Arh. hig. rada, *28* (1977) 29.

THE EFFECT OF NITROGEN OXIDES AND PRODUCTS OF THEIR NEUTRALIZATION BY AMMONIA ON THE HEMATOPOIETIC SYSTEM IN GUINEA PIGS

S. Kośmider, A. Misiewicz and A. Węgiel

II Clinic of Internal Diseases, Clinic of Occupational Diseases and Clinical Toxicology, and Institute of Internal Diseases, Silesian School of Medicine, Katowice, Poland

(Received for publication May 25, 1975.)

The effect of nitrogen oxides on the hematopoietic system was studied. The experiments were carried out on 90 guinea pigs. The animals were divided into three equal groups: a control group (A), a group (B) exposed to nitrogen oxides at a concentration of 1 mg/m³, eight hours a day for a period of 120 days and a third group (C), which was exposed to the reaction products of nitrogen oxides and ammonia for 120 days.

It was shown that nitrogen oxides stimulate erythropoiesis which resulted in a marked accumulation of erythrocytic cells in the bone marrow. The proportion of the erythropoietic to the leukocytic system was 1 : 1, while in the control group it was 1 : 2. At the same time an increased number of red cell reticulocytes and an elevated hemoglobin level were observed. The observed increase of ALA-D activity in erythrocytes with a simultaneous decrease in the urinary elimination of ALA and an increased elimination of coproporphyrins was quite an interesting phenomen. These data might be indicative of an intensified hem synthesis. The neutralization of nitrogen oxides by gaseous ammonia led to the formation of reaction products and under their influence the picture of the peripheral blood and bone marrow became similar to that observed in the control group.

Nitrogen oxides are among main gaseous contaminants of the atmospheric air. The development of industry and transport brings about an increased emission of these gases into the atmosphere. So far much attention has been paid to the lesions of the respiratory system pro-

This work was supported by the U. S. Environmental Protection Agency, Contract No. 5-533-1

duced by nitrogen oxides (1—7). Toxic impairment of the mucous membranes of the bronchial tree and alveoli, though it can be easily observed, marks only the beginning of the effect of nitrogen oxides upon the entire system.

Methemoglobinemia has long been known to be due to the action of nitrogen oxides; its level and duration are associated with the intensity of concentration and duration of exposure (1, 8). Thus in addition to the respiratory system, blood is another organ which is easily affected by nitrogen oxides. However, to our knowledge this problem has attracted little attention in literature. A single exposure of animals to a high NO_2 concentration had no effect on blood platelet count and hematocrit index (9).

Similar results were obtained in some cases of chronic exposure (10). No changes in hemoglobin concentration or erythrocyte count were observed either (11).

Other investigations performed in different animal species revealed that only after a few weeks of exposure to nitrogen dioxide at a concentration of 2 ppm, a marked increase of erythrocyte count and hemoglobin level were found (12).

It should be mentioned that children living in the areas contaminated by nitrogen oxides showed an increased osmotic resistance of erythrocytes and a higher number of reticulocytes (13).

The few reports quoted above refer only cursorily to the effects produced on some of the components of peripheral blood. The results are difficult to compare as the investigations were carried out on different animal species to different NO_2 concentrations for a different length of time. The precision of NO_2 dosing and several other factors also varied.

Taking all this into account we studied the reaction of the hematopoietic system in animals exposed to nitrogen oxides for several months. At the same time we succeeded in evaluating, from the toxicological point of view, the binding of nitrogen oxides to gaseous ammonia.

MATERIAL AND METHODS

The experiments were carried out in guinea pigs. A total of 90 animals were divided into three equal groups; group A served as control, group B was exposed to nitrogen oxides and group C was exposed to the reaction products of nitrogen oxides and ammonia.

The experimental set-up and exposure was the same as described in the previous paper (14). The following blood analyses were carried out in all the animals:

1. Hemoglobin level was estimated by means of »Haemotest« — Testa Laboratory, Copenhagen.

2. Erythrocyte and white blood cells count was estimated according to the usual technique with stirrers.

3. Hematocrit index was estimated in heparinized capillaries centrifuged in a hematocrit centrifuge of *Hawksley* make.

4. Mean volume of erythrocytes (M. V. R.), mean concentration of hemoglobin (M. C. H.) and mean weight of hemoglobin (M. W. H.) in an average erythrocyte were calculated according to commonly accepted methods (15).

5. Total white blood cell count, a percentage composition in smears dyed with May-Grünwald-Giemsa method, and an absolute number of particular cell series were estimated.

6. Reticulocytes were calculated after dyeing with cresyl diamond blue (16). The results were expressed as a mean of two counts.

7. The number of blood platelets was estimated according to Fonio.

8. Methemoglobin was estimated according to *Pehr* and *Nosek* (17).

9. The activity of delta-aminolevulinic acid dehydratase (ALD) in erythrocytes was estimated according to *Bonsignore* and coworkers (18).

Examinations of the bone marrow were also performed. The samples were taken from the femur. A percentage composition of a particular cell series and an index of maturation were estimated. The urine of the animals kept in Roth cages was collected several times within 24 hours. Nitrites and nitrates were estimated per 1 g of creatinine according to Mellete's method (19), and coproporphyrin level per 1 g of creatinine according to *Haeger-Aronsen* (20). Besides, the content of delta-aminolevulinic acid in daily urine was estimated according to Mauzeralli's method modified by *Grabecki* (21).

The results were expressed as arithmetic means (X) and standard deviations (SD) while the difference between the means was tested by Student's t-test.

RESULTS

The animals chronically exposed to NO_2 at a concentration of 1 mg/m^3 showed an increased respiration rate throughout the experiment. They showed no excitement or aggressiveness and the heart rate was not increased. The animals exposed to the reaction products of NO_2 and ammonia showed a slight increase of respiration rate during the first days of experiment.

Methemoglobin level in animals exposed to nitrogen oxides was higher as compared with the controls (Table 1). Urinary elimination of nitrates and nitrites was also increased. The binding of nitrogen oxides to ammonia resulted in a decreased methemoglobin level in the blood and in reduced urinary elimination of nitrates and nitrites. (Table 1).

ALA-D activity in the erythrocytes of the animals exposed to nitrogen oxides was significantly higher as compared with the controls. At the

Table 1

Methemoglobin level in the blood serum and daily urinary elimination of nitrates and nitrites in control and experimental animals

	Group A x ± SD (range)	Group B x ± SD (range)	Group C x ± SD (range)	Statistical difference A : B =	Statistical difference B : C =
Methemoglobin level (%)	0.080 ± 0.010	0.280 ± 0.077	0.141 ± 0.017	$P < 0.025$	NS
Nitrates in urine after 1 month of exposure in mg/1 g creatinine	1.19 ± 0.028 0.88 — 1.30)	2.53 ± 0.37 (1.60 — 2.90)	1.49 ± 0.080 (1.02 — 1.56)	$P < 0.001$	$P < 0.005$
Nitrates in urine after 3 months of exposure (in mg/g creatinine	0.080 ± 0.010	0.219 ± 0.033	0.173 ± 0.019	$P < 0.001$	NS
Nitrates + nitrites in urine after 1 month of exposure in mg/g creatinine	0.129 ± 0.032	0.441 ± 0.110	0.185 ± 0.021	$P < 0.02$	$P < 0.05$
Nitrates + nitrites in urine after 3 months of exposure in mg/g creatinine	0.129 ± 0.032	0.294 ± 0.070	0.214 ± 0.014	NS	NS

same time urinary elimination of delta-aminolevulinic acid decreased and the elimination of coproporphyrins insignificantly increased. After the exposure of animals to the reaction products of nitrogen oxides and ammonia (Group C) the values of ALA-D and coproporphyrins were similar to those observed in the control group (Table 2).

In the peripheral blood of the animals chronically exposed to nitrogen oxides the number of reticulocytes and hemoglobin level were increased. The hematocrit and dye index did not change.

At the same time a slight increase of mean hemoglobin concentration in an average erythrocyte in the animals exposed to nitrogen oxides was found. On the other hand, the mean volume of an erythrocyte and the mean weight of hemoglobin were lower. After the exposure to the reaction products of nitrogen oxides and ammonia an opposite tendency was observed towards normalization. It should be pointed out that hemoglobin level, mean concentration of hemoglobin in an average erythrocyte and the number of erythrocytes decreased below the values observed in the control group. This phenomenon was accompanied by blood dilution manifested by a lower hematocrit level and a higher mean volume of an average erythrocyte. No essential differences in the white cells between particular groups of animals were found in reference to total white cell count or to reciprocal percentage values of particular cellular systems. A tendency to an increased percentage number of lymphocytes could only be noted after the exposure to nitrogen oxides and a decreased percentage of neutrophilic cells with a divided nucleus and acidophilic cells among granulocytes was found (Tables 3, 4).

An increased accumulation of cells in the erythropoietic system was found in the bone marrow of the animals exposed to nitrogen oxides. The ratio of the erythropoietic system to that of leukocytes was 1 : 1; in controls it was 1 : 2. The percentage number of proerythroblasts and other cells (basophilic erythroblasts) in the erythropoietic system was increased. An increased maturation index (the proportion of polychromatophilic erythroblasts) was also found in animals chronically exposed to nitrogen oxides as compared with the control group. The percentage number of the cells in the granulopoietic system in the bone marrow of animals exposed to nitrogen oxides was lower than in controls. The number of myelocytes, metamyelocytes and granulocytes with a divided nucleus was most markedly decreased. The percentage number of eosinophilic granulocytes in the animals exposed to nitrogen oxides was decreased, whereas no essential changes in the reticulum texture were found (Table 5).

After the cessation of exposure to the reaction products of nitrogen oxides and ammonia the marrow picture of erythroblasts completely normalized. This concerned not only the total value of the cells of this system but also particular series of erythrocyte precursors. A similar normalization was also found in the majority of cellular series of marrow texture of leukocytes (Table 5).

Table 2

ALA-D activity in erythrocytes and daily urinary elimination of ALA and coproporphyrins in control and experimental animals

	Group A $\bar{x} \pm SD$ (range)	Group B $\bar{x} \pm SD$ (range)	Group C $\bar{x} \pm SD$ (range)	Statistical difference A : B =	Statistical difference B : C =
ALA-D activity (enzymic units/hr/ml erythrocytes)	29,28 ± 11.5 14.88 — 60.81	88.06 ± 27.29 47.79 — 143.75	34.84 ± 12.07 20.83 — 70.31	$P < 0.001$	$P < 0.001$
ALA (mg/l)	1.30 ± 0.75	0.71 ± 0.35	1.43 ± 0.89	$P < 0.01$	$P < 0.01$
ALA mg/1 g creatinine)	2.18 ± 1.27	1.21 ± 0.58	2.71 ± 1.31	$P < 0.01$	$P < 0.001$
Coproporphyrins (mg/1 g creatinine)	17.68 ± 1.53	21.10 ± 5.95	18.88 ± 3.24	NS	NS

Table 3

Peripheral blood in control and experimental animals

	Group A $\bar{x} \pm SD$ (range)	Group B $\bar{x} \pm SD$ (range)	Group C $\bar{x} \pm SD$ (range)	statistical difference A : B =	statistical difference B : C =
Hemoglobin (g%)	12.85 ± 0.70 11.20 — 13.76	13.04 ± 0.82 12.0 — 14.72	10.69 ± 1.97 9.92 — 12.80	N. S.	$P < 0.001$
Hematocrit %	41.6 ± 3.12 38.0 — 47.0	42.13 ± 2.87 38.0 — 50.0	39.03 ± 2.17 36.00 — 43.0	N. S.	$P < 0.001$
Erythrocytes (1000/mm³)	3988.71 ± 250.0 3520.0 — 4360.0	4209.3 ± 284.4 3650.0 — 4620.0	3512.0 ± 224.6 3200.0 — 4150.0	$P < 0.005$	$P < 0.001$
Index	1.01 ± 0.02 0.98 — 1.05	1.00 ± 0.07 0.95 — 1.01	0.99 ± 0.02 0.96 — 1.02	N. S.	N. S.
Leukocytes (in 1 mm³)	8293.0 ± 3058.0 3000.0 — 14000.0	8296.0 ± 3301.0 3400.0 — 14000.0	9672.0 ± 3532.0 4200.0 — 16800.0	N. S.	N. S.
Lymphocytes (%)	88.3 ± 3.83 84.0 — 97.0	90.13 ± 5.41 73.0 — 96.0	91.21 ± 4.85 72.0 — 98.0	N. S.	N. S.
Segments (%)	7.63 ± 3.21 4.0 — 13.0	6.58 ± 4.39 1.0 — 22.0	5.78 ± 3.21 1.0 — 12.0	N. S.	N. S.
Band forms (%)	0.06 ± 0.25 0.0 — 1.0	0.103 ± 0.30 0.0 — 1.0	0.12 ± 0.29 0.0 — 1.0	N. S.	N. S.
Basophils (%)	0.033 ± 0.182 0.0 — 1.0	0.06 ± 0.25 0.0 — 1.0	0.04 ± 0.23 0.0 — 1.0	N. S.	N. S.
Eosinophils (%)	3.53 ± 2.72 1.0 — 7.0	2.517 ± 1.87 0.0 — 7.0	2.48 ± 1.63 1.0 — 8.0	N. S.	N. S.
Monocytes (%)	0.447 ± 0.62 0.0 — 2.0	0.71 ± 0.74 0.0 — 2.0	0.37 ± 0.49 0.0 — 1.0	N. S.	N. S.
Reticulocytes (%)	6.0 ± 3.0 4.0 — 9.0	9.0 ± 2.0 6.0 — 11.0	8.0 ± 3.0 6.0 — 10.0	$P < 0.001$	N. S.
Thrombocytes (in 1 mm³)	60.425 ± 24.293 28.140 — 85.250	36.729 ± 12.431 23.100 — 78.480	35.186 ± 14.547 17.500 — 73.200	$P < 0.005$	N. S.

Table 4

Mean concentration of hemoglobin, mean weight of hemoglobin and mean volume of an erythrocyte in control animals (Group A), in animals exposed to nitrogen oxides (Group B) and in animals exposed to the reaction products of nitrogen oxides and ammonia (Group C).

	Group A $\bar{x} \pm SD$ (range)	Group B $\bar{x} \pm SD$ (range)	Group C $\bar{x} \pm SD$ (range)	Statistical difference A : B —	Difference between means $m_A - m_B$	Student's t-test t	Statistical difference B : C —
Mean hemoglobin concentration (%)	30.9 ± 1.6 (27.2 — 34.2)	31.1 ± 0.9 (29.4 — 33.0)	28.0 ± 1.6 (25.5 — 30.2)	N. S.	3.1	10.3	$P < 0.001$
Mean volume of erythrocyte (um^3)	104.1 ± 6.55 (94.5 — 110.0)	99.9 ± 3.7 (96.0 — 108.0)	111.2 ± 8.2 (99.0 — 125.0)	$P < 0.005$	11.3	6.64	$P < 0.001$
Mean hemoglobin weight p. g.	32.2 ± 0.9 (30.3 — 33.9)	31.0 ± 0.8 (30.0 — 32.0)	31.3 ± 0.8 30.0 — 32.6)	$P < 0.001$	0.3	1.5	N. S.

Table 5

Percentage values of bone marrow cells in the control and experimental groups

		Group A $\bar{x} \pm SD$	Group B $\bar{x} \pm SD$	Group C $\bar{x} \pm SD$ (range)	Statistical difference A : B —	Statistical difference B : C —
Erythropoetic system	Proerythroblasts	0.4 ± 0.1	1.2 ± 0.2	0.5 ± 0.2	$p < 0.001$	$p < 0.001$
	Basophilic erythroblasts	5.6 ± 2.4	8.6 ± 2.8	6.2 ± 2.0	$p < 0.001$	$p < 0.005$
	Polychromatic erythroblasts	10.0 ± 3.2	13.8 ± 4.8	10.9 ± 4.2	$p < 0.005$	$p < 0.05$
	Orthochromatic erythroblasts	12.4 ± 3.6	16.0 ± 5.2	13.2 ± 4.0	$p < 0.005$	$p < 0.05$
	Total	28.4 ± 6.2	39.6 ± 7.4	30.8 ± 6.8	$p < 0.001$	$p < 0.001$
	Index of maturation of erythrocytes system, polychromatophilic & orthochromatic erythroblasts	0.803 ± 0.16	0.872 ± 0.21	0.817 ± 0.19		
Myeloblasts		1.2 ± 0.5	1.1 ± 0.6	1.0 ± 0.4	N. S.	N. S.
Promyeloblasts		2.8 ± 1.2	2.5 ± 1.6	2.2 ± 1.4	N. S.	N. S.
Leukop. system	Myelocytes	9.2 ± 2.4	6.8 ± 2.4	8.8 ± 2.2	$p < 0.005$	$p < 0.01$
	Metamyelocytes	6.8 ± 1.8	5.2 ± 1.6	7.2 ± 2.0	$p < 0.005$	$p < 0.001$
	Rod neutrophils	5.8 ± 1.8	6.3 ± 2.4	6.4 ± 3.0	N. S.	N. S.
	Filamented neutrophils	15.2 ± 4.6	11.1 ± 3.8	12.4 ± 3.9	$p < 0.005$	N. S.
	Eosinophils	2.6 ± 1.6	4.2 ± 2.2	2.8 ± 1.8	$p < 0.01$	$p < 0.05$
Total		43.6 ± 7.4	37.2 ± 6.8	40.8 ± 7.2	$p < 0.01$	N. S.
Lymphatic texture		18.4 ± 8.2	12.4 ± 5.2	16.2 ± 6.6	$p < 0.01$	N. S.
Reticular texture		9.6 ± 4.4	10.8 ± 4.2	12.2 ± 5.6	N. S.	N. S.

DISCUSSION

The investigations carried out have demonstrated distinct differences in the reaction of the hematopoietic system in control animals in those chronically exposed to nitrogen oxides and in the group exposed to reaction products of nitrogen oxide and ammonia. The changes observed concerned both the peripheral blood as well as the hematogenic marrow texture. Methemoglobinemia occurred in all animals chronically exposed to nitrogen oxides and in some cases methemoglobin level amounted to 3%. The lowest methemoglobin level in animals exposed to nitrogen oxides markedly exceeded the highest methemoglobin values observed in controls. Methemoglobinemia which occurs after the exposure to nitrogen oxides is not a new phenomenon, but it is worth mentioning because it appears and is maintained after the exposure to comparatively low concentrations. Further changes in the peripheral blood consisted in an elevated erythrocyte count, an increased mean hemoglobin concentration in an average erythrocyte, and in an increased leukocyte number. We have not estimated the volume of the circulating blood and circulating erythrocytic mass, but with the same hematocrit index one can suppose that chronic exposure of animals to nitrogen oxides may lead to some hyperplethoric features. This is quite acceptable since the changes in the peripheral blood were due to the stimulation of erythropoiesis in the marrow. A large accumulation of cells of the erythroblastic system, mainly proerythroblasts, was found in the marrow of animals exposed to nitrogen oxides. This is compatible with a higher maturation index i. e. the proportion of polychromatic to orthochromatic erythroblasts.

The changes found in erythrocytes of the peripheral blood are in accordance with the results of the investigations in which only after a few weeks of exposure to nitrogen oxides, hyperplethoric features have been found (12). The question remains what the mechanism of the changes is, and first of all in what way erythroblastic action in the marrow is stimulated by nitrogen oxides. It seems to us that, to some extent, this phenomenon can be associated with the stimulation of the marrow by chronic hypoxia brought about by pathological changes of the respiratory organs (15).

It is known that the exposure to nitrogen oxides leads to bronchitis, impairment of pulmonary supporting tissues, atelectasis and emphysema with all the consequences (2—7, 21) leading to secondary hypoxia. Lactic acid which results from tissue hypoxia is an activating factor of adenyl cyclase which contributes to an increased level of cyclic AMP. In its presence an activation of protein kinase takes place and accelerates the synthesis of renal erythropoietic factor or leads to its activation (22).

There are other factors of which little can be said at present such as elevated activity of delta-aminolevulinic acid dehydratase (ALA-D) in erythrocytes and a decreased elimination of ALA with urine in animals

intoxicated with nitrogen oxides. However, they could be indicative of certain disturbances in the rate of the complex process of hemoglobinopoiesis. At present we cannot give a satisfactory explanation of the mechanism of erythropoiesis stimulated by low concentrations of nitrogen oxides.

In the light of literature and on the basis of clinical observations (23) it is possible to suggest that chronic action of nitrogen oxides at low concentrations may bring about an overproduction of erythrocytes which is associated with the stimulation of normoblastic erytropoiesis. Disturbances of the hematopoietic system can be prevented by binding nitrogen oxides to gaseous ammonia. The exposure of animals to the reaction products of nitrogen oxides and ammonia brought the investigated phenomena back to normal, and the results obtained were similar to those of the control group. This was observed in the picture of peripheral blood in the marrow, as well as in methemoglobinemia and the activity of delta-aminolevulinic acid dehydratase of erythrocytes. However, a decrease in the hemoglobin level decreased mean hemoglobin concentration in an average erythrocyte and erythrocyte number in 1 mm^3 of blood in animals exposed to the reaction products require some explanation. The values obtained which are lower than those of the control group might suggest that the reaction products of nitrogen oxides and ammonia exert a toxic effect on the hematopoietic system. It is only a seeming phenomenon resulting from an increase of the liquid part of the vascular bed and the dilution of morphotic elements in the blood. This is supported by a smaller hematocrit number, increased mean volume of an average erythrocyte and finally by normalization of the erythroblastic system in the marrow. No substantial changes were observed in the leukocytic system of the peripheral blood under the influence of nitrogen oxides in reference to total white cell count or mutual percentage values of particular cell systems. There was a certain tendency of the percentage number of leukocytes to increase and of the percentage of neutrophilic cells among granulocytes to decrease. These slight changes in the periphery stand to some extent in contrast with the shifts of new forms of marrow granulocytes. Nitrogen oxides led to a marked decrease in the number of myelocytes, metamyelocytes and granulocytes with a divided nucleus, and at the same time brought about an increase in the percentage of marrow eosinophiles. Besides, the toxic effect of nitrogen oxides resulted in a marked decrease in the number of cells in lymphatic texture. However, we can assume that the production and outflowing of white cells were normal. This is supported by a normal leukocyte number in the peripheral blood and by their normal morphological state. On the other hand, we cannot exclude the possibility that long-term exposure to nitrogen oxides leads to a pathological weakening of the generation of cells of leukocytic series. Neutralization of nitrogen oxides by gaseous ammonia, similarly to the erythropoietic system, restores normal quantitative

relationships between cell series and elements of white cells in the marrow texture. This is yet another proof of biologically beneficial effect of nitrogen oxides neutralized by ammonia.

CONCLUSIONS

1. A long-term action of nitrogen oxides upon the hematopoietic system stimulates erytropoiesis.

2. Nitrogen oxides do not exert a greater effect on the production and outflow of white blood cells.

3. Neutralization of nitrogen oxides by gaseous ammonia tends to restore normal functioning of the erythroblastic system.

4. Nitrogen oxides activate some of the enzymes taking part in the hem synthesis.

References

1. *McCord, C. D., Harrold, G. C., Meek, S. F.:* J. Ind. Hyg. Toxicol., *23* (1941) 200.
2. *Henry, M. C., Ehrlich, R., Blair, W. H.:* Arch. Environ. Health, *18* (1969) 580.
3. *Kleinerman, J., Cowdrey, C. R.:* Yale J. Biol. Med., *40* (1968) 579.
4. *Kośmider, S., Misiewicz, A., Felus, E., Drozdz, M., Ludyga, K.:* Int. Arch. Arbeitsmed., *31* (1973) 9.
5. *Kośmider, S., Luciak, M., Zajusz, K., Misiewicz, A., Szygula, J.:* Pat. Pol., *1* (1973) 107.
6. *Stephens, R., Freeman, G., Grance, S. C.:* Exp. Molec. Path., *14* (1971) 1.
7. *Thomas, H. V., Mueller, P. K., Wright, G.:* J. Air Pollut. Contr. Ass., *17 (1967)* 33.
8. *Mc Quiddy, E. L.:* J. Ind. Hyg. Toxicol., 23 (1941) 134.
9. *Carson, T. R.:* Amer. Ind. Hyg. Ass. F., *23* (1962) 457.
10. *Wagner, W. D.:* Arch. Environ. Health., *10* (1965) 455.
11. *Fenters, J. D., Findlay, J. C., Curtis, D., Ehrlich, R., Coffin, D. L.:* Arch. Environ. Health., *27* (1973) 85.
12. *Freeman, G., Grance, S. C., Stephens, R. J.:* Arch. Environ. Health., 18 (1969) 609.
13. *Petr, F., Schmidt, P.:* Z. Gesamte Hyg. Grenzgeb., *13* (1967) 34.
14. *Kośmider, S.:* Arh. hig. rada, 28 (1977) 17.
15. *Lawkowidzowie, W. I.:* Application of hematological investigations in the clinic, PZWL, Warszawa, 1970.
16. *Kokot, F.:* Methods of laboratory investigations used at the clinic. PZWL, Warszawa, 1970.
17. *Pehr, F., Nosek, J.:* Biol. Clin. Bchemsl., *1* (1972) 35.
18. *Bonsignore, D., Calissano, P., Cartasegno, C.;* Med. Lav., *3* (1965) 199.
19. *Melette, S. J., Brodsky, A., Palmer, L.:* J. Lab. Clin. Med., *41* (1953) 963. quoted from: Z. Medizinische Chemie für klinischen und theoretischen Gebrauch, Hirsberg K. Lang. Urban Schwarzenberg, München, Berlin, Wien, 1957.

20. *Haeger-Aronsen, B.:* Scand. J. Clin. lab. Invest., *12* (1960) 37.
21. *Grabecki, J., Haduch, I.:* Chemia Analit., *10* (1965) 1311.
22. *Kuratowska, Z.:* Pol. Arch. Int. Med., *53* (1975) 299.
23. *Hager-Malecka, B., Romańska, K., Szczepański, Z., Rusiecka, A., Jonczyk, K., Sychlowy, A., Zolnierczyk, Z.:* Materials of VI Congress of Polish Association of Hygiene, P. T. H., Warszawa, *20* (1973).
24. *Blair, W. H., Henry, M. C., Ehrlich, R.:* Arch. Environ. Health., *18* (1969) 186.

Sažetak

UTJECAJ DUŠIKOVIH OKSIDA I PRODUKATA NJIHOVE NEUTRALIZACIJE S AMONIJAKOM NA HEMATOPOETSKI SUSTAV

Ispitivan je utjecaj dušikovih oksida na hematopoetski sustav. Eksperimenti su vršeni na 90 zamorčadi. Životinje su podijeljene u tri skupine: kontrolnu je skupinu (A) sačinjavalo 30 zamorčadi, u drugoj (B) bilo je 30 životinja koje su bile izložene dušikovim oksidima u koncentraciji 1 mg/m³ 8 sati dnevno u toku 120 dana. Treća je skupina (C) bila izložena produktima reakcije dušikovih oksida i amonijaka u jednakom trajanju kao i skupina (B). U svih je životinja određivan sastav krvi. Primijećeno je da dušikovi oksidi stimuliraju eritropoezu, jer je došlo do znatne akumulacije eritrocita u koštanoj srži. Odnos eritrocita i leukocita bio je 1 : 1, a u kontrolnoj 1 : 2. U isto vrijeme opažen je povećan broj retikulocita i povišena vrijednost hemoglobina. Zanimljivo je da je primijećena povećana aktivnost ALAD u eritrocitima, dok se istovremeno smanjilo izlučivanje ALAD putem urina, a povećalo se izlučivanje koproporfirina. Ovi bi podaci mogli biti pokazatelji pojačane sinteze krvi. Neutralizacija dušikovih oksida s amonijakom u plinovitom stanju dovodi do formiranja produkata reakcije, pod čijim utjecajem nastaje slična slika periferne krvi i koštane srži onoj u kontrolnoj skupini.

II interna klinika, Klinika za profesionalne bolesti i kliničku toksikologiju i Institut za interne bolesti. Šleska medicinska škola, Katowice

Primljeno 25. V 1975.