Monitoring cobalamin inactivation during nitrous oxide anesthesia by determination of homocysteine and folate in plasma and urine

The effects of nitrous oxide–induced cobalamin inactivation on homocysteine and folate metabolism have been investigated. Plasma levels of cobalamin, folate, homocysteine, and methionine were determined in 40 patients before and after operation under nitrous oxide anesthesia (range of exposure time, 70 to 720 minutes). Twelve patients anesthetized with total intravenous anesthesia served as control subjects (range of exposure time, 115 to 600 minutes). Postoperative plasma levels of folate and homocysteine increased ($p < 0.001$) up to 220% and 310%, respectively, in nitrous oxide–exposed patients, whereas plasma levels of methionine decreased ($p < 0.025$). Response occurred after 75 minutes of nitrous oxide exposure. The percentage increase of plasma folate and homocysteine correlated significantly with exposure time ($p < 0.025$ and $p < 0.0001$, respectively). In eight patients receiving nitrous oxide anesthesia plasma homocysteine levels had not returned to preoperative levels within 1 week ($p < 0.01$). Urinary excretion of folate and homocysteine increased during and after nitrous oxide exposure ($p < 0.01$ and $p < 0.002$, respectively) and correlated with exposure time ($p < 0.01$ and $p < 0.005$, respectively). It can be concluded that disturbance of homocysteine and folate metabolism by nitrous oxide develops with little delay and return to normal levels requires several days. Elevation of plasma homocysteine levels may therefore be used for monitoring nitrous oxide–induced cobalamin inactivation. (CLIN PHARMACOL THER 1991;49:385-93.)

Anton A. M. Ermens, PhD, Helga Refsum, MD, Joseph Rupreht, MD, Lidwien J. M. Spijkers, BS, Anne B. Guttormsen, MD, Jan Lindemans, PhD, Per M. Ueland, MD, and Johannes Abels, MD

Rotterdam, The Netherlands, and Bergen, Norway

The widely used anesthetic gas nitrous oxide is known to oxidize the coenzyme methylcobalamin, causing irreversible inactivation of methionine synthase.[1,2] This enzyme is responsible for the methyltransfer from 5-methyltetrahydrofolate to homocysteine (Fig. 1). Exposure to nitrous oxide may therefore disturb the intracellular folate metabolism and reduce the folate-dependent thymidylate and purine synthesis, essential for DNA replication. In addition, the reduction of methionine synthase activity may result in a decrease of plasma methionine concentrations.[3]

Hematopoiesis in humans strongly depends on the function of the methylcobalamin coenzyme, and inactivation by prolonged exposure to nitrous oxide may cause megaloblastic changes of the bone marrow[1,3] similar to those observed in cobalamin deficiency. Moreover, neurologic impairment, which is well known in cobalamin deficiency, may occur after prolonged, intermittent exposure to nitrous oxide.[4] In critically ill patients[5] and folate- or cobalamin-deficient subjects[6] these effects may be provoked by nitrous oxide within a few hours. However, in the majority of surgical patients receiving nitrous oxide anesthetic the oxidation of methylcobalamin is regarded as harmless.[7]

From the Institute of Hematology and the Department of Anesthesiology, Erasmus University Rotterdam, and the Departments of Pharmacology and Toxicology and Anesthesiology, University of Bergen.

Supported by the AGA Forskningsfond.

Received for publication Sept. 20, 1990; accepted Dec. 5, 1990.

Reprint requests: Anton A. M. Ermens, PhD, Institute of Hematology Ee2202, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

13/1/27143

Studies on hematologic toxicity of nitrous oxide have focused mainly on bone marrow morphology and quantitation of the folate-dependent thymidylate synthesis in hematopoietic cells by means of the deoxyuridine suppression test.[1,3,8]

Recently, elevated serum levels of homocysteine have been reported in cobalamin- and folate-deficient subjects[9] and patients with cancer treated with high-dose methotrexate.[10] Likewise the concentration of homocysteine in plasma and urine may reflect disturbance of cobalamin-dependent folate metabolism.

In this study we determined plasma levels of homocysteine, methionine, folate, and cobalamin and renal excretion of homocysteine and folate in patients subjected to nitrous oxide anesthesia.

Our findings reveal an increase in plasma and urinary homocysteine and folate concentrations by nitrous oxide exposure within a few hours, indicating that cobalamin inactivation occurs rapidly. Among the parameters studied, plasma and urinary homocysteine are the most responsive to nitrous oxide–induced cobalamin inactivation.

METHODS

Patients. Fifty-two patients requiring plastic, otolaryngologic, or neurologic surgery who underwent anesthesia with nitrous oxide (n = 40) or total intravenous anesthesia (TIVA; n = 12) were included in this study. Their ages ranged from 18 to 81 years, and 31 patients were men (Table I). All the patients gave informed consent. The duration of nitrous oxide anesthesia varied from 70 to 720 minutes (mean, 318 ± 26 minutes) and for patients anesthetized with TIVA from 115 to 600 minutes (mean, 332 ± 49 minutes).

Two traumatized patients admitted for reconstructive surgery, who underwent several operations during a 3-day period, were exposed to nitrous oxide for a total of 24 and 28 hours, respectively. Preoperative data from these two male patients are also included in Table I (patients 41 and 42). Sixteen patients received 1 to 8 erythrocyte concentrates (200 ml/unit), and two patients also received 1 to 2 units of fresh frozen plasma (200 ml/unit) to compensate for blood loss during surgery (Table I).

Anesthesia. The group of patients exposed to nitrous oxide received thiopental (3 to 6 mg/kg) for induction and anesthesia including nitrous oxide (70%), oxygen (30%), enflurane (0.5% to 1%), and fentanyl. The control group received propofol for induction (2 to 3 mg/kg) and anesthesia including oxygen-enriched air, propofol (10 to 6 mg/kg/hr), and fentanyl. Muscle relaxation was established with pancuronium (0.1 mg/kg).

Protocol. Venous blood from all patients was collected at the start and end of anesthesia, and the following parameters were determined routinely in plasma: total cobalamin, holotranscobalamin II, folate, methionine, and total homocysteine.

For determination of urinary folate and homocysteine excretion, voided urine was collected for 24 hours after the start of anesthesia from 19 volunteers exposed to nitrous oxide and 10 volunteers anesthetized with TIVA.

Blood samples from six patients exposed to nitrous oxide (patients 6, 7, 14, 19, 25, and 38 in Table I) and four patients anesthetized with TIVA (patients 49, 50, 51, and 54 in Table I) were also collected hourly during anesthesia and repeatedly afterward during 24 hours, for the above-mentioned determinations. In addition, free homocysteine levels were determined. Fractionated urine samples were collected during 24 hours for determination of urinary folate and homocysteine excretion.

The long-term effect of nitrous oxide was studied in blood samples collected from eight patients (patients 10, 15, 17, 18, 20, 21, 26, and 27 in Table I) 1, 2, and 7 days after surgery. All these patients received nitrous oxide anesthesia for 215 to 370 minutes (mean, 290 ± 19 minutes). Six patients (patients 46 to 51 in Table I) anesthetized for 195 to 520 minutes with TIVA (mean, 313 ± 44 minutes) were used as control subjects.

Sample collection and processing. Ten milliliters of venous blood was collected into cooled, EDTA-containing tubes and immediately placed on ice. Plasma was prepared by centrifugation within 5 minutes. For determination of free, acid-soluble homocysteine, a portion (500 μl) of the sample was deproteinized immediately with perchloric acid as described.[11] The samples of deproteinized and whole plasma were stored at −20° C until analysis. Urine was collected on ice and also stored at −20° C.

Analytic methods. Total homocysteine in plasma was determined by a fully automated assay developed recently in our laboratory.[12] Free, acid-soluble homocysteine in plasma and urinary homocysteine were assayed by a radioenzymic method.[11] Protein-bound homocysteine is total homocysteine minus free homocysteine.

Methionine in plasma was determined in deproteinized plasma, by a method involving derivation of the amino acids with orthophtaldialdehyde, followed by reversed-phase liquid chromatography and fluorescence detection.[13]

Total cobalamin and cobalamin analogs in plasma were determined by a radioassay with purified intrinsic factor and salivary R-binder.[14] [^{57}Co]cobalamin and [^{125}I]pteroylglutamic acid were obtained from Becton Dickinson and Co., Cockeysville, Md. Folate in plasma was also determined by a radioassay with milk binder. Folate in urine was determined with a radioassay (Becton Dickinson and Co.). Holotranscobalamin II was determined according to van Kapel et al.[15]

Statistical evaluation. Only the patients undergoing one period of anesthesia were included in the statistical analysis. Differences between preoperative and postoperative plasma values were evaluated statistically with the Wilcoxon signed-rank test for paired observations. Urinary excretion of folate and homocysteine of the nitrous oxide and TIVA groups was tested with the Mann-Whitney *U* test. Percent changes of plasma homocysteine, folate, and methionine and urinary folate and urinary homocysteine excretion were correlated with duration of anesthesia with the Spearman rank correlation coefficient.

To evaluate the long-term effects of anesthesia, values at the start of anesthesia were tested against the values at the end of anesthesia and also 1, 2, and 7 days afterward with the Wilcoxon signed-rank test for paired observations.

Data are expressed as mean values ± SE. All *p* values are given as two tailed.

RESULTS

Preoperative plasma values. Initial values for total cobalamin, holotranscobalamin II, folate, homocysteine, and methionine are summarized in Table I. Three patients had low plasma cobalamin levels together with low holotranscobalamin II values. Two of these patients also had subnormal methionine levels. Folate concentrations were frequently below the normal range, which may reflect the moderate physical and nutritional condition of some patients undergoing surgery. Homocysteine levels above the normal range were observed in 10 patients. Plasma folate concentrations correlated inversely with homocysteine levels ($n = 52$, $r_S = -0.30$; $p < 0.025$).

Effect of anesthesia on postoperative plasma parameters. In both groups of patients, total cobalamin and holotranscobalamin II levels had not changed significantly at the end of anesthesia. Increase in plasma concentrations of cobalamin analogs was not observed.

In patients anesthetized with TIVA ($n = 12$), plasma folate levels decreased moderately without significant changes in plasma homocysteine or methionine values (Fig. 2, *right panels*) ($p < 0.05$).

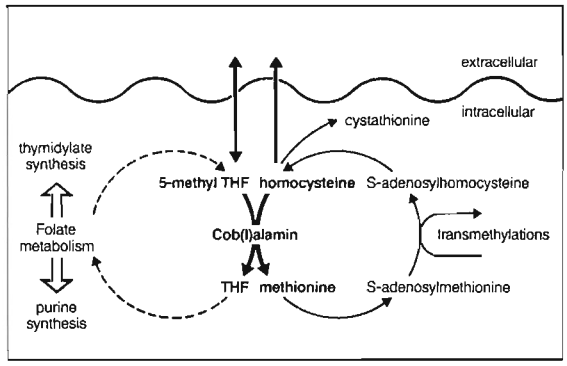

Fig. 1. Outline of the cobalamin-dependent conversion of 5-methyltetrahydrofolate (5-methyl THF) and homocysteine to tetrahydrofolate (THF) and methionine.

During nitrous oxide exposure, postoperative levels of plasma homocysteine and folate increased significantly (Fig. 2, *upper and middle panels*) ($n = 40$; $p < 0.001$).

The postoperative folate levels exceeded the normal range in only 5 of 42 patients anesthetized with nitrous oxide. In 35 of the 42 patients operated on under nitrous oxide the elevated homocysteine level surpassed the upper limit of the normal range. The percentage change in plasma homocysteine and folate levels correlated significantly with nitrous oxide exposure time ($n = 40$; $r_S = 0.73$; $p < 0.0001$ and $n = 40$; $r_S = 0.36$; $p < 0.025$, respectively). Moreover, preoperative folate values correlated inversely with the absolute increase in plasma homocysteine concentrations ($n = 40$; $r_S = 0.34$; $p < 0.025$).

Interestingly, patients 41 and 42, who had been exposed to nitrous oxide for more than 24 hours within 3 days, showed a marked increase in plasma homocysteine values from 13.6 to 134.5 μmol/L and 15.0 to 67.4 μmol/L, respectively. Plasma folate levels increased only twofold from 18.1 to 39.6 nmol/L and 16.3 to 27.7 nmol/L. After the last nitrous oxide anesthesia, bone marrow smears from both patients revealed macrocytic changes.

As reported by others,[3] we found that during nitrous oxide exposure plasma methionine levels decreased ($n = 40$; $p < 0.025$) (Fig. 2, *bottom*). The percentage change in plasma methionine levels correlated inversely with the duration of nitrous oxide anesthesia ($n = 40$; $r_S = -0.29$; $p < 0.025$)

Effect of anesthesia on urinary excretion of folate and homocysteine. During the first 24 hours after the

Table I. Patient characteristics and preoperative plasma values of parameters studied

No.	*Age (yr)*	*Sex*	*Anesthesia (min)*	*Transfusions*	*Cobalamin (pmol/L)*	*TC II (pmol/L)*	*FA (nmol/L)*	*HCY (μmol/L)*	*MET (μmol/L)*
Patients anesthetized with nitrous oxide									
1	41	M	70	—	157	50	10.7	14.2	25.5
2	26	F	75	—	147	24	5.9	16.8	21.9
3	70	M	80	—	309	90	5.6	13.8	27.3
4	50	F	130	—	719	298	21.0	7.2	14.4
5	49	F	135	—	196	34	10.6	19.0	20.9
6	32	M	135	—	254	49	5.0	12.0	19.3
7	21	F	175	—	283	113	8.6	6.8	15.0
8	26	F	175	—	184	51	3.1	16.3	24.1
9	36	M	185	—	167	29	6.0	13.5	19.1
10	24	F	215	—	435	155	6.4	7.8	20.9
11	50	M	220	—	199	58	3.3	23.8	22.5
12	50	M	220	—	612	249	16.0	11.2	19.4
13	32	M	225	—	226	46	7.8	4.6	19.7
14	74	F	225	—	224	40	4.2	14.2	23.3
15	63	M	230	—	273	44	5.7	8.8	14.3
16	33	F	230	1 EC	179	27	5.2	14.7	18.5
17	40	M	255	—	220	48	5.8	9.6	27.3
18	45	F	280	—	212	43	6.3	9.8	39.5
19	29	M	300	—	287	45	5.0	8.1	18.5
20	59	M	300	—	180	50	10.6	12.1	24.5
21	72	M	305	—	121	24	8.4	12.7	19.0
22	26	F	310	—	143	89	2.9	14.2	26.0
23	41	F	310	—	233	67	4.8	12.9	27.9
24	52	F	315	—	172	36	14.5	9.3	35.7
25	47	F	320	3 EC	515	82	2.8	8.4	24.9
26	45	M	360	—	382	71	3.8	9.2	27.7
27	46	M	370	—	315	102	16.4	11.4	25.8
28	56	M	390	1 EC	325	30	7.8	11.2	17.6
29	65	F	400	—	165	32	6.4	10.8	17.9
30	18	M	420	—	167	34	4.1	14.6	31.1
31	27	F	425	—	109	8	4.8	10.6	6.2
32	54	M	460	2 EC	185	43	4.7	8.3	18.8
33	74	F	480	1 EC	204	53	10.0	6.7	13.6
34	52	M	520	3 EC	163	56	19.7	9.0	12.7
35	71	M	555	—	174	34	8.0	10.3	16.3
36	65	M	580	4 EC	70	17	6.8	7.2	7.2
37	49	F	600	1 EC	322	86	7.7	15.9	26.7
38	39	M	615	8 EC, 2 FFP	88	21	8.3	10.9	19.9
39	27	M	660	—	279	110	3.1	33.5	30.0
40	65	F	720	6 EC, 1 FFP	272	67	12.2	7.1	11.1
41	53	M	1440*	4 EC	350	90	18.1	13.6	18.6
42	18	M	1680*	4 EC	287	49	16.3	15.0	25.7
Patients anesthetized with TIVA									
43	32	F	115	—	189	83	6.0	15.0	20.8
44	54	M	125	—	475	163	24.2	11.6	21.6
45	30	M	165	—	389	225	5.4	18.9	24.4
46	40	M	195	—	167	23	23.8	13.2	14.7
47	81	M	240	—	196	31	6.3	15.1	11.4
48	24	F	245	—	213	41	17.8	8.1	13.4
49	40	F	310	—	272	61	7.0	7.5	10.5
50	35	M	370	—	171	16	5.3	9.6	19.0
51	70	M	520	4 EC	86	16	4.7	19.6	18.4
52	18	M	540	2 EC	138	14	15.7	11.7	14.0
53	51	M	560	1 EC	184	23	14.2	16.4	21.4
54	18	M	600	4 EC	317	51	7.0	12.3	9.6

TCII, Holotranscobalamin; FA, folate; HCY, homocysteine; MET, methionine; M, male; F, female; EC, erythrocyte concentrate; FFP, fresh frozen plasma; TIVA, total intravenous anesthesia.

Normal ranges: plasma total cobalamin, 120 to 800 pmol/L; holotranscobalamin II, 20 to 220 pmol/L; folate, 7 to 25 nmol/L; homocysteine, 5 to 15 μmol/L; methionine, 10 to 55 μmol/L.

*Total exposure time during a 3-day period.

start of anesthesia, the urinary folate and homocysteine excretions (Fig. 3) of patients anesthetized with nitrous oxide were significantly higher than those of patients anesthetized with TIVA ($p < 0.002$ and $p < 0.01$, respectively). An increase of urinary concentrations of both compounds up to 20-fold was observed. The increased homocysteine and folate excretion in the patients correlated with the duration of nitrous oxide anesthesia ($n = 19$; $r_S = 0.62$; $p < 0.005$ and $n = 19$; $r_S = 0.60$; $p < 0.01$, respectively). Postoperative plasma homocysteine levels correlated with urinary homocysteine values ($n = 19$; $r_S = 0.43$; $p < 0.05$), whereas no correlation was found between postoperative plasma folate and urinary folate excretion.

Time course of induced changes. From six patients anesthetized with nitrous oxide for various time periods and four patients anesthetized with TIVA, blood samples were collected at regular intervals for 24 hours to study changes in plasma folate, homocysteine, and methionine values as a function of time. In patients anesthetized with TIVA, only minor fluctuations in the parameters studied occurred and no significant pattern evolved (Fig. 4, *right panels*).

In patients given nitrous oxide anesthetic, plasma methionine levels usually declined after a lag phase ranging from 3 to 6 hours but returned to normal within 24 hours. Plasma folate and homocysteine values increased progressively within 1 hour after start of nitrous oxide anesthesia. In most patients, plasma homocysteine values reached a maximum at the end of anesthesia, whereas plasma folate levels peaked a few hours after termination of anesthesia. Then plasma folate and homocysteine values declined gradually. The ratio between free and protein-bound homocysteine (Fig. 4, *left panel*) remained unchanged during the entire observation period.

Urinary excretion of homocysteine followed roughly the time course of the plasma homocysteine level, whereas urinary folate excretion reached a maximum in the period of declining plasma folate (Fig. 4, *left panel*).

In patients anesthetized with TIVA only diurnal variations in urinary folate and homocysteine excretion occurred (Fig. 4, *right panel*).

Long-term effects. From eight patients operated on under nitrous oxide anesthesia and six patients operated on under TIVA, plasma homocysteine and plasma folate levels were determined 1 day, 2 days, and 1 week after anesthesia (Table II). No specific patterns developed in the group of patients anesthetized with TIVA. In patients anesthetized with nitrous oxide, elevated plasma folate levels usually returned

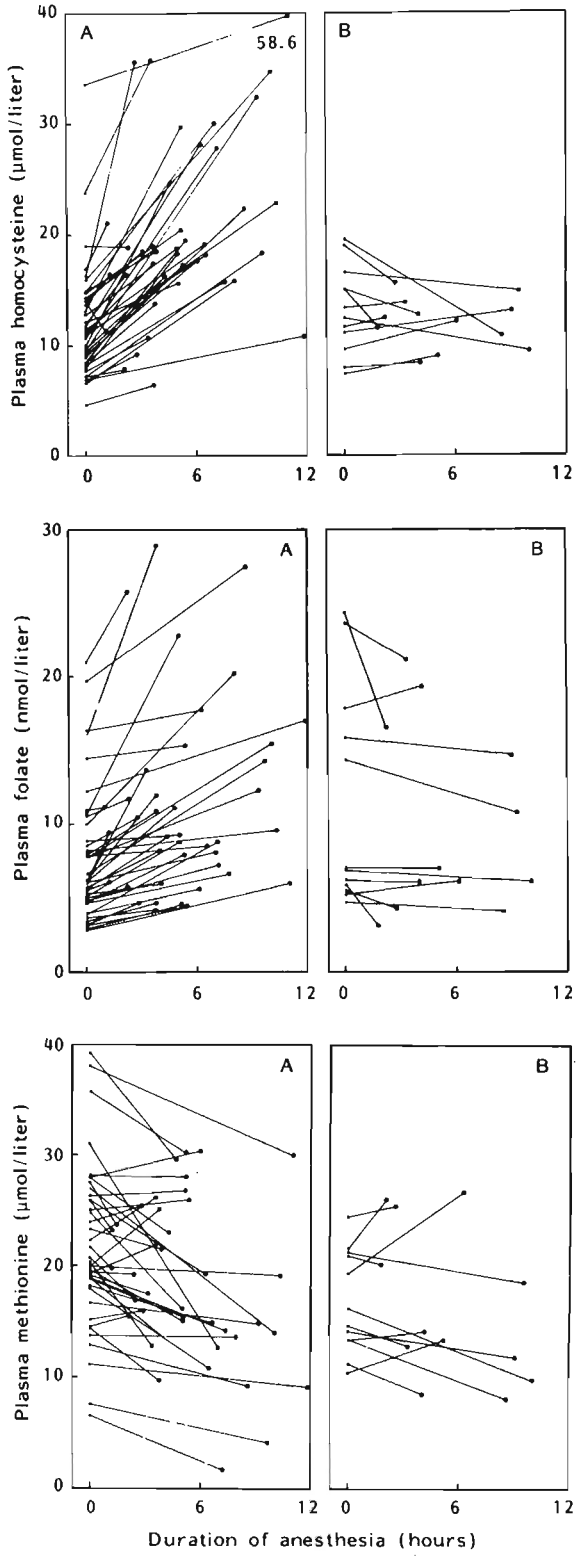

Fig. 2. Preoperative and postoperative levels of plasma homocysteine (normal range, 5 to 15 μmol/L), folate (normal range, 7 to 25 nmol/L), and methionine (normal range, 10 to 55 μmol/L). **A,** Patients anesthetized with nitrous oxide ($n = 40$). **B,** Patients anesthetized with TIVA ($n = 12$).

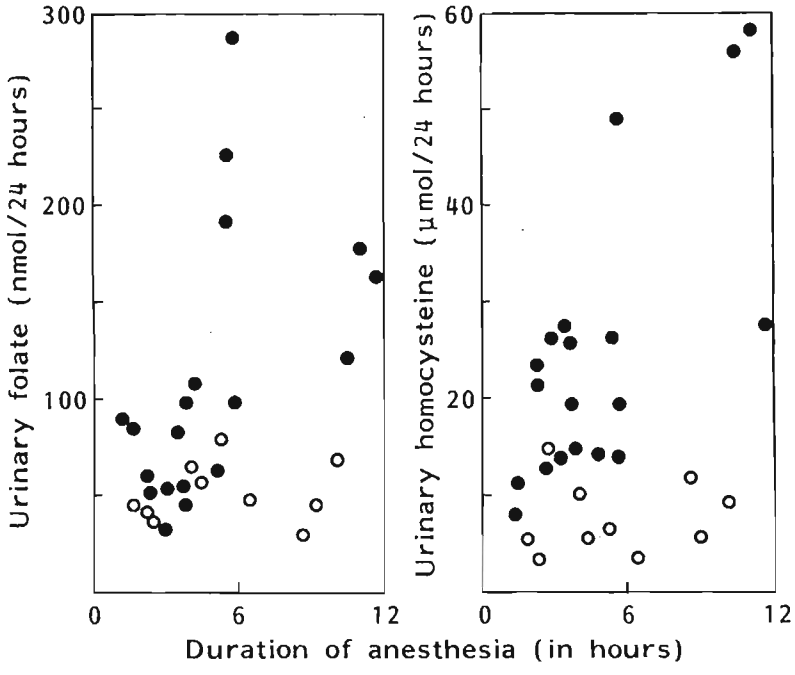

Fig. 3. Urinary excretion of folate and homocysteine during the first 24 hours after start of anesthesia. *Closed circles,* Patients anesthetized with nitrous oxide (n = 19); *open circles,* patients anesthetized with total intravenous anesthesia (n = 10). Urinary folate excretion in 10 healthy volunteers was less than 75 nmol/24 hr. Normal urinary homocysteine excretion, 3.5 to 9.5 μmol/24 hr.

Table II. Plasma folate and homocysteine levels 1, 2, and 7 days after anesthesia with nitrous oxide (N_2O) (n = 8) or total intravenous anesthesia (TIVA) (n = 6)

Group	*Folate (nmol/L)*		*Homocysteine (μmol/L)*	
	N_2O	*TIVA*	*N_2O*	*TIVA*
Start of anesthesia	7.9 ± 1.3	10.8 ± 3.0	10.2 ± 0.6	13.1 ± 1.8
End of anesthesia	11.7 ± 1.9*	9.0 ± 2.3	18.0 ± 1.9*	11.1 ± 0.8
1 Day after anesthesia	10.5 ± 1.2*	9.2 ± 2.6	18.4 ± 2.1*	8.9 ± 1.1
2 Days after anesthesia	8.0 ± 1.1	10.3 ± 3.0	15.6 ± 1.4*	12.0 ± 1.9
1 Wk after anesthesia	6.4 ± 1.3	ND	15.1 ± 1.6*	ND

ND, Not determined.
*$p < 0.01$.

to preoperative values within 2 days, whereas plasma homocysteine values were increased even 1 week after anesthesia (n = 8; $p < 0.01$).

DISCUSSION

Nitrous oxide is the only compound known to inactivate the methylcobalamin coenzyme of methionine synthase, which is an important enzyme of the folate metabolism. Possible side effects of this interaction are clinically relevant, in view of the widespread application of nitrous oxide in anesthesia.

Earlier studies have shown that in fit surgical patients disturbance of the folate-dependent thymidylate synthesis occurs after 5 to 6 hours of nitrous oxide anesthesia.[16] Megaloblastic bone marrow changes occur in fit patients after exposure to nitrous oxide for 12 to 24 hours.[1,3] The risk that routine nitrous oxide anesthesia becomes myelotoxic is generally considered to be small because duration of nitrous oxide exposure is usually too short to interfere seriously with the cobalamin-dependent folate metabolism. However, the rapid rise of plasma homocysteine and folate levels

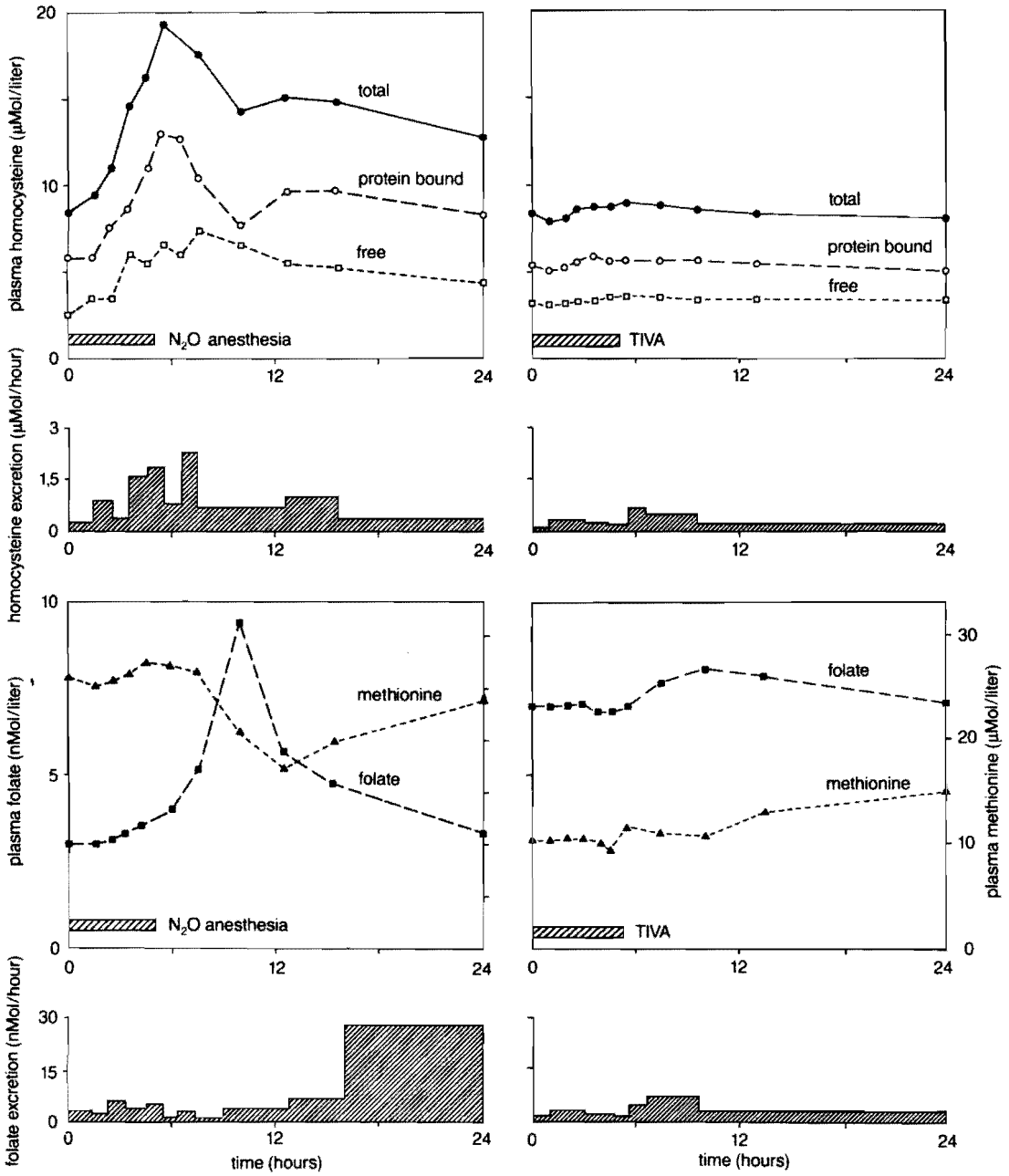

Fig. 4. Time course of changes in plasma and urinary parameters studied in a patient (No. 25) anesthetized with nitrous oxide **(left)** and a patient (No. 49) anesthetized with total intravenous anesthesia **(right).**

during nitrous oxide anesthesia found in our study indicates that perturbation of folate and homocysteine metabolism develops with little delay. The megaloblastic bone marrow changes seen in critically ill patients after less than 2 hours of exposure to nitrous oxide[5] and the highly increased bone marrow toxicity of methotrexate when administered after nitrous oxide anesthesia may well be attributed to this phenomenon.[17,18]

The observed differences in the individual response to nitrous oxide–induced cobalamin inactivation concurs with the observation of Royston et al.,[19] who

showed that the rate of methionine synthase inhibition during nitrous oxide anesthesia is variable. In addition, this phenomenom may be related to the cobalamin and folate status of the patient, because it is shown that low preoperative plasma folate levels may aggrevate the rise of plasma homocysteine values during nitrous oxide anesthesia.

Plasma homocysteine levels after nitrous oxide exposure frequently rose above normal. In several patients the plasma homocysteine levels were comparable with those observed in cobalamin deficiency.[9] These findings further support the suggestion that plasma homocysteine may be a useful parameter to measure disturbances of the cobalamin-dependent folate metabolism.[9]

Excretion of homocysteine into urine may also be a good indicator because the enhanced renal excretion of this amino acid correlates strongly with the duration of nitrous oxide anesthesia. The observed increase in urinary folate values corresponds to similar observations in rats,[20] but is difficult to explain because folate plasma values rarely became abnormal. The reuptake of urinary folates by renal tubuli cells is possibly also affected by the inhibition of methionine synthase causing elevated excretion.

In most tissues the cobalamin-dependent methylation of homocysteine to methionine is an important pathway of homocysteine disposal.[21] This is demonstrated by the homocystinuria observed in patients with reduced methionine synthase activity.[22,23] Our finding of a marked elevation of plasma homocysteine levels after cobalamin inactivation is in accordance with the quantitative importance of the homocysteine remethylation. We found that plasma homocysteine levels are still elevated 1 week after nitrous oxide anesthesia. This implies that cobalamin-dependent methionine synthesis recovers very slowly and may explain that increase of plasma homocysteine values was most pronounced in our two patients who underwent multiple nitrous oxide exposures. Notably, subjects who have been exposed repeatedly to nitrous oxide within a short period become particularly vulnerable to development of megaloblastosis.[24,25] The finding that the increased plasma folate levels returned to preoperative values by 2 days after nitrous oxide exposure could be related to both elevated excretion[20] and reuptake by tissues (e.g., because of enhanced folate polyglutamation).[26]

In summary, this study demonstrates that during nitrous oxide anesthesia cobalamin-dependent methionine synthesis becomes seriously compromised and this effect can be monitored by the determination of plasma homocysteine levels. In addition, it shows that nitrous oxide anesthesia perturbs cobalamin-dependent folate and homocysteine metabolism at a faster rate than hitherto recognized and complete recovery of methionine synthase activity probably requires several days, even after short exposure.

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