katchewan. The depth of the deposit is 1600 m and the calcium content is less than 0.1 percent. A significant portion of that calcium is contained in clay seams that can be separated from the KCl. The thorium and uranium contents have not yet been measured, but values on the order of 1 ppb have been found for similar deposits (*13*).

Some aspects of the chemistry are also favorable. Very elegant accelerator mass analysis techniques, in which $^{41}Ca$ atoms are fully stripped, can be applied. We anticipate that a discrimination of $10^{16}$ with 1 percent efficiency may be attainable (*14*), although this has not yet been established experimentally. For a marine deposit we may need to deal with up to 1 kg of natural calcium extracted from 1.3 tons of KCl in order to obtain the $10^4$ atoms of $^{41}Ca$ needed for such an analysis. Since optimal sample sizes for accelerator mass analysis are 10 to 15 mg, a preconcentration of $^{41}Ca$ relative to natural calcium by $10^5$ is required, and the initial quantity of calcium must be increased in proportion to the inverse of the efficiency of this preconcentration. This may be the major experimental difficulty. Since the calcium concentration in marine deposits may be far from homogeneous, careful selection of salt samples may be important. We are currently analyzing samples from several layers of the Regina deposit and are also exploring possibilities for designing crude, high-amperage mass separators that might be capable of processing a 1-kg sample. We believe that an order of magnitude improvement in the amperages that can be achieved with present technology is required. Alternatively, suitable nonmarine sources of potassium might be found that are significantly more free of calcium, which would simplify the requirements for preconcentration. We would welcome any suggestions on these points.

Although geologic experiments sensitive to other components of the solar neutrino flux have been proposed (*15*), we believe that the experiment discussed here is the first one to be viable from the standpoints of both backgrounds (*3*) and the reliability of the nuclear cross section. We also believe that production of $^{41}Ca$ may be the only tool nature has provided for probing the long-term $^8B$ solar neutrino luminosity, and thus for probing small variations in the solar core that may have occurred during the past $10^5$ years.

Finally, in view of tentative laboratory evidence for neutrino oscillations (*16*) and for a massive electron neutrino (*17*), one other aspect of the proposed experiment should be stressed. Although the reported neutrino mixing is not sufficient to resolve the large discrepancy between theory and Davis's experiment, the possibility that additional mixing mechanisms could operate over larger oscillation lengths is open. Solar neutrinos thus present a unique opportunity for extending present terrestrial experiments. The $^{37}Cl$ results are ambiguous in that they can be explained by the absence of high-energy $^8B$ neutrinos, predicted by various nonstandard solar models, or by an overall reduction of all components of the solar neutrino flux, which could result from mixing of several neutrino species. Since the $^{41}Ca$ experiment will test only $^8B$ neutrinos, this measurement, in combination with Davis's results, could help distinguish between these two possibilities. Independent of the question of secular variations in the sun's core, this provides an important motivation for mounting new solar neutrino experiments.

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## Anesthetics as Teratogens: Nitrous Oxide Is Fetotoxic, Xenon Is Not

Abstract. *Exposure of pregnant rats to the anesthetic nitrous oxide on the ninth day of gestation causes fetal resorption, skeletal anomalies, and macroscopic lesions including encephalocele, anophthalmia, microphthalmia, and gastroschisis. The inert gas xenon, which has anesthetic properties similar to those of nitrous oxide, does not cause teratogenic effects under the same experimental conditions.*

There has been increasing concern that inhalation anesthetics may cause teratogenic and other harmful effects. Physicians and nurses who work in operating rooms polluted by waste anesthetic gases have a higher incidence of spontaneous abortions and fetal malformations than control groups (*1*). However, other factors, including differences in occupational stress and working conditions, may be implicated (*2*). Such factors may be excluded from studies of dentists and their assistants, because the dental population can readily be divided into groups that differ only in the use of inhalation anesthetics for analgesia. A recent survey of dental health professionals in the United States (*3*) strongly suggests that


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the occupational use of nitrous oxide and halothane is associated with disease, including teratological problems.

For obvious ethical and practical reasons, investigations of the teratogenicity of anesthetics and the mechanisms involved must depend on animal studies. The results have been conflicting; variations in species and exposure protocols may explain the lack of effects reported in some studies (*4*). Prolonged exposure to high concentrations has caused teratogenic effects (*5*), but the absence of variation in the effects of different anesthetics, coupled with the negative reports, has led to suggestions that the observed effects may be associated with general anesthesia and caused by stress or the presence of other pollutants rather than by specific pharmacologic effects of anesthetic agents (*4, 6*).

A wide variety of molecular structures—from elemental gases to halogenated ethers—produce inhalation anesthesia. We investigated the possibility that the teratogenic effects of anesthetics, if any, may be related to specific pharmacologic effects rather than to the intrinsic mechanisms of anesthesia. Such effects might include changes in uterine blood flow mediated through the autonomic nervous system, changes in nucleic acid synthesis and metabolism, and the formation of toxic metabolites through biotransformation. We compared the teratogenic effects of nitrous oxide and the inert gas xenon, an anesthetic as potent as nitrous oxide. Our results show that whereas nitrous oxide has significant teratogenic effects on Sprague-Dawley rats, xenon does not.

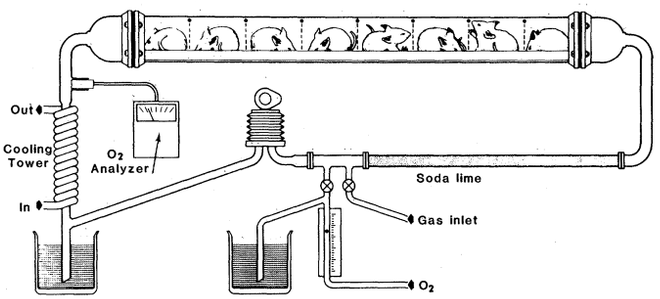


Fig. 1. Schematic of the exposure system. The rats are housed on a steel trough in a glass tube (5 inches in diameter) and are separated from each other by steel mesh partitions. Gases are introduced through a check valve; a continuous supply of oxygen is provided for metabolic needs. The gases are pumped counterclockwise through the chamber by an electric pump with flexible steel bellows and carbon dioxide is absorbed by soda lime. Oxygen concentration is measured with a polarographic electrode, and temperature and humidity are regulated by a cooling tower. The pressure within the chamber is maintained at 1 cm of water above atmospheric pressure by a water manometer. In use, the chamber is sealed by external compression joints with Teflon gaskets. The chamber is purged with oxygen for 30 minutes to displace nitrogen before the addition of either nitrogen (group B), nitrous oxide (group C), or xenon (group D). Oxygen concentration is maintained at between 25 and 30 percent for 24 hours, when the animals are returned to their cages. All the animals are deprived of food and water for the duration of the 24-hour treatment period.

We were able to complete this investigation with 100 liters of xenon by restricting the exposure to xenon in each experiment to a single treatment period and by using a closed exposure system to conserve this precious gas (Fig. 1). Three identical chambers were constructed from glass, steel, and Teflon components to avoid contamination by such pollutants as vinyl chloride. Our preliminary experiments with this exposure system confirmed a previous study (*7*) showing that administration of 70 percent nitrous oxide for 24 hours on the ninth day of pregnancy causes significant teratogenic effects.

A group of 32 Sprague-Dawley rats (Charles River) was used for each experiment. The day they were placed with the males was designated day 0; on day 9 they were randomly divided into groups of eight for the control and anesthetic treatments. Group A remained in standard laboratory cages; groups B, C, and D were placed in sealed chambers (Fig. 1) and exposed to 25 to 30 percent oxygen with either 70 to 75 percent nitrogen (group B), 70 to 75 percent nitrous oxide (group C), or 70 to 75 percent xenon (group D). The purpose of the group B controls was to detect any difference (compared with group A controls) that might be attributed to the stress of the exposure system. After 24 hours of exposure the animals were returned to their cages, and on day 20 they were killed. The fetuses were removed by cesarian section (*8*).

We completed four experiments, yielding 122 litters and 1270 fetuses for examination. There were no deaths from disease, but six animals were not pregnant. The incidence of fetal loss by resorption was similar in the control groups and was not increased by treatment with xenon, but nitrous oxide caused a fourfold increase in the number of resorptions (Table 1). Likewise, the incidence of macroscopic organ anomalies was similar in the control groups (3 and 1 percent) and the groups treated with xenon (1 percent), but was 15 percent in groups exposed to nitrous oxide. The anomalies included encephalocele, hydrocephalus, anophthalmia, microphthalmia, gastroschisis, and gonadal agenesis. Delays in maturation of the skeletal system and anomalies including fused ribs were more common in the nitrous oxide–treated groups than in the xenon-treated or control groups.

The absence of teratological effects with xenon is as significant as the posi-

Table 1. Teratogenicity of nitrous oxide and xenon in rats.

| Item | Control groups | | Experimental groups | |
|---|---|---|---|---|
| | A (Air) | B ($N_2$) | C ($N_2O$) | D (Xe) |
| Litters | 31 | 31 | 30 | 30 |
| Fetuses | 328 | 333 | 286 | 323 |
| Resorptions | 15 | 12 | 54* | 12 |
| *Fetuses with malformations* | | | | |
| Fetuses examined | 156 | 163 | 138 | 160 |
| Malformed fetuses | 4 | 1 | 20* | 1 |
| Encephalocele | 0 | 0 | 4 | 0 |
| Hydrocephalus | 1 | 0 | 2 | 0 |
| Anophthalmia | 0 | 0 | 9 | 0 |
| Microphthalmia | 0 | 0 | 3 | 0 |
| Cleft lip/palate | 1 | 0 | 0 | 0 |
| Gastroschisis | 0 | 0 | 4 | 0 |
| Gonadal agenesis | 0 | 1 | 3 | 1 |
| Undescended testis | 1 | 0 | 1 | 0 |
| Shortened limb | 1 | 0 | 0 | 0 |
| *Fetuses with skeletal anomalies* | | | | |
| Fetuses examined | 170 | 170 | 148 | 163 |
| Fetuses with anomalies | 13 | 20 | 55* | 14 |

*$P < .0001$.

tive effects with nitrous oxide. While these experiments do not establish the mechanism for the teratogenicity of nitrous oxide, it is clearly not related to the intrinsic mechanisms of anesthesia, since xenon is as potent as nitrous oxide. Such factors as stress and the presence of additional atmospheric pollutants may also be excluded.

Why, then, is nitrous oxide teratogenic? First, it is chemically reactive and may undergo biotransformation (*9*) to metabolites that could be toxic; this cannot occur with xenon. Second, changes in uterine and fetal blood flow due to the cardiovascular effects of nitrous oxide could affect fetal development. Finally, nitrous oxide has peculiar effects on hematopoiesis. Prolonged administration of nitrous oxide to humans causes leucopenia and megaloblastic anemia (*10*). Nitrous oxide inactivates vitamin $B_{12}$ by oxidizing cobalamin (*11*), inhibiting the conversion of homocysteine to methionine (*12*). This in turn affects thymidine (and perhaps DNA) synthesis. The clinical implications of the possibility that nitrous oxide may affect both hematopoiesis and organogenesis by inhibiting vitamin $B_{12}$-dependent reactions are especially challenging.

Should xenon replace nitrous oxide for clinical anesthesia? Calculations indicate that the atmosphere does not contain enough xenon for general clinical use with current techniques of anesthesia. As Cullen (*13*) said of xenon in 1951, "although it may not by virtue of its cost of manufacture prove to be a satisfactory agent commercially, it may materially assist in solving one of the important theoretical problems of anesthesia."

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## Cellular Transplantation in the Treatment of Experimental Hepatic Failure

Abstract. *The survival of Lewis rats with D-galactosamine-induced fulminant hepatic failure was prolonged if they were given intraperitoneal injections of single-cell suspensions of liver or bone marrow cells from normal rats. Suspensions of liver cells were also effective in prolonging the survival of rats with ischemia-induced hepatic necrosis. The liver cells did not act by repopulating the recipient liver.*

There is no satisfactory treatment for fulminant hepatic failure in man (*1*, *2*). The variability of the disease and hence difficulties with controlled trials in humans makes it necessary to find a suitable animal model of the disease in which to assess potential therapy aimed at maintaining hepatic function until hepatic regeneration occurs. Here we describe the efficacy of hepatocellular and bone marrow cell transplantation in the reversal of acute hepatic necrosis induced in rats.

A consistent model of lethal hepatic necrosis was produced in 2- to 4-month old inbred male Lewis rats (*3*) weighing 280 to 330 g by the intraperitoneal injection of D-galactosamine hydrochloride (Sigma). When 50 animals were injected with D-galactosamine (2.6 g per kilogram

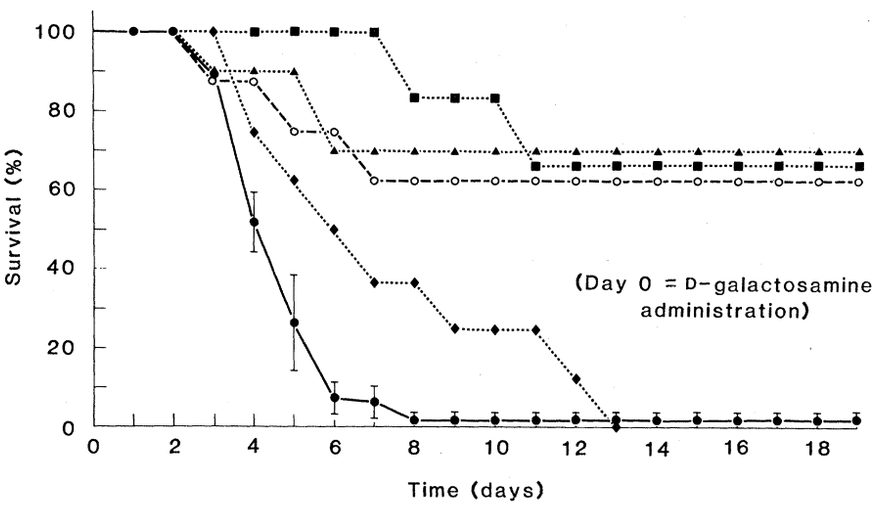


Fig. 1. Percentage of survival of D-galactosamine-injected Lewis rats after they received syngeneic liver cells. Single-cell suspensions of liver cells ($4 \times 10^7$ cells per rat) were injected intraperitoneally at 24, 48, and 60 hours after toxin administration. Viability of the liver cells, as determined by trypan blue exclusion, was 85 ± 5 percent. Although hepatocytes given at 24 hours (◆) prolonged survival, all animals succumbed by day 13 ($N = 16$). When hepatocytes were given at 48 hours (▲) ($N = 16$) and at 60 hours (■) ($N = 12$), 70 and 66 percent of the rats survived, respectively ($P < .001$). By day 14 these animals showed normal liver function and structure. Also shown here is the influence of nonreplicating liver cells on survival after D-galactosamine administration. Single-cell suspensions of syngeneic liver cells received 10,000 rads from a cesium source and were then injected intraperitoneally ($4 \times 10^7$ cells per rat; 50 percent viability by trypan blue exclusion) 48 hours after toxin administration. These nonreplicating cells increased survival to 62.5 percent (○) ($N = 16$, $P < .001$). The control curve (●) shown here represents the mean (± standard error) survival for three separate experiments ($N = 82$).


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