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SERUM METHIONINE AND HEPATIC ENZYME ACTIVITY IN ANAESTHETISTS EXPOSED TO NITROUS OXIDE

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SUMMARY

Normal serum concentrations of methionine, leucine, isoleucine and valine have been found in 10 anaesthetists using nitrous oxide under their regular working conditions without scavenging of patients' exhaled gas. Mean inhaled concentrations of nitrous oxide ranged from 150 to 400 p.p.m. The results indicate either that there was no significant inhibition of methionine synthase (attributable to oxidation of vitamin B_{12} by nitrous oxide) or that methionine concentrations were maintained by dietary intake or by the alternative betaine pathway of methylation of homocysteine. In either case, anaesthetists working under these conditions should not be at risk from reduced methionine concentrations. We also report normal serum activities of aspartate transaminase and gamma glutamyl transpeptidase.

Epidemiological studies of the hazards of working in operating theatres are prone to biased reporting, but nevertheless an increased rate of abortion is a consistent finding (Vessey and Nunn, 1980) and there is some evidence that this is related to exposure to nitrous oxide (Cohen et al., 1980; Lane et al., 1980). A few hours of exposure to concentrations of nitrous oxide greater than about 1% results in oxidation of vitamin B_{12}, causing inactivation of methionine synthase, of which it is the co-factor (Deacon et al., 1978; Koblin et al., 1981). Inhibition of this enzyme seems likely to be responsible for the derangement in folate metabolism that interferes with the synthesis of thymidine (Amess et al., 1978; Chanarin, 1980).

Animals chronically exposed to lower concentrations of nitrous oxide (0.1–0.5%) have depressed hepatic methionine synthase activity (Nunn and Sharer, 1981). These concentrations are rather higher than those of time-weighted mean samples drawn from the front of masks worn by anaesthetists working without scavenging devices for 2-h periods under routine operating theatre conditions (Davenport et al., 1980). However, peak concentrations are much higher than the time-weighted mean values (Hillman et al., 1981) and may have a greater effect because of the irreversible chemical action of nitrous oxide on the cobalamin, which is in contrast to the weak reversible bonding that is characteristic of most other actions of inhalation anaesthetics. It is not known, however, whether prolonged exposure to trace concentrations of nitrous oxide has any effect on vitamin B_{12} of operating theatre staff.

The most sensitive index of the action of nitrous oxide on vitamin B_{12} is methionine synthase activity itself. However, since this is an intracellular enzyme, there are difficulties in studying its activity in operating theatre staff. We have therefore measured the serum concentration of methionine, which would tend to decrease if an enzyme involved in its synthesis were inhibited. This has already been shown to occur in rats exposed to 50% nitrous oxide (Deacon et al., 1981), and in surgical patients whose anaesthetic regimen included nitrous oxide and whose dietary intake was restricted in the perioperative period (Parry, Blackmore and Roberts, 1981; P. O. Skacel, personal communication).

In addition we measured the serum concentration of two amino acids that utilize another B_{12}-dependent enzyme (methyl malonyl CoA mutase) in their catabolism, namely isoleucine and valine. The programme used for the analysis of these amino acids allowed us to measure leucine concurrently. We also took the opportunity of measuring hepatic enzyme activities in view of reports of an increased frequency of hepatic disorders in anaesthetists (American Society of Anesthesiologists, 1974).

METHODS

Subjects

We selected 10 members of the operating theatre staff (table I) from two hospitals where we had

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TABLE I. *Details of subjects*

No	Age (yr)	Sex	Grade	Exposure to nitrous oxide during previous 80 h (h)
Hospital A				
1	27	M	Technician	26
2	30	F	Sister	26
3	31	M	Anaesthetist (Reg.)	16
4	55	M	Anaesthetist (Cons.)	17
5	26	M	Anaesthetist (SHO)	16
6	20	F	Receptionist	—
7	54	M	Technician	—
8	33	F	Radiographer	—
9	60	F	Nurse	—
10	25	F	Nurse	—
Hospital B				
11	24	F	Anaesthetist (SHO)	28
12	27	M	Anaesthetist (Reg.)	10
13	46	M	Anaesthetist (Cons.)	20
14	25	F	Anaesthetist (SHO)	30
15	29	F	Anaesthetist (Reg.)	13
16	38	M	Nurse	—
17	31	F	Sister	—
18	28	F	Nurse	—
19	38	F	Sister	—
20	30	F	Nurse	—

already established that anaesthetists were exposed to high concentrations of nitrous oxide (Davenport et al., 1980). No scavenging systems were in use, but hospital A used a charcoal adsorption canister which removes volatile agents but not nitrous oxide. Samples were taken between 1.30 and 3.30 p.m. on Thursday of a typical working week. Control subjects were sampled simultaneously and comprised of hospital staff who did not work in an environment where anaesthetics were used. Venous blood was taken from the antecubital fossa, allowed to clot and stored at 4 °C during transport to the Clinical Research Centre.

Nitrous oxide concentrations

The mean concentration of nitrous oxide in the operating theatre atmosphere was determined using a Wright personal sampling system (Davenport et al., 1976). Samples were taken for periods of 1 h from the area of the anaesthetist's nose. Nitrous oxide was detected by mass spectrometry (Medishield multigas monitor MS2).

Amino acid analysis

Serum samples were prepared for analysis by deproteinization with an equal volume of 10% aqueous salicylsulphonic acid. Nor-leucine was added as an internal standard. Amino acid analysis was carried out using a Technicon model TSM analyser with an accelerated program designed to resolve and quantitate methionine. With this program it is possible to determine, in addition, valine, isoleucine and leucine.

RESULTS

There was no significant difference between the mean values found for the serum methionine concentration of the operating theatre staff and the control group, nor was there any degree of correlation between these values and the number of hours of exposure during the previous 3½ days (fig. 1). The other serum amino acid concentrations measured and the hepatic function enzyme activities showed no significant difference between the two groups, although the aspartate transaminase activity was slightly above the reference range for

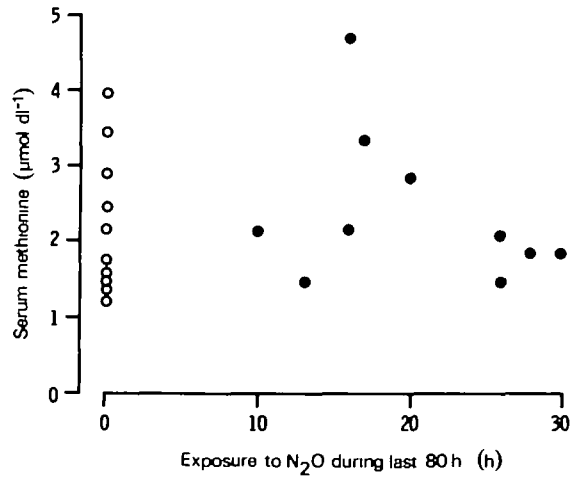

FIG. 1. Serum methionine, plotted against duration of exposure to nitrous oxide. Open circles = controls; closed circles = operating staff personnel.

one exposed subject (table II). Mean nitrous oxide concentrations in air breathed by the operating staff personnel ranged from 150 to 400 p.p.m.

DISCUSSION

This study provides no evidence of abnormality in the serum concentrations of methionine, valine, isoleucine or leucine in 10 operating theatre staff working in unscavenged environments with high ambient trace concentrations of nitrous oxide. This contrasts with the reported decreases in methionine concentrations following acute exposure to greater concentrations (Deacon et al., 1981; Parry, Blackmore and Roberts, 1981; P.O. Skacel, personal communication).

While these results are reassuring from the point of view of one potential hazard to those working in unscavenged operating theatres, it is difficult to interpret them in terms of the synthesis of methionine. This is because serum methionine is derived from at least three sources. As an essential amino acid, all the methionine in the mammalian body is ultimately of dietary origin. However, its turnover in donating methyl groups exceeds the dietary intake and therefore methionine concentrations are maintained by the remethylation of homocysteine by enzymic transfer of methyl groups from either 5-methyl tetrahydrofolate or betaine (fig. 2). Our results may mean that either methionine synthase is not significantly inhibited by the concentrations of nitrous oxide reaching the active site on the molecule under these conditions or, alternatively, that reduced methionine synthesis by this route can be compensated either by an adequate dietary intake of the amino acid or by methylation of homocysteine by the betaine pathway.

In spite of the normal amino acid concentrations

TABLE II. *Results*

	Serum amino acids (μmol dl^{-1})				Hepatic enzymes (iu $litre^{-1}$)	
	Methionine	Valine	Isoleucine	Leucine	Gamma GT	Asp. trans.
Exposed subjects						
1	2.05	17.85	5.55	11.25	22	23
2	1.45	15.40	3.95	10.40	8	21
3	4.70	23.55	8.45	15.70	44	44
4	3.35	24.20	8.50	16.60	23	17
5	2.15	25.05	8.20	15.60	15	20
11	1.85	—	4.45	9.65	14	15
12	2.15	20.55	4.90	12.30	10	16
13	2.85	34.10	7.35	13.55	10	20
14	1.85	22.35	5.40	15.90	8	16
15	1.45	13.90	3.30	7.35	14	18
Mean	2.39	21.88	6.01	12.83	16.8	21.0
SD	1.00	6.03	1.96	3.14	10.91	8.47
SEM	0.32	2.01	0.62	0.99	3.45	2.68
Controls						
1	1.75	14.70	3.85	8.80	19	19
7	1.55	24.95	6.50	14.30	17	19
8	2.45	22.25	4.80	10.60	9	12
9	2.90	18.15	7.90	15.50	10	14
10	3.45	27.40	12.45	14.75	18	21
16	3.95	23.40	7.90	15.30	16	23
17	2.15	19.90	4.80	8.65	6	21
18	1.25	20.75	4.60	12.70	10	13
19	1.50	10.15	3.45	6.20	5	15
20	1.40	13.55	4.10	7.35	11	17
Mean	2.24	19.52	6.04	11.39	11.10	17.4
SD	0.93	5.42	2.76	3.52	4.72	3.78
SEM	0.29	1.71	0.87	1.11	1.49	1.19
t	0.35	0.90	0.03	0.97	1.51	1.23
Normal range	1.3–3.9	12–33	3.5–10.0	6.9–16.0	up to 50	10–35

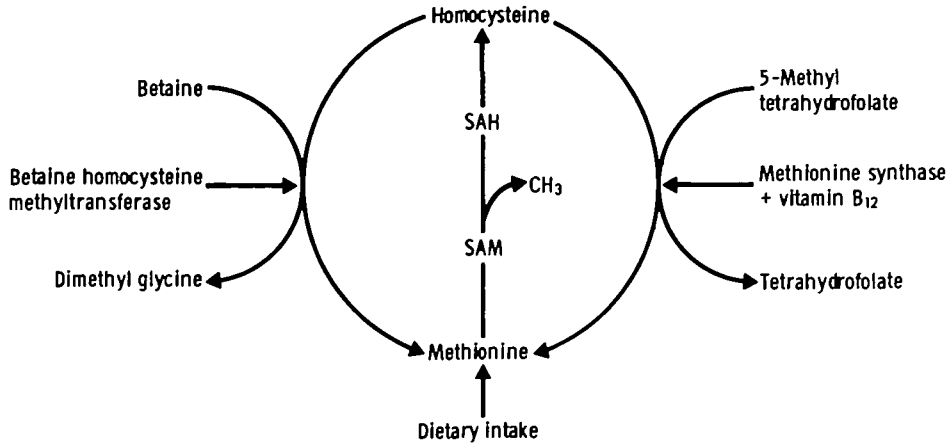

FIG. 2. Pathways in maintenance of methionine concentrations. SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine

found in this study, there remain two special circumstances under which exposure to nitrous oxide may be dangerous and in which B_{12} inactivation is probably implicated. First, abuse by those with professional access to the gas is known to cause demyelination of the central nervous system, giving rise to a condition not unlike sub-acute combined degeneration of the cord (Layzer, 1978). This appears to be similar to a condition associated with demyelination of the cord which develops in primates exposed to nitrous oxide (Dinn et al., 1978), and which can be prevented by supplementation of the diet with methionine (Scott et al., 1981). Second, Lane and others (1980) have shown that 75% nitrous oxide causes significant fetotoxicity in rats when administered at the critical stage of pregnancy.

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CONCENTRATION SERIQUE DE METHIONINE ET ACTIVITE ENZYMATIQUE DU FOIE CHEZ LES ANESTHESISTES EXPOSES AU PROTOXYDE D'AZOTE

RESUME

Des concentrations normales de méthionine, de leucine, d'isoleucine et de valine ont été retrouvées chez 10 anesthésistes qui utilisaient du protoxyde d'azote de façon régulière dans leur travail sans évacuer les gaz expirés par le patient Les concentrations moyennes inhalées de protoxyde d'azote allaient de 150 à 400 volumes pour 1 million. Les résultats montrent qu'il n'y a pas d'inhibition sigificative de la méthionine synthétase (qu'on pourait attribuer à l'oxydation de la vitamine B_{12} par le protoxyde d'azote) ou que les concentrations de méthionine sont préservées par l'apport alimentaire ou par la voie alterne de la bétaine par méthylation de l'homocystéine Dans l'un et l'autre cas, les anesthésistes qui travaillent dans ces conditions ne devraient pas courrir de risques du fait de concentrations faibles de methionine Nous avons également retrouvé des concentrations sériques normales d'aspartate transaminase et de gammaglutamyl transpeptidase.

SERUM METHIONIN UND LEBERENZYM-AKTIVITÁT BEI ANÁSTHESISTEN, DIE LACHGAS AUSGESETZT WAREN

ZUSAMMENFASSUNG

Normale Serumkonzentrationen von Methionin, Leuzin, Isoleuzin und Valin wurden bei 10 Anàsthesisten gefunden, die bei ihrer taglichen Arbeit Lachgas benutzten ohne dass eine Gasabsaugeanlage vorhanden war Die durchschnittlich eingeatmete Lachgasmenge reichte von 150–400 Teile pro millionen Die Ergebnisse zeigen, dass entweder die Methionin-Synthase nicht signifikant gehemmt wird (wegen einer Oxidation des Vitamin B_{12} durch Lachgas oder aber dass die Methioninspiegel durch die Nahrungszufuhr oder durch den alternativen Betain-Stoffwechselweg durch Methylisierung von Homozystein aufrecht erhalten werden. In jedem Fall sind Anasthesisten, die unter diesen Bendingungen arbeiten nicht von erniedrigten Methioninspiegeln gefahrdet. Ausserdem fanden wir normale Serumspiegel von Aspartat-Transaminase und Gammaglutamyl-transpeptidase

ACTIVIDAD LE LA METIONINA DE SUERO Y DE LA ENCIMA HEPATICA EN LOS ANESTESISTAS EXPUESTOS AL OXIDO NITROSO

SUMARIO

Se han encontrado concentraciones de metionina, leucina, isoleucina y valina en el suero de los anestesistas que usan óxido nitroso regularmente en sus ambientes de trabajo en los que no se efectúa el barrido de los gases de espiración del paciente. Las concentraciones medias de óxido nitroso inhalado oscilaron entrè 150 y 400 p.p.m Los resultados indican que no hubo una inhibición significativa de la sintasa de metionina (atribuible a la oxidación de la vitamina B_{12} por el óxido nitroso) o que las concentraciones de metionina se mantuvieron por entrada dietética o por la ruta betaina alternativa de metilación de la homocisteina. Sea como fuere, los anestesistas que trabajan bajo estas condiciones no sufren riesgo alguna a causa de las concentraciones de metionina Asimismo informamos de que las concentraciones de aspartata trasnsaminasa y de transpeptidasa gama de glutamil en el seuro son normales.