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Cobalamin (Vitamin B_{12}) Analogs Are Absent in Plasma of Fruit Bats Exposed to Nitrous Oxide[1] (41482)

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Abstract. Fruit bats are an animal model for the neurologic damage which occurs in vitamin B_{12}-deficient humans. Cobalamin (vitamin B_{12}) analogs were not detected in the plasma of fruit bats treated with nitrous oxide (N_2O), which inactivates cobalamin. This observation does not lend support to the suggestion that the neurological changes associated with cobalamin inactivation by N_2O and/or cobalamin deficiency per se may be related to the accumulation of cobalamin analogs. However, although the plasma of control fruit bats lacked analogs, we did find analogs in their livers, at levels about 10% of total liver corrinoids, similar to human liver analog levels.

It was recently suggested that physiologically inactive cobalamin (vitamin B_{12}) analogs are present in mammalian plasma (1) and tissues (2). If present, these analogs may have considerable importance, for they could exacerbate the effects of true cobalamin deficiency (3), such as the neurological changes. Kondo *et al.* (4) reported that exposure of rats to nitrous oxide (N_2O), which inactivates cobalamin (5), also results in the conversion of cobalamin to analogs in the liver, but that the animals do not develop neurological changes. When fruit bats are fed a cobalamin-free diet, they develop neurological changes similar to those seen in human cobalamin deficiency, within 9 to 12 months (6), but this is not associated with the presence of cobalamin analogs (7). As gross neurological changes occur rapidly in fruit bats exposed to N_2O (our unpublished data), the purpose of the present study was to determine whether cobalamin analogs played a role in the development of the N_2O-induced neurological changes in these animals.

Materials and Methods. *Experimental animals.* Fruit bats (*Rousettus aegyptiacus*) were captured in the wild and rendered cobalamin-deficient on an all-fruit diet (6). To prevent other vitamin deficiencies from developing, 0.2 ml of a cobalamin-free oral vitamin preparation (Abidec, Parke-Davis) was administered every 2 weeks in a dose containing 100 IU vitamin D, 0.25 mg thiamine, 0.01 mg riboflavin, 0.12 mg niacin, and 12.5 mg ascorbic acid. Bats maintained on this diet became cobalamin-deficient after 9 to 12 months. Control bats received intramuscular injection of 0.5 μg cyanocobalamin per 100 g body weight every 2 weeks.

Exposure to nitrous oxide. Bats were exposed to an atmosphere of 50% O_2/50% N_2O for 90 min every day for 3 weeks in a specially constructed cabinet in which CO_2 and water vapor were controlled. For 22.5 hr a day, the bats breathed room air in their usual aviary, in which they had ample room to fly.

Measurement of cobalamin analogs. Blood was drawn by cardiac puncture into heparinized tubes. The plasma was separated by centrifugation and stored at −20°. Plasma cobalamin analogs were determined by the Lau *et al.* coated charcoal radioisotope dilution technique (8) as modified by Kolhouse *et al.* (1), using salivary R-binder as ligand to measure total corrinoids, and pure intrinsic factor (IF) as

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ligand to measure intact cobalamins, based on the facts that IF has a very low affinity for cobalamin analogs other than intact cobalamins, whereas the affinity of R-binders for these analogs is so much greater that it binds the totality of intact cobalamins plus other corrinoids (1, 9).

The R-binder used was human saliva, and the IF used was a gift from Becton-Dickinson Immunodiagnostics (Orangeburg, N.Y.) of pure hog IF prepared by affinity chromatography. The process for extraction from serum of cobalamin and analogs was that used by Kolhouse *et al.* (1), as was the amount of cyanide and BSA (bovine serum albumin) added. Separation of bound from free cobalamin was with albumin-coated charcoal prepared as described by Lau *et al.* (8). The reference standard was USP (United States Pharmacopeia) Cyanocobalamin Standard, purchased from the USP (Washington, D.C.). We have used this identical methodology to study cobalamin and analog levels in human serum, red cells, brain, liver, and bile (10–12). The amount of analogs present is thus represented by subtracting from the total corrinoids detected when R-binder is used as ligand at pH 9, the quantity of intact cobalamins measured at pH 9 with IF as ligand (1).

Results. The results are shown in Table I.

The group of four cobalamin-replete fruit bats received intramuscular injections of cyanocobalamin every 2 weeks during the 2 months they were in captivity. Serum cobalamin levels were normal, varying from 1536 to 2781 pg/ml when assayed with IF as ligand. No significant amounts of cobalamin analog were present in the plasma of these bats, the analog ranging from 0 to 126 pg/ml.

The bats rendered cobalamin deficient had received the cobalamin-free diet for 12 to 21 months and all were severely cobalamin deficient (plasma cobalamin = 1–91 pg/ml). No significant amounts of cobalamin analog could be detected in deficient animals exposed to N_2O (range = 0–18 pg/ml), or those not so exposed (range = 0–33 pg/ml).

Discussion. In all fruit bats studied, cobalamin analogs were absent from the plasma or present in negligible amounts only. In the bats rendered cobalamin deficient by dietary means only, the results were similar to those reported by Green and Jacobsen (7). Cobalamin analogs were also not detected in the plasma of bats exposed to N_2O, which suggests that analogs do not play a role in the development of the severe neurological changes that accompany N_2O exposure in the bat. Furthermore, cobalamin analogs were detected in small amounts

TABLE I. PLASMA LEVELS OF COBALAMINS AND ANALOGS (pg/ml) IN FRUIT BATS[a]

Group.	No.	Cobalamins	Total corrinoids	Analogs
Cobalamin-replete	1	2610	2713	103
	2	2781	2786	5
	3	1536	1490	0
	4	2747	2873	126
Cobalamin-deficient	5	19	0	0
	6	26	59	33
	7	16	0	0
	8	58	77	19
Cobalamin-deficient exposed to N_2O	9	1	0	0
	10	23	41	18
	11	91	60	0
	12	26	1	0

[a] Assayed by radioisotope dilution assays using as ligand pure intrinsic factor (IF) for cobalamins and salivary R-binder for total corrinoids (cobalamins + analogs).

in only two of four fruit bats cobalamin replete from intramuscular cobalamin injections every 2 weeks; this slightly differs from the finding by Green *et al.* (13) of quantity of analogs present in serum of fruit bats injected with 100 ng cyanocobalamin weekly (but not in other fruit bats).

It is not clear why cobalamin analogs appear in the tissues of N_2O-treated rats (4) but not in the plasma of bats. This may represent a species difference, for there is evidence that the bat and the rat respond differently to N_2O exposure: N_2O causes severe neurological changes in the bat but none in the rat, and the deoxyuridine (dU) suppression test (14) is abnormal in the N_2O-exposed rat (15) but not in the bat (our unpublished observations). Another possibility is that the length of exposure to N_2O may be critical, for Kondo and co-workers (4) exposed rats continuously to N_2O for periods ranging from 30 min to 38 hr, while the bats in the present study were exposed for 90 min daily for 3 weeks. It is also possible that analogs present in tissues may be cleared more rapidly from the plasma of the bat than of the rat.

The term "analog" as used in the present study does not refer to any specifically isolated form or forms of corrinoids, but is used to describe molecules which have a corrin nucleus and therefore attach to R-binders as does vitamin B_{12}, but in addition have a lesser affinity than cyanocobalamin for IF and/or a much greater affinity than cyanocobalamin for R-binder.

When we studied the livers of 4 control and 6 nitrous-oxide treated bats, we found the control bats had a mean of 65.8 ± 16.1 ng total corrinoid/g liver, a mean of 59.9 ± 15.7 ng cobalamin/g liver, and a mean of 5.9 ± 2 ng analog/g liver. Thus, although normal fruit bats lack cobalamin analogs in their serum, such analogs are present in their livers, suggesting more rapid clearance of analogs from serum into liver in bats than in humans (10–12). Livers from the 6 N_2O-treated bats had a mean of 1.3 ± 1.3 ng total corrinoid/g liver, a mean of 1.5 ± 0.5 ng cobalamin/g liver, and no measurable analog in their livers.

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