time, how nitrogen, captured from the atmosphere and fixed in the soils of the earth, is ultimately returned to the atmosphere at the conclusion of the nitrogen cycle:

a) Nitrous oxide appears in the soil as a decomposition product of the fixed nitrogen compounds.

b) The nitrous oxide escapes into the atmosphere.

c) Nitrous oxide diffusing into the upper atmosphere is decomposed photochemically by $\lambda < 2{,}000$ A into N_2, O_2, and NO; and at these high atmospheric levels NO is also decomposed photochemically into nitrogen and oxygen by $\lambda < 2{,}000$ A.

d) Presumably, the nitrous oxide accumulates above the earth's surface until the rates of accumulation and decomposition are equal.

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An Analog of Vitamin B_{12}

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In the chemistry of the coordination compounds, it is well known that group replacements may occur within the coordination sphere. It also has been established that vitamin B_{12} is a cobalt coordination compound (*1*) which we shall represent as follows:

$$\begin{bmatrix} -- & CN^- \\ Co^{+++} & \\ 0\ 0\ 0 & \end{bmatrix}$$

where the minus signs denote negative charges and the zeros denote a neutral group or groups coordinated to cobalt by dative bonds. All the zeros and minus signs, with the exception of the CN^- ion, may occur in a single molecular aggregate (moiety) or be divided among several molecular aggregates without regard to the number of minus signs and zeros in any one of the several molecular aggregates. Obviously some of these aggregates will be ions. If all the foregoing is the case, the possible existence of analogs of vitamin B_{12} resulting from replacement of the CN^- ion with other ions or uncharged groups may be expected.

Vitamin B_{12} is converted to vitamin B_{12a} by photolysis[2] in acidic solution with evolution of hydrogen cyanide (*2*) or by the catalytic action of platinum and hydrogen in neutral solution (*3*) with evolution of methyl amine and the formation of an apricot-colored intermediate. Subsequent air oxidation of this effects the final conversion to vitamin B_{12a}, which is a weak base of PKa 6.9, as was determined by potentiometric titration. The introduction of the uncharged group H_2O into the complex would produce such a cation. The addition of cyanide ion readily reconverts vitamin B_{12a} to vitamin B_{12}. No detectable amount of cyanide ion was observed in equilibrium with the regenerated vitamin B_{12} in this system when it was examined polarographically. These polarographic results, however, do not preclude the existence of the following equilibrium:[3]

$$\underset{\text{Vitamin } B_{12a}}{\begin{bmatrix} -- & (H_2O) \\ Co^{+++} & \\ 0\ 0\ 0 & \end{bmatrix}} + CN^- \overset{K}{\rightleftharpoons} \underset{\text{Vitamin } B_{12}}{\begin{bmatrix} -- & CN^- \\ Co^{+++} & \\ 0\ 0\ 0 & \end{bmatrix}} + H_2O$$

but only demonstrate that the concentrations of cyanide ion and of vitamin B_{12a} ion are subdetectable in this type of measurement and that therefore the equilibrium constant, K, must be very large.

In more general form, then:

$$\begin{bmatrix} -- & (H_2O) \\ Co^{+++} & \\ 0\ 0\ 0 & \end{bmatrix} + X^- \rightleftharpoons \begin{bmatrix} -- & X^- \\ Co^{+++} & \\ 0\ 0\ 0 & \end{bmatrix} + H_2O$$

Thus, by using other groups more strongly bound than H_2O, the preparation of analogs of vitamin B_{12} should be possible. This is indeed the case (*4*). We have found it possible to prepare the thiocyanate analog of vitamin B_{12} in which the cyanide ion of vitamin B_{12} is replaced by the thiocyanate ion. As was expected, this new analog of vitamin B_{12} is fully active biologically, thus further multiplying the number of known bioactive forms of vitamin B_{12}.

Vitamin B_{12a} prepared catalytically (*3*) and excess potassium thiocyanate in the molar ratio of 10:1 at a vitamin B_{12a} level of 3–5 mg/ml are allowed to react in water at room temperature for a few hours. A 20-vol excess of acetone is added, and the system held at 5° C for 24 hr; whereupon the thiocyanate analog of vitamin B_{12} crystallizes as dark purple-red needles. Vitamin B_{12} in contradistinction is bright red.

The thiocyanate analog, like vitamin B_{12a}, is microbiologically equivalent to vitamin B_{12}, i.e., 11,000 units/μg by the *Lactobacillus lactis* cup assay (*5*). The thiocyanate analog and vitamin B_{12a} when tested by the *L. lactis* (A) and the *L. leichmannii* "unpro-

[1] We are indebted to G. A. Emerson, M. Zanetti, and M. Bingemann, of the Merck Institute for Therapeutic Research, for the acute toxicity and bioassay work; to D. Hendlin, J. A. Lally, and associates for various microbiological assays; to R. W. Walker for the infrared measurements.

[2] This photolysis was first observed in our laboratories by A. Holland and J. C. Rickards.

[3] J. B. Conn, of our laboratories, has demonstrated polarographically the existence of a mobile equilibrium between vitamin B_{12a} and chloride ion.

tected" (B) titrimetric assay methods (*6*) also respond in a like manner, distinct from vitamin B_{12}. The term "unprotected" indicates that the medium is not protected with reductants (Table 1).

TABLE 1

Sample	Units/μg A*	Units/μg B†
Thiocyanate analog	2,300	6,200
Vitamin B_{12a}	1,800	6,800
Vitamin B_{12}	11,000	11,000

* *L. lactis.*
† *L. leichmannii* "unprotected" titrimetric assay.

Bioassays in rats reveal the same order of activity as vitamin B_{12}. Acute toxicity tests on mice failed to reveal any detectable toxicity at the equivalent level of 3.2 mg of the analog for a 70-kg man. Preliminary clinical reports have indicated that the thiocyanate analog of vitamin B_{12} is fully active for pernicious anemia.

The ultraviolet absorption spectrum of the thiocyanate analog is practically identical with that of vitamin B_{12a} from 6,000 to 2,200A (Table 2).

TABLE 2

	E% at 3,520A	E% at 5,250A
Thiocyanate analog	174	61
Vitamin B_{12a}	174	59

The thiocyanate analog shows an absorption band in the infrared at 4.70 μ characteristic of thiocyanate compounds. Similarly, vitamin B_{12} shows an absorption band at 4.60 μ characteristic of the cyano grouping. Vitamin B_{12a}, on the other hand, shows no absorption bands in the 4–5 μ region (*7*).

TABLE 3

Distribution coefficient	Benzyl alcohol / water
Thiocyanate analog	1.66
Vitamin B_{12}	0.84
Vitamin B_{12a}	0.13

Craig countercurrent studies of the thiocyanate analog show that our material is homogeneous and of high purity. The distribution curve is theoretical with the maxima in the fourth tube of an 8-tube study.

The thiocyanate analog is hygroscopic like vitamin B_{12} and vitamin B_{12a}. The analog is not compatible with ascorbic acid at the level of 20 μg/ml of the analog to 20 mg/ml of ascorbic acid. Decolorization occurs within 24 hr. This is analogous to the observed behavior of vitamin B_{12a}, which also reacts with ascorbic acid, and in contradistinction to that of vitamin B_{12} (*8*).

The properties of the thiocyanate analog of vitamin B_{12} are shared in part by both vitamin B_{12} and vitamin B_{12a} (Table 3). Thus in the ultraviolet and in its reaction with ascorbic acid it resembles vitamin B_{12a}, whereas in the 4- to 5-μ region of the infrared and in its distribution behavior in the benzyl alcohol–water system it resembles vitamin B_{12}. Therefore, in the characterization of a related unknown, care must be exercised to examine as many properties as possible before any reasonably certain conclusions can be drawn regarding its relationship to known vitamin B_{12} analogs.

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Pulmonary Edema and Hemorrhage Induced by Hypothalamic Lesions in Rats[1]

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Pulmonary edema, often fatal, is a puzzling complication in a wide variety of clinical conditions. These include not only cardiovascular and pulmonary diseases but also allergy, thyroid crisis, beriberi, cirrhosis and degeneration of the liver, carcinoma, blood dyscrasias, septicemia, drowning, shock, heat stroke, head injuries, and brain tumors. In experimental animals, pulmonary hemorrhage and edema are reported to follow such seemingly unrelated and nonspecific procedures as epinephrine injection (*1*), thiourea poisoning (*2, 3*), feeding ammonium salts (*4*), insulin shock (*5*), cerebral concussion (*6*), increased intracranial pressure (*7*), intracarotid saline infusions (*8*), cisternal injection of veratrin (*9*), bilateral vagotomy (*10*), ligation of the aorta or compression of the left ventricle (*11*), positive pressure respiration (*12*), hyperthermia plus positive pressure respiration (*13*), and war-gas poisoning (*14*). Although the diversity of the clinical and experimental states leading to pulmonary edema suggests multiple causative mechanisms, a neural mechanism is thought by some to be the common denominator of some types of lung edema. The evidence is mostly indirect, since it is largely based upon the protective effect of autonomic blocking agents, narcotics, or surgical attacks on the autonomic nervous system. There is little uncontested evidence of pulmonary edema produced by peripheral or central neural lesions. The following experiments demonstrate that such a "neurogenic"

[1] Aided by a grant from the Washington State Research Fund for Biology and Medicine.