

Consumption of Vitamin B₁₂ during Sodium Nitroprusside Administration in Humans

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In view of evidence suggesting possible participation of cobalamin in cyanide metabolism, the effects of sodium nitroprusside (SNP) infusion on blood cyanide and plasma thiocyanate levels and serum vitamin B₁₂ levels were examined in ten patients undergoing major orthopedic procedures. Whole blood cyanide concentrations increased significantly ($P < 0.001$) from 3.6 ± 1.1 to 65.7 ± 16.2 $\mu\text{g}/\text{dl}$, and total serum B₁₂ values decreased significantly ($P < 0.005$) from 482 ± 56 to 267 ± 42 pg/ml , three hours after SNP therapy. Plasma thiocyanate did not change. Cyanide released from SNP converts hydroxocobalamin to cyanocobalamin, which is readily excreted in urine. These variables were not changed in six patients (control) who received trimethaphan. *In vitro* experiments revealed that none of the serum factor(s) nor nitroprusside interfered with the assay of B₁₂. Hydroxocobalamin may be an appropriate adjunct during SNP therapy. (Key words: Anesthetic techniques: hypotension, induced, nitroprusside; trimethaphan. Pharmacology: nitroprusside; serum B₁₂. Toxicity: cyanide; thiocyanate.)

INCREASED EXPERIENCE with sodium nitroprusside (SNP) has led to the realization that its toxic potential is greater than previously believed. Metabolic acidosis,¹ toxicity, and death^{2,3} have been reported with its use. These complications have been related to direct effects of cyanide. Animal^{4,5} and clinical studies⁶ have suggested the possible participation of cobalamin (vitamin B₁₂) in the metabolism of cyanide. The present clinical study was designed to measure the effects of SNP infusion not only on cyanide and thiocyanate levels, but also on serum levels of vitamin B₁₂.

Materials and Methods

Sixteen patients scheduled for major orthopedic procedures under general anesthesia and controlled arterial hypotension were studied.† Their ages ranged from 16 to 39 years (mean 34 years) and their mean (\pm SEM) weight was 58 ± 4 kg. None had clinical or laboratory evidence of cardiovascular, renal,

hepatic, endocrine or metabolic disorder. They were randomly allocated into two groups according to the hypotensive drug used: ten patients received SNP and six were given trimethaphan (TMP). The groups were comparable with regard to age, sex distribution and weight. Nitroprusside (Nipride,[®] Roche) was used as a 0.01 per cent solution and trimethaphan (Arfonad,[®] Roche) as a 0.1 per cent solution.

A standard technique of anesthesia was employed for both groups of patients. Premedication consisted of morphine, 0.1 mg/kg, and scopolamine, 0.3 mg/70 kg, given intramuscularly one hour before induction of anesthesia with thiopental sodium, 5 mg/kg. Succinylcholine, 1 mg/kg, was given intravenously to facilitate endotracheal intubation. Anesthesia was maintained with 60 per cent nitrous oxide in oxygen, and intravenous morphine sulfate as required (average dose 24 mg/patient). *d*-Tubocurarine, 0.4 mg/kg, was used to provide muscle relaxation. Ventilation was controlled to maintain arterial carbon dioxide partial pressure (Pa_{CO_2}) between 35 and 40 torr, as determined by repeated measurements. The electrocardiogram (Lead II), arterial pressure (radial artery catheter); and body (esophageal) temperature were monitored continuously. Lactated Ringer's solution was infused at $7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. Albumin, 5 per cent in saline solution, and packed erythrocytes were administered to replace measured blood loss determined by weighing sponges and measuring the volume of blood in suction bottles.

Arterial hypotension was induced before incision of the skin by continuous intravenous infusion‡ of SNP or TMP. The infusion rate was adjusted to maintain mean arterial blood pressure at 55 torr in an attempt to decrease blood loss. Drug infusion was discontinued, and arterial pressure allowed to return to prehypotension values before wound closure to secure hemostasis.

Arterial blood samples were withdrawn prior to anesthetic induction; before, during (every hour for 3 hours), and two hours after SNP or TMP administration. Samples were analyzed in duplicate for whole blood cyanide, plasma thiocyanate, total serum B₁₂ (cobalamin) concentration, blood lactate and pyruvate, serum electrolytes, P_{O_2} , P_{CO_2} and *pH*. Cyanide and

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† All patients gave informed consent to the study and the protocol was approved by the Subcommittee on Human Studies at the Massachusetts General Hospital.

‡ Volumetric Infusion Pump, IMED Corporation.

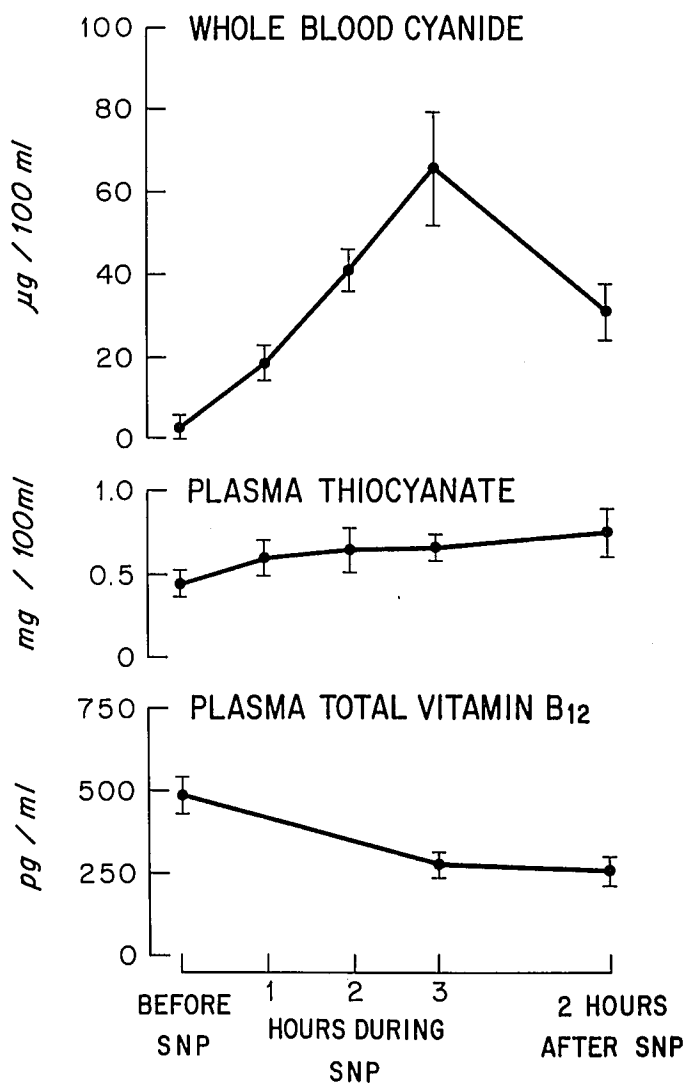


FIG. 1. Changes (mean \pm 1 SEM) in concentrations of whole blood cyanide, plasma thiocyanate and total plasma vitamin B₁₂ during (3 hours) and after (2 hours) sodium nitroprusside infusion in ten patients.

thiocyanate concentrations were determined by colorimetric methods,^{7,8} lactate and pyruvate by enzymatic methods,⁹ serum electrolytes by flame photometry, P_{O₂}, P_{CO₂} and pH by standard electrodes.

Total serum B₁₂ (methylcobalamin, adenosylcobalamin, hydroxocobalamin, and cyanocobalamin) was measured by a radiosorbent technique,¹⁰ which utilizes intrinsic factor as the binder. The lower limit of this technique is 25 pg/ml.

Peripheral blood smears were obtained before and after operation, and examined for evidence of macrocytosis and hypersegmentation of neutrophils.

In vitro experiments were conducted to study the possible effect of some factor(s) in the serum of patients on the assay of vitamin B₁₂. Serum from patients

who received SNP or TMP was mixed with serum from normal individuals (known B₁₂ concentration) and incubated for 30 min at 37° C. Further experiments to study the effect of SNP on the assay of B₁₂ in serum were carried out as follows: duplicate aliquots of serum obtained from normal subjects were assayed for vitamin B₁₂ content both in the absence of SNP and with SNP added in final concentrations ranging from 1–0.001 (W/V) per cent. A third set of experiments consisted of measuring B₁₂ levels before and after addition of known amounts of cyanocobalamin to samples of serum from patients treated with SNP or TMP.

A two-way analysis of variance and Student's *t* tests were used to analyze the data. A probability of less than 0.05 was sufficient to reject the null hypothesis.

Results

Cyanide was detected in blood samples obtained from all patients who received SNP. Whole blood cyanide concentration rose from a pre-infusion value of 3.6 ± 1.1 µg/dl (mean \pm SEM) to 65.7 ± 16.2 µg/dl 3 hours after SNP infusion had begun (fig. 1). This change was significant ($P < 0.001$). Two hours after cessation of infusion, whole blood cyanide levels were still significantly ($P < 0.01$) higher than pre-infusion values. Plasma thiocyanate levels tended to increase with time (fig. 1) and a small but significant ($P < 0.05$) increase was observed 2 hours after cessation of SNP infusion.

Infusion of SNP was associated with a significant ($P < 0.005$) decrease in serum B₁₂ concentration. The pre-infusion value of 482 ± 56 pg/ml decreased to 267 ± 42 pg/ml, 3 hours after start of infusion, and to 252 ± 46 pg/ml, 2 hours after SNP was discontinued. A significant negative correlation ($r = -0.798$, $P < 0.01$) between whole blood cyanide and serum B₁₂ concentrations was found.

There were no significant differences between the two groups in whole blood cyanide, plasma thiocyanate or serum B₁₂ values before initiation of hypotension. No changes in whole blood cyanide or plasma thiocyanate were detected in patients who received TMP. Serum B₁₂ concentrations did not change significantly in the trimethaphan-treated patients. Mean pre-infusion, post-infusion and post-operative (2 hours) values were 502 ± 47 , 486 ± 39 , and 479 ± 41 pg/ml, respectively.

Arterial P_{O₂} remained above 100 torr in all patients during periods of observation. Acid-base balance, serum electrolytes, blood lactate and pyruvate did not change significantly during observation periods in either group.

The total dose of SNP per patient was 50.2 ± 9.1 mg (0.89 ± 0.16 mg/kg), whereas that of TMP was 365 ± 46 mg (6.18 ± 0.79 mg/kg). Infusion rates of SNP and TMP were 4.3 ± 0.9 and 28.3 ± 5.1 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively. There was no significant difference in durations of hypotension which were 207 ± 11 min for SNP and 218 ± 19 min for TMP.

No significant difference in blood replacement between the groups existed. The nitroprusside-treated group received 490 ± 45 ml packed erythrocytes and 275 ± 40 ml albumin, whereas the trimethaphan-treated patients had 475 ± 40 ml red cells and 400 ± 50 ml albumin. None of the patients experienced persistent hypotension, electrocardiographic changes, excessive blood or fluid losses. There was no evidence of anemia, macrocytosis or hypersegmentation of the neutrophils in any of the subjects studied.

Dilution of serum from subjects receiving SNP or TMP with serum from normal individuals produced B₁₂ values predicted by the dilution ratio. Addition of nitroprusside at concentrations ranging from 1–0.001 per cent did not affect B₁₂ concentrations in serum of normal individuals. When serum was assayed before and after addition of 300–800 pg/ml of cyanocobalamin to each ml of serum, recoveries of added cyanocobalamin ranged from 90 to 97 per cent, indicating no interference with B₁₂ assay by serum of the patients.

Discussion

The present study demonstrates that *in vivo* administration of therapeutic doses of nitroprusside (acutely administered) is associated with the release of cyanide and a significant decrease in serum B₁₂ concentration. The total dose (0.89 mg/kg) and infusion rate ($4.3 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) of SNP employed in this study group are below the recommended maximum doses of 1.5 mg/kg¹¹ and $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$,¹² respectively, for short-term use of SNP. Neither general anesthesia nor controlled arterial hypotension *per se* could have influenced the results since no changes were observed in control patients receiving trimethaphan. Furthermore, it is unlikely that anesthetic drugs employed in this study produced quantitative or qualitative changes in the metabolic degradation of SNP, since blood cyanide levels were similar in unanesthetized patients receiving SNP for treatment of medical emergencies.¹³ Absence of hematological changes in the peripheral blood of all patients, together with the insignificant changes in serum B₁₂ levels of trimethaphan-treated patients, strongly suggest that nitrous oxide did not interfere with the results of the present study.

Nitroprusside reacts non-enzymatically with hemoglobin liberating five cyanide radicals.¹⁴ Approximately two-thirds of the cyanide is converted enzymatically in the presence of thiosulfate (sulfur donor) to thiocyanate by liver and tissue rhodanese.¹⁵ The progressive and significant increase in whole blood cyanide concentration with SNP infusion and its decline following cessation of administration (fig. 1) suggests a relatively rapid release of cyanide from SNP. The observed elevations in blood cyanide concentration are in agreement with the reports of Vesey *et al.*¹¹ in humans, and Michenfelder's results with dogs.¹⁶ Furthermore, they are much lower than the minimum lethal blood cyanide concentration of 340 $\mu\text{g}/\text{dl}$ suggested by Gettler and Baine.¹⁷

Changes in plasma thiocyanate concentration are relatively small, however. Merrifield and Blundell⁹ and Davies *et al.*,² using short-term SNP infusions, were unable to find any significant increase of plasma thiocyanate in their patients who died from SNP (cyanide) toxicity. Thus, plasma thiocyanate concentration is not a valuable indicator of short-term exposure to cyanide.

An important finding reported here is the significant decrease in total serum B₁₂ concentration associated with SNP administration. The mechanism by which serum B₁₂ values are reduced in these patients is not immediately apparent. Possibilities include the following: enhanced tissue avidity; any factor(s) which interfere with the radio-isotope dilution assay for the vitamin; and increased excretion.

For significant renal excretion of B₁₂ to occur, serum B₁₂ will either have to exceed the binding capacity of serum, or be readily dissociated from the binding proteins, or be in the form of cyanocobalamin. Cyanocobalamin is less tightly bound to nonspecific protein binders than hydroxocobalamin, and is thus easier to eliminate in urine. Studies of healthy smokers¹⁸ and patients with neuro-ophthalmological disorders (Leber's optic atrophy, optic atrophy, and tobacco amblyopia)¹⁹ have shown an inverse relationship between the concentration of serum B₁₂ and cyanide, together with an increased proportion of cyanocobalamin. In a comparative study between smokers (high cyanide intake) and nonsmokers, Linnell *et al.*¹⁸ found that smokers had elevated plasma concentration of cyanide and thiocyanate, decreased total serum B₁₂ concentration, and a tendency for the level of cyanocobalamin (normally present in trace amounts, if any) to rise in plasma. Urinary excretion of vitamin B₁₂ was increased. These authors attributed their findings to conversion of tissue cobalamins to cyanocobalamin, which does not bind well to protein and is relatively more readily excreted in urine.

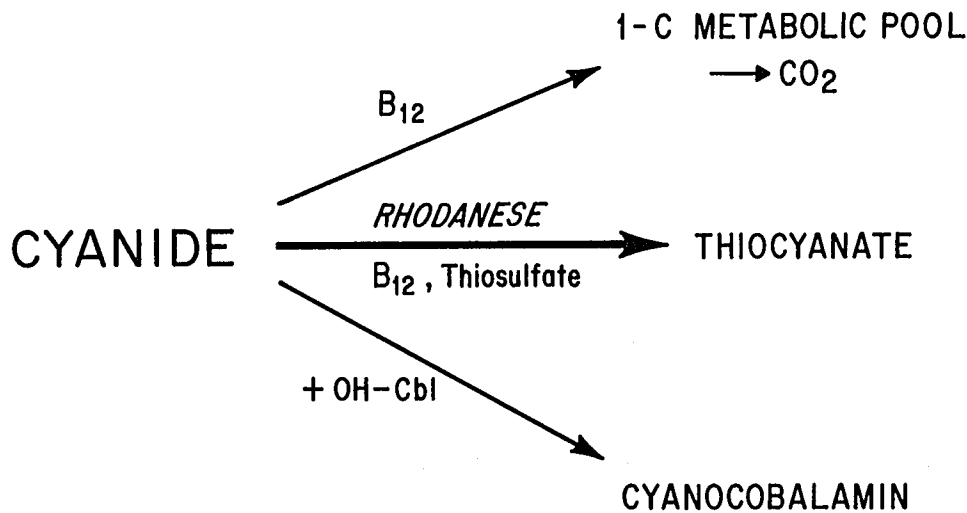


FIG. 2. Role of vitamin B₁₂ in cyanide metabolism. Diagram is based on reports in the literature. (See text for references.)

Hydroxocobalamin, an important constituent of total plasma cobalamin, takes up cyanide avidly to form cyanocobalamin,²⁰ and can thereby help protect animals and humans against cyanide toxicity.²¹ Furthermore, examination of plasma and liver cobalamins in patients with tropical ataxic neuropathy,²² due to consumption of cassava (a rich source of cyanide) has revealed that their plasma contained more than six times as much cobalamin as cyanocobalamin than plasma from control subjects. Cyanocobalamin was also detected as an increased portion of total cobalamin in the majority of liver biopsies from these patients, but not in controls. The cyanide load resulting from SNP infusion can, therefore, be expected to increase conversion of hydroxocobalamin (and possibly other cobalamins) to cyanocobalamin (fig. 2), which being less firmly bound to plasma proteins, is more readily excreted than other forms of vitamin B₁₂. This would decrease plasma B₁₂ concentration. Cyanide administration has also been shown to decrease liver reserves of vitamin B₁₂. Injection of sublethal doses of cyanide to rats caused a significant depletion of liver stores of vitamin B₁₂ and suggested that this store is an important detoxifying agent during cyanide poisoning.²³ Thus, the negative correlation between blood cyanide and serum B₁₂ concentration observed in our patients could be a result of B₁₂ depletion due to the high cyanide load imposed by SNP administration.

In addition to its consumption during cyanide metabolism, vitamin B₁₂ has been speculated to have other roles (fig. 2). Wokes and Picard²⁴ suggested that vitamin B₁₂ is important for the conversion of cyanide to thiocyanate by rhodanese, and Smith and Duckett⁵ have presented some data supporting this hypothesis. Wokes and Picard²⁴ found that urinary thiocyanate excretion in B₁₂-deficient patients was lower than in

healthy controls and that cobalamin treatment rapidly increased this excretion. Smith and Duckett⁵ compared thiocyanate excretion in three groups of rats treated, respectively, with cyanide, with cyanide and cyanocobalamin, and with cyanide and hydroxocobalamin. Relatively high doses of cyanide were used, and a marked increase in thiocyanate excretion after cyanide administration was observed. Excretion was highest in the group treated with both hydroxocobalamin and cyanide, but there was little difference between the other two groups. Boxer and Rickards⁴ suggested that vitamin B₁₂ may also act as an intermediary in the incorporation of cyanide into the I-C metabolic pool, perhaps as formate, with eventual formation of carbon dioxide.

Although the effects on serum vitamin B₁₂ levels and on B₁₂ metabolism of disciplined short-term administration of SNP are unlikely to be important in normal subjects, such may not be the case in patients whose vitamin B₁₂ status may be impaired. These include patients with low plasma B₁₂ levels, inborn errors of B₁₂ or its related binding proteins, impaired liver function, Leber's optic atrophy, and tobacco amblyopia. Until the significance of the decrease in serum B₁₂ is determined, these conditions may represent a contraindication to the use of SNP. Finally, administration of hydroxocobalamin may be an appropriate adjunct during SNP therapy; it not only raises plasma cobalamin levels, but also acts as a cyanide antidote,²¹ thereby decreasing the potential toxicity of nitroprusside.

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