prove useful in the detection of enzymatic blocks in any of the first three steps of the phosphorylation pathway of galactose in a variety of cells. The rationale employed here of inhibiting alternate routes of metabolism in order to isolate the route of interest should be generally applicable to the study of other inborn errors of metabolism.

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Fluid Shifts Associated with Gas-Induced Osmosis

Abstract. *It has been proposed that equilibration of nitrous oxide with blood plasma increases osmotic pressure and thereby causes hemodilution. However, calculations show that the 250-torr osmotic gradient produced by 0.7 atmosphere of nitrous oxide dissolved in blood plasma (separated from the other body fluids by a membrane permeable only to water) would be eliminated by a water shift which would dilute the plasma by only 4 percent. Permeability of the membrane to nitrous oxide would further reduce the shift. In vivo measurements confirmed the smallness of any osmotically induced shifts by demonstrating no significant transient changes in hematocrit value when 0.7 atmosphere of nitrous oxide was added to or removed from an inhaled anesthetic mixture in man. These results cast doubt on the suggestions that gas-induced osmosis is an important factor in dysbarism or in clinical anesthesia.*

Kylstra *et al.* (*1*) proposed that exposure to hyperbaric breathing mixtures containing helium or nitrogen causes transient osmotic gradients in man during the period of uptake or output of these gases. Such gradients have been implicated as causing hemoconcentration, capillary stasis, and impaired exchange of gases in tissues (*1*); dry joints (*2, 3*); aseptic bone necrosis (*2*); and urticaria (*4*). Further, Hills (*2, 5*) has suggested that gas-induced osmosis is an initiating mechanism for anesthesia. Because of the high solubility of nitrous oxide relative to both nitrogen and helium, the increase in osmolarity of an aqueous phase caused by 1 atm of nitrous oxide is approximately equal to that caused by 30 atm of nitrogen or 50 atm of helium. Thus, it has been inferred that large osmotic gradients result from nitrous oxide administration during clinical anesthesia (*2*).

However, these suggestions appear to ignore the background of osmotic pressures due to proteins and salts in physiological fluids. The osmotic pressures due to dissolved gases are superimposed on this background and form only a small fraction of the total osmotic pressure. This is particularly important when fluid shifts are considered which would remove the potential osmotic gradients. We report here the calculation of such shifts and the in vivo measurement of transient changes in hematocrit value when 0.7 atm of nitrous oxide was added to or removed from an anesthetic mixture inhaled by man. We calculated a maximum theoretical 4 percent fluid shift in the blood plasma and we could not demonstrate any significant change in hematocrit value.

Table 1. Effect on hematocrit values of adding and of eliminating 70 percent nitrous oxide. The changes are given as mean percentages of the control hematocrit value; S.E.M., standard error of the mean.

Time (min)	Change (%) ± S.E.M.	
	N_2O added	N_2O removed
1	100 ± 0.6	100 ± 0.3
2	100.2 ± .5	100.4 ± .5
3	99.1 ± .4	101.1 ± .4
4	99.4 ± .3	99.7 ± .5
5	99.8 ± .4	99.3 ± .5
10	98.7 ± .6	98.3 ± .3
15	96.5 ± .9	99.1 ± .7

We calculated the theoretical maximum change in blood volume produced by solution of nitrous oxide at 0.7 atm, assuming a membrane between the cardiovascular system and the other body fluids permeable only to water. Since the solubilities of nitrous oxide in blood and in water are similar (*6*), we assumed that the osmolarity of nitrous oxide in blood equals its molarity. Therefore 0.7 atm of nitrous oxide increases the osmolarity by 13 milliosmoles per liter (which from the van't Hoff equation is equivalent to an osmotic pressure of 250 torr). Addition of 13 milliosmoles per liter to the 310 already present in plasma and other body fluids (*7*) produces only a 4.2 percent increase in osmolarity. Since plasma contains 7 percent of the total body water, a shift from the remaining water stores (interstitial and intracellular fluid) sufficient to increase the plasma volume by 4 percent eliminates the osmotic gradient between plasma and other body water. That is, a 4 percent increase in plasma water content, resulting from the transfer of water from interstitial and cell fluid, would eliminate any osmotic gradient induced by the presence of nitrous oxide in plasma. In this calculation we assume that no nitrous oxide diffuses (or leaks) from plasma to interstitial or cell fluid, and find the maximum transient fluid shift that could occur. However, since nitrous oxide has a high permeability, such diffusion does occur and thereby decreases the osmotic gradient and fluid shifts. The calculation of osmotic pressures across a "leaky membrane" involves a correction factor in the van't Hoff equation called the Staverman reflection coefficient, σ ($0 \leq \sigma \leq 1$) (*8*). In the above calculation we have assumed that σ equals 1 for both nitrous oxide and the solutes in the blood. Hills (*2*) found that for nitrous oxide σ is less than .04, which indicates a 25-fold

reduction in the theoretical osmotic pressure gradient. The other solutes in the blood can also diffuse across the membrane, but their rates of penetration are slow relative to that of nitrous oxide (*9*). The true transient fluid shift is probably at least an order of magnitude less than the calculated maximum figure.

Thus, our prediction conflicts with the suggestion that gas-induced osmosis can cause transient changes in hematocrit value. To test this argument, we measured hematocrit values in eight surgical patients before and after the addition of 70 percent nitrous oxide to anesthetic mixtures of halothane and oxygen or isoflurane (Forane) (*10*) and oxygen (Table 1). In six other patients, anesthetized for a minimum of 2 hours with mixtures of isoflurane or halothane in 70 percent nitrous oxide, we measured the hematocrit value immediately before and after the elimination of nitrous oxide (Table 1). A nonrebreathing system was used in both experiments. There was a nearly instantaneous change in the concentration of the inspired gas (*11*). The corresponding change in alveolar gas required at least several breaths during which the partial pressure of nitrous oxide in the arterial blood only gradually increased. However, the time constant of this change was much smaller than the time constants of the processes for which gas-induced osmotic effects have been postulated (*1–5*). Hemoconcentration during nitrous oxide elimination or hemodilution during nitrous oxide inhalation would have supported the argument that gas-induced osmosis is important. We were unable to demonstrate any significant transient increase or decrease in hematocrit value for either experiment.

Since nitrous oxide appears to produce alpha sympathetic stimulation (*12*), shifts in the body fluid due to the cardiovascular effects of nitrous oxide may have masked those due to osmotic effects. However, the cardiovascular effects would be expected to occur more slowly than the transient osmotic effects. Conversely, a change in erythrocyte volume due to nitrous oxide would have magnified any change in hematocrit.

The in vivo results, together with our calculation of the maximum potential fluid shift, cast doubt on the importance of gas-induced osmosis during either hyperbaric exposure or clinical anesthesia.

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Menkes' Kinky Hair Disease: Further Definition of the Defect in Copper Transport

Abstract. *Duodenal mucosa obtained from two patients with Menkes' syndrome contained abnormally large amounts of copper. The defect in copper absorption in this disease must lie in the process of intracellular handling or of transport across the serosal cell membrane. Fibroblastic cells cultured from the skin of patients and of heterozygous females show intense metachromasia in primary culture which disappears in subculture. These cells may be useful for the study of copper transport in vitro and for the identification of heterozygotes in affected families.*

Danks *et al.* (*1*) have demonstrated a defect in the intestinal absorption of copper in the X-linked inherited degenerative brain disease described by Menkes *et al.* (*2*). The arterial degeneration, cerebral degeneration, hypothermia, hair changes, and bone lesions seen in the disease can all be explained by copper deficiency (*3*).

We here report the results of further studies which show that the defect in copper absorption lies not in the uptake by the gut epithelium, but in the release of the copper from these cells, and suggest that a defect in copper transport may also exist in fibroblastic cells cultured from the skin of patients and of heterozygotes.

Samples of duodenal mucosa were obtained by an oral suction technique, with a Watson pediatric capsule being passed into the fourth part of the duodenum that had been treated with quinalbarbitone and metoclopramide. The mucosa samples weighed 2 to 3 mg (wet weight), and the copper content was determined by atomic absorption spectroscopy with the use of a carbon rod atomizer (*4*). The coefficient of variation in replicate samples was 6 percent. Samples were obtained from 17 babies and young children aged 10 months to 5 years during the investigation of mild persistent diarrhea. Only five of the biopsies showed any histological abnormality—celiac disease in two and giardiasis in four. The copper content of these samples ranged from 7.7 to 29.0 μg per gram (dry weight), the mean being 18.5 ± 6.3 (S.D.) μg per gram; the five that were microscopically abnormal were scattered throughout this range.

Three samples of duodenal mucosa, obtained from patient J.T. (*5*) at 6 and 9 months, contained 50, 60, and 76 μg per gram (dry weight). A sample from patient M.M. aged 10 months (*6*) contained 58 μg per gram (dry weight). Oral copper therapy had been withheld for at least 4 weeks before the samples were taken, and the levels are clearly abnormally high.

Considering this finding along with the previously published data showing defective absorption of radioactive copper from the intestine (*1*), we conclude that the defect in absorption lies not in uptake by the cells (that is, not in the brush border), but in transport within the cells or across the membrane on the serosal aspect of the cells. Very little is known about the intestinal absorption of copper (*7*). There is evidence of an active transport system in the upper intestine of the rat and a copper-binding protein has been demonstrated in the mucosal cells of the duodenum of the chick (*8*). If a similar protein exists in man it could be affected by this mu-