Effects of Nitrous Oxide Inhalation on Hemopoiesis in Rats

Cloid D. Green, M.D., and Douglas W. Eastwood, M.D.

The deleterious effect of prolonged nitrous oxide anesthesia on the bone marrow of man has been known since 1956, when Lassen and his associates reported the development of granulocytopenia and thrombocytopenia. They studied 13 patients with tetanus, six of whom had developed hematologic changes and had also received nitrous oxide. The occurrence of such changes only in the individuals who received nitrous oxide has discouraged further use of prolonged nitrous oxide analgesia for persons requiring sedation. The only study of the changes occurring in cells exposed to prolonged nitrous oxide tensions is that of Kieler, who studied the effects of nitrous oxide on embryonic mouse heart myoblasts in tissue culture. The toxicity of 50 per cent nitrous oxide in 50 per cent oxygen was greater than 50 per cent nitrous oxide, 30 per cent nitrogen, and 20 per cent oxygen. It was concluded that nitrous oxide was a "mitotic" poison preventing interphase cells from entering mitosis and causing spindle destruction and chromosomal abnormalities in dividing cells.

Nitrous oxide has been used without detailed studies of its action upon various organ systems. In studies on intact animals Goldman reported the intermittent administration for two months of nitrous oxide with acetylene and ethylene and oxygen to mice and rats. He observed no deleterious effects but performed no studies of the hemopoietic system and does not report the frequency or concentrations of administration.

Methods

Rats from a mixed strain albino colony were used as experimental animals. They were confined in groups of two in chambers constructed of metal and plexiglass. Gases were circulated through the chambers by a bellows ventilator or an electrically driven fan. The gases were cooled, and water precipitated from them by a modified unit from an oxygen tent. Carbon dioxide was absorbed by a carbon dioxide absorber from an anesthetic machine. Oxygen and carbon dioxide tensions were determined by sampling at frequent intervals. The pressure within the chamber was maintained approximately 5 mm. greater than the ambient pressure to minimize leaks of air into the chamber. Animals were confined within the chamber for two, four, and six days. At the end of each 48 hours the chambers were opened for cleaning and for the replacement of food and water. Air was circulated through the chamber to the control animals, and for experiment, 80 per cent nitrous oxide and 20 per cent oxygen. The experimental rats appeared "intoxicated" by the nitrous oxide. They slept more than control animals and consumed less food and water.

At two, four, and six days, groups of animals were sacrificed by giving 25 mg. thioental sodium intraperitoneally in 1 ml. of solution. When the animal was asleep, but before death, the thorax was opened and a blood sample drawn into a heparinized syringe from the heart. This blood sample was used for determination of hematocrit, white blood cell count, and to make a smear for white blood cell differential counts and studies. A section of the upper portion of the femur was fixed in formalin, decalcified, subsequently sectioned, and stained for bone marrow study.

Analysis of nitrous oxide used in our experiments, performed by the National Bureau of Standards, indicated an absence of any oxide of nitrogen other than nitrous oxide and no impurity. The method of analysis was a combination of mass spectrometric and infrared techniques. The sensitivity was such that
Table 1. Comparative Data Between White Blood Cell Counts of Control and Experimental Animals

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<th>After Two Days</th>
<th>After Four Days</th>
<th>After Six Days</th>
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<td>C</td>
<td>3</td>
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<td>4</td>
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<td>7</td>
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<td>$P &gt; 0.05$</td>
<td>$P &lt; 0.005$</td>
<td>$P &lt; 0.005$</td>
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C = Control animals  
E = Experimental animals

$-$ = Below the median  
$+$ = Above the median

The control animals were exposed to air, the experimental animals to 80 per cent nitrous oxide, 20 per cent oxygen. No significant difference was found in the white blood cell counts after two days. A highly significant difference was found after four and six days.

nitrogen dioxide at concentrations of 0.001 per cent (10 p.p.m.) would have been detected.

Results

Studies were completed on 57 animals, 32 of which were control animals and 25, experimental. No deaths prior to sacrifice occurred in the control group. One-half of the animals that survived five days in 80 per cent nitrous oxide died before the sixth day of exposure was completed. Because the data regarding white blood cell counts were not normally distributed and hence preclude the use of a routine parametric test, the Fisher "exact probability" test was used for the analysis. The comparative data between the white blood counts of control and experimental animals taken at two, four, and six days are summarized in table 1 and shown in figure 1. No significant difference existed at two days, but a highly significant difference was found at four and six days. The normal median figure of 15,500 white blood cells per cubic millimeter dropped to 1,300 by the sixth day of exposure to nitrous oxide. The difference was significant at $P < 0.005$.

The control animals demonstrated a normal distribution of polymorphonuclear cells and lymphocytes for the rat. In each control group 73 or 74 per cent of the total number of cells present were lymphocytes. The experimental animals demonstrated an approximately normal distribution, 67.4 per cent lymphocytes on the second day, although the

![N2O Effects on Rats](image)

**Fig. 1.** The median of the total and differential white blood cell counts of control and experimental animals are compared. Inhalation of 80 per cent nitrous oxide, 20 per cent oxygen resulted in an absolute decrease of lymphocytes and disappearance of all granulocytes by the sixth day.
Fig. 2. (A) The femoral bone marrow of a normal, mature white rat (×115).
(B) The femoral bone marrow of a white rat after two days of 80 per cent nitrous oxide, 20 per cent oxygen inhalation. The number of empty spaces is greatly increased. There is an apparent increase of megakaryocytes. All other cell types are reduced in number (×115).
(C) The femoral bone marrow of a white rat after six days of 80 per cent nitrous oxide, 20 per cent oxygen inhalation. The majority of cellular material is erythrocytes. Maturation of cells has apparently ceased. There are many empty spaces and lakes of fibrin. The megakaryocytes have retained their histologic integrity (×115).
(D) MacCallum stain of femoral bone marrow of a white rat after six days of 80 per cent nitrous oxide, 20 per cent oxygen inhalation. The intracellular bodies seen are believed to be bacteria (×1,400).

The total number of cells was reduced. However, by the fourth day 91 per cent were lymphocytes. By the sixth day all remaining cells were lymphocytes.

The hematocrits of the control animals suggested a mildly developing hemocoagulation but within the normal limits of variation. The experimental animals showed a somewhat more marked suggestion of hemoconcentration, but again the values were within the normal range for a rat population.

The normal bone marrow of the rat femur is dense. There is little fat. A typical rat marrow from a control animal is seen in figure 2 A. Figures 2 B and C show the bone marrow of rats after two and six days' exposure to nitrous oxide. There is progressive hypoplasia seen as nitrous oxide administration is prolonged. There is an increase in the number of empty spaces found and also in the hyperemia of the marrow as it becomes hypoplastic. Reproduction of cells apparently ceases, and mitoses disappear. In the more severely degenerative marrows there are areas of hemorrhage and large lakes of fibrin. Most of the cellular material in Figure 2 C is red blood cells. No particular cell form seems to have escaped with the exception of the megakaryocytes. There is an apparent increase in megakaryocytes as the administration of nitrous oxide continues. However, it is believed that this is more apparent than real and that
the disappearance of other cell forms leaves the megakaryocytes prominent. Platelet counts of peripheral blood were not performed, but study of smears revealed platelets in near normal numbers. However, the platelets of the moribund animals were found in large clumps and not normally distributed. Besides megakaryocytes, the cells remaining in the severely hypoplastic bone marrow were plasma cells, stem cells, and other more mature forms in various stages of degeneration. In Figure 2 D is seen a group of bodies within the cytoplasm of a cell. A series of studies by differential staining techniques has demonstrated that these are bacteria.

**Discussion**

Kindred 6, 7 calculated that the production of mature granulocytes by the normal rat marrow is fifty-eight times greater than required to maintain the average peripheral count. The consistent lack of evidence of neutropenia in the face of histologic evidence of reduced granulocyte production following the injection of sulfur and nitrogen mustard was, he thought, owing to the surplus of mature granulocytes stored as well as by the marrow's surplus capacity. Although we have no quantitative studies of cell production, the changes in the peripheral blood count and in the histology of the bone marrow indicate that the production of white blood cells is reduced by the administration of nitrous oxide. The degeneration of mature forms noted both in the peripheral blood and in the bone marrow suggests that not only is the production of cells reduced but that destruction of cells may be actively induced.

The evidence suggests that the lymphoid series may be affected slightly less than the granulocytic forms. Since the spleen, thymus, and other lymph nodes all actively participate in the production of lymphocytes in the rodent, a detailed study of these organs is indicated.

The persistence of histologically normal megakaryocytes in the bone marrow is striking. The quantitative studies of Kindred 7 following injections of vesicants into rats showed no significant changes in the number of megakaryocytes. It seems that this cell type is peculiarly able to maintain its histologic integrity in the face of certain types of toxins. The fact that platelets were found in peripheral blood practically devoid of all other forms of white blood cells was also remarkable. Whether the clumping of the platelets was due to abnormality of the cell or to an alteration in the proteins or some other fraction of the serum is unknown.

The hematocrit determinations of both control and experimental groups remained remarkably constant and within normal limits. The absence of any evidence of anemia on the sixth day of the experiment is remarkable in view of the extensive bone marrow destruction. The fact that no evidence of anemia occurs in our rat experiments suggests that the erythrocyte life span is long enough to prevent occurrence of anemia or that near mature forms of red blood cells in the marrow complete their development and are released into the peripheral circulation.

The exact mode of death in the animals has not yet been determined. It is likely that bacteremia secondary to neutropenia plays a significant role. A direct toxic action of nitrous oxide on cells other than the hematopoietic system is possible. All of the animals had severe diarrhea on the fifth and sixth days, but in none of the animals was evidence of hemorrhage seen either during life or on post-mortem examination. The possibility that the effect demonstrated was due to contaminants of nitrous oxide must be considered. Reports 8, 9, 10, 11 of the presence of unidentified contaminants, inert gases, nitric acid, carbon dioxide, and ammonia are known. Though these contaminants may be dangerous, none are known to produce changes in the blood cells. The analysis of nitrous oxide from our source of supply indicated an absence of detectable contaminants. We assume that the changes noted were due to nitrous oxide.

The mechanism of nitrous oxide in producing the above alterations is unknown. It seems feasible that nitrous oxide could have the same mechanism of action in the central nervous system as it has in the bone marrow. It is possible that a thorough exploration of the action of nitrous oxide and other anesthetic agents upon the cells of the hematopoietic system will reveal information concerning the mechanisms of narcosis.
EFFECTS OF NITROUS OXIDE ON HEMOPOIESIS IN RATS

Summary

Prolonged nitrous oxide administration with normal oxygen tension is toxic to the bone marrow of the white rat and causes decrease of the peripheral white blood count. The only mature cell form of the bone marrow which appears histologically unaffected is the megalakaryocyte.

Since completion of this work, nitrous oxide has been administered to two patients having myelogenous leukemia (New Engl. J. Med. 268: 297, 1963).

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References


SUPINE HYPOTENSION Postural hypotension occurred in a pregnant woman at term when the position of the fetus changed to free breech. Blood pressure returned to normal level when the patient was placed on her left side. Anesthesia for Cesarean section was successfully accomplished by induction with cyclopropane with the patient in the left lateral position, following which the patient was placed on her back, but the table tilted to the left during the operation. (Crapnell, V. E., and Johnston, L. W.: The Supine Hypotensive Syndrome, Canad. Med. Ass. J. 87: 1335 (Dec. 22) 1962.)