Soc.* 1:17-23.
5. Sylianco, C.Y. Lim, 1978. Some Interactions Affecting the Mutagenicity Potential of Hexachlorophene, Malathion and Thiodan. *Mutation Research* **53**:271.
6. Sylianco, C.Y. Lim, 1983. Desmutagens and Antimutagens. *Acta Medica Philippina*, 19: 33-39.
7. Inoue, P., Y. Ohta, Y. Sadaeie and T. Rada, 1981. Effect of Cobaltous Chloride on Spontaneous Mutation Induction of B. subtilis Mutator Strain. *Mutation Research* 91: 41.
8. Kada, P., Cobaltous chlorine antimutagen on Trup-P-1. *Mutation Research (In Press)*.
9. Schmid, W., 1973. *Agents and Actions* 3:77-78.
10. Selkirk, J.K., 1977. HPLC, a New Technique for Studying Metabolism and Activation of Chemical Carcinogens, *Environmental Cancer*, pp. 1-23 J Hemisphere Publishing Co., Washington, U.S.A.
11. Gelboin, H.V., 1980. Benzo(a)pyrene metabolism, Activation and Carcinogenesis. *Physiol. Revs.* 60:1107-1166.
12. Tephly, T.R. and P. Hibbelin, 1971. The Effect of Cobalt Chloride Administration on the Synthesis of Hepatic Microsomal Cytochrome P-450. *Biochem. Biophys. Res. Commum.* **42**:589-595.
13. Maines, M.D. and A. Kappas, 1976. Studies on the Induction of Haem Oxygenases by Cobalt and other ions. *Biochem. J.* **154**:125-131.
14. Nemoto, N., 1975. K Region Benzo(a)pyrene 4, 5-oxide is Conjugated by Glutathione S-transferase. *Nature* **255**:512.
15. Pegg. A.A., 1978, Enzymatic Removal of 0-6 methylguanine from DNA by Mammalian Cell Extracts. *Biochem. Biophys. Res. Commun.* **84**:166-173.