the ascitic fluid, with appropriate diminution of the inhibitors to give concentrations of crystal violet of 1:100,000 and of potassium tellurite of 1:35,000. As PPLO produce little or no visible growth in liquid media, growth in the enrichment broth has to be ascertained by inoculating the enrichment broth cultures onto the surface of solid medium in Petri dishes. The medium employed for this purpose was that described by Morton, Smith, and Leberman (*8*). A few cultures in which some bacterial growth occurred were subcultured onto the solid medium containing 0.006 g crystal violet and 0.53 ml Bacto-Chapman tellurite solution per liter.

In the case of 11 individuals a sterile cotton swab was rubbed over the tonsillar areas and the posterior portion of the throat. The swab was then placed directly into a tube of 5 ml of enrichment broth and incubated at 37° C for 2 days. Growths in the tubes were inoculated onto Petri plates of Bacto-PPLO Agar, Experimental (*8*), enriched with ascitic fluid and incubated aerobically for 2 days at 37° C. In the case of 103 medical students, the throat swabs were placed in test tubes containing 1 ml of extract broth. After a suspension of the material on the swabs was made in the broth, the swab was discarded, and 0.5 ml of the suspension in each case was transferred to a tube of the enrichment broth. These were then treated as described above.

The Petri dish cultures were examined under the low power of the microscope (100 ×) for characteristic colonies. Verification of the colonies of PPLO may be made by staining according to the method of Dienes, which has been described fully elsewhere (*6*).

The throat cultures of 6 of the 11 individuals were positive for pleuropneumonialike organisms. In addition, the throat cultures of 32 of the 103 normal, healthy, medical students were positive for PPLO. A total of 38, or 33.6%, of 114 throat cultures yielded pleuropneumonialike organisms. This incidence is lower than that of PPLO in human saliva, PPLO having been detected in the saliva of 46 of 100 individuals (*6*). The colonies of pleuropneumonialike organisms isolated from throat cultures are similar to those isolated by us from the genitourinary tract of humans (*9*) and to a known culture isolated from a human by Dienes and to cultures of human and rat origins furnished by I. G. Schaub, of Johns Hopkins Hospital.

By means of improvements in the cultivation of PPLO, these organisms have been detected in saliva and throat cultures of individuals and in a frequency exceeding that reported for the normal female (*10, 11*) and male (*12*) genitourinary tracts. However, if some pathology exists in the genitourinary tract, the incidence of isolation of PPLO is greater (*10–13*).

References

1. Sabin, A. B. *Science,* **90**, 18 (1939).
2. Sabin, A. B., and Johnson, B. *Proc. Soc. Exptl. Biol. Med.,* **44**, 565, 569 (1940).
3. Morton, H. E., Smith, P. F., and Leberman, P. R. *Ibid.,* **72**, 328 (1949).
4. Dienes, L., *et al. New England J. Med.,* **238**, 509 (1948).
5. Smith, P. F., Morton, H. E., and Leberman, P. R. *Proc. Soc. Exptl. Biol. Med.,* **74**, 552 (1950).
6. Morton, H. E., *et al. J. Dental Research* (in press).
7. Smith, P. F., and Morton, H. E. *J. Bact.,* **61**, 395 (1951).
8. Morton, H. E., Smith, P. F., and Leberman, P. R. *Am. J. Syphilis, Gonorrhea, Venereal Diseases* (in press).
9. Leberman, P. R., Smith, P. F., and Morton, H. E. *J. Urol.,* **64**, 167 (1950).
10. Randall, J. H., Stein, R. J., and Ayres, J. *Am. J. Obstet. Gynecol.,* **59**, 404 (1950).
11. Klieneberger-Nobel, E. *Lancet,* **2**, 46 (1945).
12. Reveridge, W. I. B., Campbell, A. D., and Lind, P. E. *Med. J. Australia,* **1**, 179 (1946).
13. Morton, H. E., Smith, P. F., and Leberman, P. R. *Am. J. Syphilis, Gonorrhea, Venereal Diseases,* **35**, 14 (1951).

Atmospheric Nitrous Oxide and the Nitrogen Cycle

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The author's discovery of nitrous oxide (*1*) in the earth's atmosphere has received numerous confirmations (*2–4*), both in this country and abroad. The amount present is comparable with that of atmospheric ozone, about 3 mm N.T.P. (*5*).

It was subsequently discovered[1] that nitrous oxide is one of the most abundant constituents of soil air (*7*), whereupon it was suggested by the present author that escaping soil air might well be a principal source of the atmospheric nitrous oxide (*8*).

It is the purpose of this note to summarize several lines of evidence, recently adduced, which lend strong support to this early hypothesis regarding the gas's origin, which make it seem probable that the nitrous oxide "layer" is "adjacent" to the earth's surface, and which strongly suggest the idea that atmospheric nitrous oxide is an important phase in the nitrogen cycle.

a) The effective radiation temperature of atmospheric nitrous oxide is about 10° C, as deduced from observations of the atmosphere's infrared emission spectrum (*9*).

b) A British Admiralty group, investigating atmospheric transmission in the infrared, has found large concentrations of nitrous oxide in air paths parallel and close to the surface of the land and sea (*4*).

c) An inspection of solar spectra recorded aboard high-flying aircraft reveals a greatly diminished absorption by nitrous oxide in the 7.8-μ region (*10*).

d) Finally, a recent mass spectrographic analysis has revealed that in the atmosphere near ground level the concentration of nitrous oxide is about $5 \times 10^{-5}\%$ by volume (*11*).

Apropos of the foregoing evidence, it appears very likely that nitrous oxide escapes from the soil into the atmosphere, and the conclusion cannot be avoided that this escape constitutes an important phase of the nitrogen cycle. It thus becomes clear, for the first

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[1] The discovery of nitrous oxide in soil air has been confirmed by Taylor *et al.* (*6*). They find it the most abundant constituent of soil air, constituting more than one fifth, by volume.

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.

References

1. Adel, A. *Astrophys. J.*, **90**, 627 (1939); **93**, 509 (1941).
2. Shaw, J. H., Sutherland, G. B. B. M., and Wormell, T. W. *Phys. Rev.*, **74**, 978 (1948).
3. Migeotte, M. In G. P. Kuiper (Ed.), *Atmospheres of the Earth and Planets.* Chicago: Univ. Chicago Press, 284 (1949).
4. Gebbie, H. A., *et al.* Admiralty Research Laboratory, T. R. E./T. 2129 (Dec. 1949).
5. Adel, A. In G. P. Kuiper (Ed.), *Atmospheres of the Earth and Planets.* Chicago: Univ. Chicago Press, 272 (1949).
6. Taylor, R. C., *et al. Anal. Chem.*, **20**, 396 (1948).
7. Kriegel, M. W. *Geophysics*, **9**, 447 (1944).
8. Adel, A. *Science*, **103**, 280 (1946).
9. ———. *Astron. J.*, **55**, 69 (1950).
10. Progress Report on High Altitude Infrared Transmission of the Atmosphere. Johns Hopkins (ONR) Contract N5ori-166. Task Order V (Jan. 10, 1949).
11. Slobod, R. L., and Krogh, M. E. *J. Am. Chem. Soc.*, **72**, 1175 (1950).

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:

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

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]

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

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:

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

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-

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[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.