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New, Man-Made N<latex>$_2$</latex>-Fixing Systems [and Discussion]

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New, man-made N_2-fixing systems

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The major inputs of fixed N into the global nitrogen cycle are assessed and compared as indicators of both the need for and the likely basis of new, complementary, man-made N_2-fixing processes. The development, since 1964, of the purely chemical, highly reactive systems for the reduction of N_2, including those driven electro- and photochemically, is traced, along with the parallel efforts to synthesize metal–N_2 complexes (the first step in any likely fixation process) and subsequently protonate them to produce hydrazine or ammonia. These experimental approaches are convergent. Successful cycling or catalysing of some of these N_2-binding systems has been achieved. The advantages and limitations of the more successful systems are noted. Approaches to this problem via direct modelling of the nitrogenase active site are outlined, as is the one successful use of such complexes in achieving N_2 reduction. This wealth of effort on the reductive approaches contrasts vividly with the almost complete absence of research on N_2 oxidation. Currently, only a re-evaluation of the arc discharge process is continuing. Finally, the author's studies of the extruded molybdenum-containing prosthetic group of nitrogenase, the enzymic N_2-reducing site, are described in relation to future N_2-fixing systems.

1. Introduction

Atmospheric nitrogen must be fixed, before it can be used by plants, either by natural non-biological and biological processes or by industrial ammonia production. The natural non-biological processes (lightning, volcanic activity and combustion) contribute about 30 megatonnes (Mt) of nitrogen annually. Industrial processes contribute about 50 Mt a^{-1} for fertilizer use, the importance of which is unquestioned for productivity in the agriculturally advanced countries. The major source of fixed nitrogen is of biological origin at about 122 Mt a^{-1}, with about 90 Mt a^{-1} occurring in soils used for agriculture. Because increases in land area devoted to agriculture may well be small in the future, any gain in food production must depend on increased crop yield per unit land area. Thus, the various processes for fixing atmospheric N_2 hold the key to the long-term solution of increasing the global food supply (Newton 1981).

Will the present commercial process be able to satisfy the increased need for fertilizer N in the future? Although about half of the 3% annual increase in world cereal-grain production in the past 25 years has been assigned to the exponential increase in commercially produced fertilizer nitrogen, major drawbacks exist. Modern ammonia plants rely on natural gas or liquid hydrocarbons to supply both H_2 and energy; the questionable availability and variable cost of natural gas present serious problems. Perhaps a more important problem, especially in developing nations, is the tremendous capital investment required for the construction of such facilities, possibly reaching U.S. $300M by the 1990s. In addition, because Haber–Bosch plants operate all year round, but fertilizer application is both seasonal and geographically localized, significant expense is involved in product storage and transportation. Thus, new complementary processes will have to be developed. Ideally, they would be less energy-intensive and

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would operate at ambient temperature and pressure with renewable energy sources (e.g. wind, solar or hydroelectric power) and be adaptable for use by an individual farmer or local community.

Ideal models for such a process are the biological catalysts, the nitrogenases, because they fix N_2 under exactly these conditions and use either sunlight captured directly or photosnthetically produced sugars for nitrogen fixation (Newton 1981; Evans *et al.* 1985). Biological nitrogen fixation also has the advantage of operating where and when needed. Because of these advantages, nitrogenases have become a centre of scientific interest as the basis of efforts to produce new non-biological N_2-fixing systems. However, success based on this approach retains two inherent shortcomings related to all topically applied nitrogen fertilizers: leaching into groundwater and streams and gaseous losses through denitrification. In fact, on average, only about one third of topically applied fertilizer nitrogen is assimilated by plants. These drawbacks are minimal in biological systems, implying that strategies for increasing and controlling biological nitrogen fixation itself should be developed concurrently.

New, man-made, nitrogen-fixing systems (or the bases thereof), currently under study as potential complements to both modern commercial nitrogen fertilizer manufacture and the natural biological process, range from purely chemical oxidative and reductive processes of varying sophistication (and relevance to the natural systems), through those efforts at modelling structural aspects of the putative active site of nitrogenases, to studies of natural components of these enzymes. Examples of all these approaches will be discussed in terms of their catalytic propensity and potential for input into agriculture when applied in the appropriate global niche.

2. Conceptualization of N_2-fixing systems

The major approaches used to develop non-biological systems to fix N_2 divide broadly into two groups: (i) the purely chemical approach of examining the properties of the N_2 molecule to determine the requirements for its activation and subsequent fixation; and (ii) investigations of the reactivity properties of nitrogenase, and the structure of the prosthetic groups contained therein, as indicators of the N_2-fixation process evolved in Nature under the constraints imposed by our ambient conditions. This division is somewhat artificial, as even the 'purely chemical' approach has much of its basis on nitrogenase chemistry, extending back to the discovery in 1930 that traces of molybdenum were essential for the growth of bacteria on N_2 (Bortels 1930). This observation implied that molybdenum played an important role in the biological fixation process and led to the speculation that N_2 might bind to molybdenum in conventional nitrogenase and be subsequently reduced to ammonia. Until recently, only circumstantial evidence supported this speculation (Smith 1977), but genetical evidence (Hawkes *et al.* 1984) pinpoints a cluster of molybdenum, iron and sulphur atoms (Shah & Brill 1977), the so-called iron–molybdenum cofactor (FeMoco), as the site of N_2 reduction in Mo-nitrogenase.

The chemistry of N_2 also indicates involvement of transition metals in N_2 activation. N_2 is very stable and consequently very inert (Mulliken 1959). The highest-energy occupied molecular orbital, at -15.6 eV, is so low in energy that no oxidant can remove its electrons under ambient conditions; the lowest unoccupied molecular orbital is, at -7 eV, too high in energy for all but the most electropositive elements, such as lithium, to donate into and so effect reduction. Even so, certain transition metal ions in appropriate oxidation state and orientation accept electrons from filled, low-energy donor orbitals of molecules such as carbon monoxide and cyanide, while

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synergistically donating electrons to a vacant, high-energy acceptor orbital; these processes are conceivably analogous to the N_2 situation. These analogies extend to the reactions of nitrogenase: 'alternative substrates' to N_2 include acetylene (Dilworth 1966; Schollhorn & Burris 1967), hydrogen cyanide (Hardy & Knight 1967; Li *et al.* 1982), methylisonitrile (Kelly *et al.* 1967), azide (Schollhorn & Burris 1967; Hardy & Knight 1967; Dilworth & Thorneley 1981), cyclopropene (McKenna *et al.* 1976) and nitrous oxide (Lockshin & Burris 1965), plus carbon monoxide as an inhibitor. All of these molecules form complexes with transition metals in lower oxidation states.

Most materials capable of reducing N_2 are also likely to react preferentially with water or O_2 under the same conditions. The key to successful man-made processes, then, is to develop sytems that either bind N_2 in preference to reaction with water or are compatible with aprotic media. Both types have been developed and with different degrees of success in the reduction of N_2.

A third option that has been pursued is a reconsideration of the Birkeland–Eyde process of nitrogen oxidation in an electric arc discharge (Treharne *et al.* 1978). Here, the major needs are small scale, economy and simplicity of design, construction and operation such that manufacture by local artisans for installation on remote farm sites is possible. Minimal capital investment, use of renewable resources and localized fertilizer production are the obvious advantages of this system. Its low efficiency of conversion of electrical energy into product is its major drawback. Developing countries with limited fossil fuel resources are the most likely beneficiaries from such systems in the near-term future.

3. CHEMICAL N_2-REDUCING SYSTEMS

The impetus for the development of new systems of N_2 reduction dates back to the 1930s. Since then, many fruitless attempts have been made to produce an active cell-free extract of an N_2-fixing organism, an isolated complex containing N_2 as a ligand or an active, non-biological N_2-fixing system, but none was successful until the early 1960s, when all were accomplished. Because the first report of a chemical N_2-reducing system concerned one which operated in aprotic media, such systems will be described and discussed first.

N_2-*reducing systems in aprotic media*

The first N_2-reducing system was based on titanium tetrachloride or bis(cyclopentadienyl)-titanium dichloride, $(C_5H_5)_2TiCl_2$, in diethyl ether with ethylmagnesium bromide or lithium naphthalenide as reducing agent under 100–150 atm pressure of N_2 (Vol'pin & Shur 1964). Partial inhibition of the reaction occurred with CO, C_2H_2, olefins, H_2 and strongly solvating solvents (Vol'pin & Shur 1966). The mechanism of these reactions remains unclear, and although these systems are difficult to probe owing to their high reactivity, 'Ti–N_2' complexes of Ti^{II} are undoubtedly produced in a sequence of reactions resulting probably in Ti–N formation. Solvolysis is required to liberate ammonia, but results in a loss of all activity, and therefore of all potential for catalysis. Similar systems, based on $Ti(OPr^i)_2Cl_2$ in glyme with sodium as reductant and isopropyl alcohol for controlled solvolysis, may be cycled five times before all activity is lost (van Tamelen *et al.* 1968). Titanium also acted as a catalyst for the nitriding of aluminium when $TiCl_4$, Al metal and $AlBr_3$ were heated at 130 °C under 100 atm N_2 to produce 284 mol NH_3 per mol $TiCl_4$ after hydrolysis (Vol'pin *et al.* 1968*a*).

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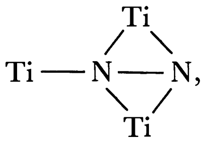
A similar system, but operated electrochemically, produced 6 mol NH_3 per mol Ti after 11 days (van Tamelen & Seely 1969). Nitrogen-containing organic compounds can be produced by these same Ti-based systems (Vol'pin *et al.* 1968*b*, 1971; van Tamelen & Rudler 1970).

Ti–N_2 complexes can be isolated from such reactions but, although these (C_5H_5)–Ti systems appeared constrained by the limited coordination possibilities, the early promise of easy insight into structure and reaction pathways was unexpectedly confounded. For example, the $(C_5H_5)_2TiCl_n$ ($n = 1, 2$)–N_2–reductant system produces a number of N_2-containing reaction products, all of which exhibit an intense blue colour ($\lambda_{max} \approx 600$ nm) in solution (Borod'ko *et al.* 1972, 1973; Marvich & Brintzinger 1971; van Tamelen *et al.* 1969). Unfortunately, the relationships among this variety of products remain unresolved, but are likely to resemble those elucidated in the reactions of $(C_5H_5)_2TiR$ (R = aryl, benzyl) with N_2 (Teuben 1973; van der Weij & Teuben 1976). Here, reaction produces initially $(C_5H_5)_2(R)Ti{-}N{\equiv}N{-}Ti(R)(C_5H_5)_2$, a blue complex with N_2 resistant to protonation. If reductant is added, one (C_5H_5) ring is lost and bridging N_2 is reduced to the $(N_2)^{2-}$ state to give $(C_5H_5)(R)Ti{-}N{=}N{-}Ti(R)(C_5H_5)$, followed on further reduction to the $(N_2)^{4-}$ level as $[(C_5H_5)(R)Ti{-}N{-}N{-}Ti(R)(C_5H_5)]^{2-}$, from which N_2H_4 is released on protonation. On warming above −78 °C, N–N cleavage occurs to give $[(C_5H_5)(R)Ti{=}N]^-$, which protonates to give NH_3.

The tendency of the (C_5H_5) rings to react either to form fulvalene ligands, which bridge dimers, or undergo C–H insertion with Ti^{II} is an added complexity. The structure of one of several related products of the fulvalene type, all of which bind N_2, has been deduced from a mixed crystal. The complex contains four Ti atoms, three of which bind to the single N_2 ligand. One Ti atom binds in the usually observed end-on (or sigma) mode (M–N=N); two others bind side-on (or pi-wise), i.e.



to produce an elongated N–N bond length of 1.31 Å†. Surprisingly, only N_2 is evolved on HCl treatment while an *ca.* 90% yield of ammonia plus hydrazine occurs with water (Pez *et al.* 1982). Other completely documented examples of side-on bonded N_2 are two nickel complexes (Kruger & Tsay 1973, Jonas *et al.* 1976), each of which has two nickel atoms bound at right angles (pi-wise) to the single N_2 ligand, which is coordinated further by lithium atoms. About 35% of the bound N_2 appears as NH_3 on hydrolysis. The same bonding mode has been suggested for N_2 both on a single Zr atom in $(C_5H_5)_2Zr(N_2)[CH(SiMe_3)_2]$ (Jeffery *et al.* 1979) and as an intermediate in the scrambling of N_2 on Ru in $[Ru(NH_3)_5(N_2)]^{2+}$ (Armor & Taube 1970).

Some of these ring-based problems have been overcome by full methyl substitution of the rings. With a system composed of $(C_5Me_5)_2TiCl_2$–N_2–reductant, two distinct, interconvertible N_2 complexes are produced in toluene (eqn. 1) (Manriquez *et al.* 1978):

$$2(C_5Me_5)_2Ti \underset{>0\,^{\circ}C}{\overset{<0\,^{\circ}C}{\rightleftharpoons}} [(C_5Me_5)_2Ti]_2(N_2) \underset{-40\,^{\circ}C}{\overset{-80\,^{\circ}C}{\rightleftharpoons}} [(C_5Me_5)_2Ti(N_2)]_2(N_2). \qquad (1)$$

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† 1 Å = 10^{-10} m = 10^{-1} nm.

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The dinuclear, mono-N_2 product has a linear Ti–N=N–Ti arrangement with a slightly elongated N–N bond length of 1.16 Å (Sanner *et al.* 1976*b*). The tri-N_2 complex of Ti is unstable above −80 °C, unlike its stable Zr analogue, which has a similar linear Zr–N=N–Zr bridge (N–N = 1.18 Å) plus one end-on terminal N_2 ligand (N–N = 1.115 Å) on each zirconium atom (Manriquez *et al.* 1976; Sanner *et al.* 1976*a*).

These mono-N_2 complexes, like those described above, do not produce hydrazine or ammonia directly, but only in the presence of excess reductant (Bercaw *et al.* 1972). In contrast, $[(C_5Me_5)_2M(N_2)]_2(N_2)$ (M = Ti, Zr) reacts directly with hydrogen chloride to liberate two N_2 molecules and hydrazine (0.9 mol mol^{-1}) (Manriquez *et al.* 1976; Sanner *et al.* 1976*a*). This dinuclear zirconium complex shows no unique structural properties either among its terminal and bridging N_2 ligands or on comparison with other metal–N_2 complexes (most of which do not produce hydrazine or ammonia on protonation) to indicate any structural requirement for N_2 reduction. Labelling with $^{15}N_2$ has, however, given insight into the reduction process. Only the terminal N_2 ligands exchange with $^{15}N_2$ to give $[(C_5Me_5)_2Zr(^{15}N_2)]_2(N_2)$, but this product yields hydrazine on protonation containing some nitrogen-15 label. The terminal and bridging N_2 ligands must then scramble through the symmetrical intermediate, $(C_5Me_5)_2Zr(N_2H)_2$, on protonation as shown in equations 2 and 3:

$$[(C_5Me_5)_2Zr(N_2)]_2(N_2) \xrightarrow{HCl} (C_5Me_5)_2Zr(N_2H)_2 + (C_5Me_5)_2ZrCl_2 + N_2; \qquad (2)$$

$$(C_5Me_5)_2Zr(N_2H)_2 \xrightarrow{HCl} (C_5Me_5)_2ZrCl_2 + N_2 + N_2H_4. \qquad (3)$$

This concept gains support from the observation that no hydrazine is formed with $[(C_5Me_5)_2Zr(CO)]_2(N_2)$ (Manriquez *et al.* 1976, 1978; Sanner *et al.* 1976*a*). In contrast, the closely related, mono-N_2 zirconium(III) compound, $(C_5H_5)_2Zr[CH(SiMe_3)_2](N_2)$, gives 0.2 mol hydrazine per mole directly on solvolysis, whereas $(C_5H_5)_2Ti(CH_2SiMe_3)$ does not even bind N_2 (van der Weij *et al.* 1977; Jeffery *et al.* 1979).

Concurrent with the investigations of the mechanism and intermediates in these N_2-reducing systems, a variety of mostly very well-defined metal–N_2 complexes was synthesized. The first such complex (Allen & Senoff 1965) was not prepared directly from N_2, but from hydrazine and $RuCl_3$ in water (equation (4)):

$$RuCl_3 + N_2H_4 \rightarrow [Ru(NH_3)_5(N_2)]^{2+}. \qquad (4)$$

The apparent reactivity of this complex also promised fulfilment of the second key step in nitrogen fixation, that of reduction of ligated N_2, because *ca.* 35% yield of NH_3 was reported on treatment with sodium borohydride. Unfortunately, it was found later that a contaminating hydrazine complex was responsible for the NH_3 (Chatt *et al.* 1968).

A great many M–N_2 complexes, involving almost all of the transition metals, are now known (Chatt *et al.* 1978; Henderson *et al.* 1983; Hidai 1985). One or more of these complexes duplicates one or more of the essential requirements of a nitrogen-fixation catalyst. They demonstrate that N_2 can compete: (i) with water for a metal site (Harrison *et al.* 1968) (equation (5)); (ii) with protons, which would produce undesirable H_2 (Sacco & Rossi 1967) (equation (6)); and (iii) with the product NH_3 to continue the cycle (Yamamoto *et al.* 1971)

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(equation (7)). However, only a very few of the more than 100 known complexes react to reduce their coordinated N_2 ligands

$$2[Ru(NH_3)_5(H_2O)]^{2+} \xrightarrow{1\,atm} [\{Ru(NH_3)_5\}_2(N_2)]^{4+} \xrightarrow{100\,atm} 2[Ru(NH_3)_5(N_2)]^{2+}. \quad (5)$$

$$CoH(PPh_3)_3 + N_2 \rightarrow CoH(N_2)(PPh_3)_3 + H_2. \quad (6)$$

$$CoH(NH_3)(PPh_3)_3 + N_2 \rightarrow CoH(N_2)(PPh_3)_3 + NH_3. \quad (7)$$

The mononuclear, tertiary phosphine complexes of molybdenum and tungsten are the best-studied examples of this ability. The complex, *trans*-$M(N_2)_2(DPPE)_2$ (M = Mo or W; DPPE = 1,2-bis(diphenylphosphino)ethane) (Hidai *et al.* 1972) produced only the hydrazido(2−) ($N\text{–}NH_2$) stage (equation (8)) (Chatt *et al.* 1974):

$$trans\text{-}Mo(N_2)_2(DPPE)_2 + 2\ HBr \rightarrow Mo(NNH_2)Br_2(DPPE)_2 + N_2. \quad (8)$$

However, a mixture of $Mo(N_2)_2(DPPE)_2$ and $Mo(NNH_2)Br_2(DPPE)_2$, when heated in *N*-methylpyridone with aqueous HBr, formed some ammonia (Brulet & van Tamelen 1975). In separate experiments, where $PPhMe_2$ or PPh_2Me was used to form *cis*- and *trans*-$M(N_2)_2(PR_3)_4$, respectively (Chatt *et al.* 1974), followed by treatment with acid, much higher ammonia yields were obtained. For M = W, about 2 mol ammonia per mole of complex were produced (9):

$$cis\text{–}W(N_2)_2(PMe_2Ph)_4 \rightarrow N_2 + 2NH_3 + W^{VI}\ \text{oxides} + 4PMe_2Ph. \quad (9)$$

With M = Mo, only 0.7 mol ammonia was produced (Chatt *et al.* 1977*a*). Similar treatment of $M(NNH_2)X_2(PMe_2Ph)_3$ provided similar yields of ammonia, indicating the intermediacy of $=N\text{–}NH_2$. These and related data have formed the basis for a suggested stepwise sequence for the reduction and protonation of N_2 on a single molybdenum atom in Mo-nitrogenase.

The early drawbacks with this system were that the metal-containing products beyond $M{=}N\text{–}NH_2$ had not been characterized (Henderson 1982, 1984) nor had the system been cycled. Further insight has been gained by taking advantage of the stabilizing effect of the chelating phosphines and the lability of the simple phosphines. Thus, $Mo(N_2)_2(PPh_3)$(triphos), where triphos = $PhP(CH_2CH_2PPh_2)_2$, reacts with anhydrous HBr (equation (10)) (Baumann & George 1980; George 1983; Baumann *et al.* 1985) to produce $MoBr_3$(triphos), which is the starting material for the preparation of the metal–N_2 complex, and so the system can be cycled.

$$2Mo(N_2)_2(PPh_3)(\text{triphos}) + 8HBr \rightarrow 2MoBr_3(\text{triphos}) + 2NH_4Br + 3N_2 + 2PPh_3. \quad (10)$$

However, the sequence of events between the $=N\text{–}NH_2$ stage and isolation of $MoBr_3$(triphos) remains unclear, except that PPh_3 is lost. Similar mono-N_2 complexes, $Mo(N_2)(\text{triphos})(L_2)$ ($L_2 = PR_3$, AsR_3) also produce NH_3 and N_2H_4 (*ca.* 75% yield) on protonation (George & Tisdale 1985).

These ammonia- and hydrazine-forming reactions appear to go under mild conditions. However, the reducing power of sodium or magnesium metal ($E_0 = -2.4$ V) is already built in during the preparation of the metal–N_2 complexes. Significantly milder reducing agents for these systems must be found if they (or related systems) are to assume any real commercial significance.

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The complete degradation of the simple phosphine complexes of Mo and W during ammonia formation did not hold much promise for eventual catalysis. But, by taking advantage of the robust nature of the compounds with chelating disphosphines, the good 'leaving group' properties of the tosyl anion ($TsO^- = p\text{-}CH_3C_6H_4SO_3^-$) and the acid–base properties of the hydrazido(2 –) group, an electrosynthetic reduction of bound N_2 in $W(N_2)_2$ (DPPE)$_2$ at Hg has been achived (Pickett & Talarmin 1985) as outlined in equations (11–14).

$$W(N_2)_2(DPPE)_2 + 2TsOH \xrightarrow{-N_2} [W(NNH_2)(TsO)(DPPE)_2]^+; \qquad (11)$$

$$[W(NNH_2)(TsO)(DPPE)_2]^+ \begin{array}{l} \xrightarrow{+2e^-} W(NNH_2)(DPPE)_2 + TsO^-; \qquad (12) \\ \longrightarrow W(NNH)(TsO)(DPPE)_2 + H^+; \qquad (13) \end{array}$$

$$W(NNH_2)(DPPE)_2 + H^+ \rightarrow [WN(DPPE)_2]^+ + NH_3. \qquad (14)$$

The key steps are loss of TsO^- on $2e^-$ reduction to give an intermediate highly reactive towards protons (12) and the production of those protons by the sacrificial deprotonation of the hydrazido^{2-} ligand (13). Yields of ammonia approach 25 mol % per W atom. The product of this series of reactions under N_2 at *ca.* -2 V is $W(N_2)_2(DPPE)_2$, which can be made to enter another cycle of NH_3 production by the stoichiometric addition of TsOH.

trans-$M(N_2)_2(DPPE)_2$ (M = Mo, W) also undergoes carbon–nitrogen bond formation on reaction with alkyl or acyl halides as does the $Mo(NNH_2)$ unit with ketones (Day *et al.* 1975; Chatt *et al.* 1977*b*; Hidai *et al.* 1976). Similarly, successive treatments of a $Mn(N_2)$ complex with CH_3Li and $[(CH_3)_3O]BF_4$ produce dimethylhydrazine, which can be displaced by N_2 to form a cyclic process (Sellman & Weiss 1977, 1978). Such systems may have significant industrial importance in the future for producing organic nitrogen compounds from N_2 instead of from ammonia.

Many other examples of chemical N_2 reduction, based on Va, Cr or Fe, in less well-defined systems have been reported (Chatt *et al.* 1978). Of note is the system based on $MoCl_4$ (DPPE)–Mg–alkyl bromide, which is reported to absorb 106 mol N_2 per Mo atom and to liberate diazene on hydrolysis. Possibly, Mo acts as a catalyst for magnesium nitride production (Sobota & Jezowska-Trzebiatowska 1978).

A rather different series of complexes, containing a metal–N–N–metal system, has been isolated from the reaction of $M(CHCMe_3)(THF)_2Cl_3$ [M = Ta^V or Nb^V] with hydrazines (Rocklage & Schrock 1982). An X-ray structure (Churchill & Wasserman 1982*a*, *b*) shows a phosphine derivative to be $(PR_3)Cl_3(THF)Ta{-}N{=}N{-}Ta(THF)Cl_3(PR_3)$, which is described as a hydrazido^{4-} bridged complex with a N–N bond length of 1.28 Å. Such compounds are also produced directly from N_2 and Ta^{III} (Rocklage *et al.* 1982). Hydrazine is produced quantitatively (*ca.* 85 %) On acidification of all these complexes. This chemistry has been extended to tungsten in high oxidation states, to produce bridged hydrazido^{4-} derivatives of W^{VI}. In some examples, the bridging hydrazido^{4-} group assumes a planar arrangement with a bent (*ca.* 131°) and protonated $W{-}\overset{H}{N}{-}\underset{H}{N}{-}W$ bridge and N–N = 1.41 Å (Churchill *et al.* 1984); in others, unprotonated hydrazido^{4-} bridges are present (Schrock *et al.* 1984). These are the first examples of highly oxidized metal ions binding N_2, a sharp contrast to those of Mo^0 discussed above. These new complexes may have common features to the reactions of Zr^{II} and to Va^{II} in protic media.

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N_2-*reducing systems in protic media*

Many strong reducing agents in the presence of derivatives of transition metals have been reported to produce minute amounts of ammonia from N_2 in aqueous solution. However, spurious results are obtained easily and only those systems that have been reasonably substantiated are considered.

Aqueous systems may reduce dinitrogen to either hydrazine or ammonia as the major product. Hydrazine was initially produced by using a molybdenum or vanadium salt in the presence of reductant ($TiCl_3$) and Mg^{2+} at pH 14, 25 °C and 1 atm N_2. At 50–100 °C and 50–150 atm N_2, yields of hydrazine reach 100 mol per mol Mo; at even higher temperatures, some ammonia is produced. Vanadium(II) or chromium(II) are equally effective as reductant in this heterogeneous reaction mixture of hydroxide-bonded polynuclear entities, which furnishes the reducing capacity of Ti^{III} through Mo^{III} to N_2 (Shilov *et al.* 1971). Vanadium(II) can replace both molybdenum and titanium but Cr^{II} is much less effective (Burbo *et al.* 1982). At alkaline pH, lower temperature and 100 atm N_2, reduction (0.22 mol hydrazine per mol Va) occurs within minutes. A four-electron reduction is proposed via a tetramer of vanadium(II) ions (Denisov *et al.* 1973; Nikonova *et al.* 1980):

$$(Va^{2+})_4 + N_2 + 4H_2O \rightarrow 4Va^{3+} + N_2H_4 + 4OH^-. \qquad (15)$$

At room temperature or higher, ammonia and hydrogen are produced by further reaction of the hydrazine with Va^{2+}. The rate of N_2 reduction is faster with vanadium than with molybdenum. Both systems reduce acetylene to ethylene plus ethane and are poisoned by carbon monoxide (Nikonova *et al.* 1972). Independent reappraisals of these systems confirm N_2 reduction but suggest an alternative mechanism (with the same overall stoichiometry and involving diazene) based on a monovanadium or monomolybdenum entity (Zones *et al.* 1978; Liu *et al.* 1984).

A related Mo^{III}-based system in methanol using sodium amalgam also produces only N_2H_4, although in lower yields. However, it operates under ambient pressure and temperature. The system is stimulated sharply by phosphatidylcholine (Didenko *et al.* 1983).

A homogeneous aqueous–alcoholic system, composed of vanadium(II) complexes of catechol and its derivatives, reduces N_2 to ammonia stoichiometrically with concomitant H_2 evolution. Only catecholates are active in this system, which is very sensitive to pH and has been likened to nitrogenase. Again, a tetramer of Va^{2+} ions is involved in a sequence of two successive four-electron reductions (Nikonova *et al.* 1975/6). A series of aqueous N_2-reducing systems, based originally on nitrogenase composition and the use of combinations of iron, molybdenum, sulphide and thiols, is reported to produce NH_3. About one micromole of NH_3 per nanomole of Mo is produced with $NaBH_4$ in borate buffer (pH 9.6) under 135 atm N_2 (Schrauzer 1975). In the absence of molybdenum, no ammonia is produced. Although specific stimulation by ATP is claimed, acids may produce the same effect (Schrauzer *et al.* 1974; Khrushch *et al.* 1974). Although no intermediates have been definitely characterized, the involvement of diazene has been proposed. More successful is the $[MoO(CN)_4(H_2O)]^{2-}$–$NaBH_4$ system, which gives 0.3 mol ammonia per mol complex (Moorehead *et al.* 1976), and a system of MoO_4^{2-} and insulin (6:1) plus $NaBH_4$, from which 65 mol ammonia per mol Mo is claimed to be evolved in 30 min at 23 °C under 1 atm N_2 (Weathers *et al.* 1979).

The aqueous systems are extremely difficult to characterize in detail and the various

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mechanisms proposed are often contradictory. They show unequivocally that N_2 can be reduced in aqueous media. All these reductive systems have serious inherent disadvantages, which limit their utility for the reduction of N_2 under truly mild conditions and indicate the need for considerable future development. In addition to the low yields of most systems and their sensitivity to O_2, even the most successful Va^{II} systems are limited because Va^{II} cannot be regenerated under the basic conditions necessary for efficient N_2 reduction.

Other N_2*-reducing systems*

A photochemical N_2-reducing system, based on titanium dioxide in the rutile form, has been found (Schrauzer & Guth 1977). Incompletely outgassed TiO_2, containing 0.4% chemisorbed water, yields H_2 and O_2 in a 2:1 molar ratio on photolysis during irradiation with a 360 W mercury arc lamp. Under N_2, H_2 evolution is inhibited and N_2 is chemisorbed and reduced to ammonia with traces of hydrazine. Iron doping increased yields until up to 6 μmol NH_3 was obtained in 3 h at 40 °C under 1 atm N_2 with 0.2 g TiO_2, impregnated with 0.2% Fe_2O_3. These studies have been extended to sterile samples of sands from various deserts

$$N_2 + 3H_2O \xrightarrow[TiO_2]{h\nu} 2NH_3 + \tfrac{3}{2}O_2 \tag{16}$$

throughout the world. A significant correlation of N_2-reduction efficiency was found with the Ti:Fe ratio of the non-magnetic mineral component of these sands. These results have prompted the suggestion that non-biological N_2 photofixation is part of the global nitrogen cycle in arid and semi-arid regions of the globe and might be responsible for an annual yield of 107 tonnes of fixed nitrogen (Schrauzer *et al.* 1983).

A photoelectrochemical cell for nitrogen reduction is also based on a semi-conductor material. Zinc-doped p-type gallium phosphide is used (rather than the n-type TiO_2 semiconductor) as the cathode with an aluminium anode (Dickson & Nozik 1978). The electrolyte is a glyme solution of titanium isopropoxide and $AlCl_3$; when light falls on the cathode, N_2 passing through the electrolyte is reduced to NH_3, while the Al anode is consumed as reductant. No external voltage is required and yields of NH_3 are *ca.* 0.05 mol per mol Ti present in 24 h on irradiation with a 150 W Xe lamp:

$$N_2 + 2Al + 6H^+ \xrightarrow{h\nu} 2NH_3 + 2Al^{3+}. \tag{17}$$

4. CHEMICAL N_2-OXIDIZING SYSTEMS

One of the first commercially exploited processes invented in the 1900s, was the Birkeland–Eyde process, in which N_2 and O_2 from the air were converted in an electric arc discharge to NO, then NO_2, before absorption into water to give nitric and nitrous acids. Its low efficiency (about 2% of the electricity used gave product) meant that, even in Norway, with its cheap hydropower, this process could not compete when the Haber process was introduced in the 1920s. Although the same constraints apply today, this process, among the several developed commercially during this century (Safrany 1974; Newton 1981), appears most likely to be adaptable to a small scale. Scale-down would eliminate costs associated with storage,

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transportation and distribution (*ca.* 75% of the delivered cost) and so be economically competitive with Haber-produced nitrogen fertilizer in some countries.

A compact, 3 kW, farm-size system consumes air, water and electrical energy to produce fixed nitrogen fertilizer. It becomes economically competitive where electric power is obtained either in off-peak periods from public utilities or from systems coupled directly to wind, water or solar energy sources as appropriate (Treharne *et al.* 1978). Air is pumped through a vortex-stabilized arc discharge struck between an electrode in the base of a 1 m iron pipe and the pipe wall (Schonherr 1909). This arc has a plasma core at *ca.* 2000 °C, where NO is produced. Rapid cooling of exiting gases prevents dissociation of NO before this product is fed through a holding column (where oxidation to NO_2 occurs) to a NO_2 trap. The trapping system depends on the product desired or materals available. It consists of an absorption column, with a countercurrent of water, containing an inert packing, limestone, or phosphate rock, plus wood ashes if available, yielding respectively dilute nitric acid, calcium nitrate, calcium nitrate plus phosphoric acid or a mixture containing combined N, P and K. Recycling of the dilute fixed N solution through the absorption column increases its concentration. The concentrated product is then applied either directly via an irrigation stream (or system) or is allowed to concentrate further by evaporation from a holding tank.

The largest drawback of this system is its low efficiency (2–5%) of energy conversion with the remainder appearing as high-grade heat. The overall economics improve if this heat is turned to good use, such as crop drying, water or space heating, evaporative cooling, etc. Currently, a 3 kW generator produces one tonne of fixed N for each 50000 kWh. Its advantages are that, with the exception of a high-voltage transformer, all components can be manufactured locally, it has a flexible energy-input requirement and very low-grade mineral ores can be used for absorption.

A very different system for oxidizing N_2 uses the semi-conductor properties of TiO_2 (Bickley & Vishwanathan 1979). Here, TiO_2 in the presence of surface-adsorbed hydrogen peroxide produces a bound nitrogen species on prolonged radiation under air or N_2, which yields NO on thermolysis. Although yields are low, such low-energy oxidation is an interesting complement to the high energy cost of oxidation in the plasma. Because TiO_2 was effective in the photoreduction of N_2 (Schrauzer *et al.* 1983), a re-evaluation of this phenomenon has suggested that the 'photooxidation' arises from oxidation by peroxide of the ammonia produced initially from the photoreduction of N_2 on the TiO_2 surface.

5. Modelling the biological system

Although much remains to be learned about the structure, composition, biosynthesis and functioning of the N_2-reducing, Mo-containing prosthetic group in conventional nitrogenase, a quantum leap in appreciation of its complexity has occurred since its first extraction from the MoFe protein as the iron–molybdenum cofactor (FeMoco) (Shah & Brill 1977). The subsequent identification of FeMoco as a metallocluster, which contains Mo, Fe and S and activates extracts of certain mutants lacking MoFe protein activity, prompted the first synthesis of complexes of the type $[Mo_2Fe_6S_8(SR)_9]^{3-}$ containing $MoFe_3S_4$ cubes (Christou *et al.* 1978; Wolff *et al.* 1978). Since then, a great many Mo–Fe–S-containing complexes have been synthesized, although none has yet produced a satisfactory chemical model for the structural, spectroscopic, redox and reactivity properties of FeMoco (Holm & Simhon 1985; Coucouvanis

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1984). Some have electronic and structural features approaching those of FeMoco (Friesen *et al.* 1983; Palermo & Holm 1983; Mascharak *et al.* 1983). These clusters have helped to elucidate the properties of mixed metal systems and aided the interpretation of various chemical and spectroscopic probes of FeMoco. More recently, incorporation of the two $Mo(CO)_3$ moieties onto a preformed prismane (Fe_6S_6) cluster has opened another class of clusters (Kanatzides & Coucouvanis 1986). If only one $Mo(CO)_3$ entity could be bound, the stoichiometry (at least) would approach that of FeMoco more closely.

N_2 *reduction by Mo–Fe–S models*

Just as none of the well-characterized metal–N_2 complexes has the multimetal characteristics and sulphur-based ligation of FeMoco, so none of the Mo–Fe–S–O chemical models of FeMoco is known to bind N_2 and produce characterizable complexes. However, certain Fe–S and Mo–Fe–S clusters mimic nitrogenase by catalysing the reductions of acetylene to ethylene (McMillan *et al.* 1979; Tanaka *et al.* 1981), of CH_3NC to methane and methylamine (Tanaka *et al.* 1982*a*) and even of N_2 to ammonia (Tanaka *et al.* 1982*b*). In these last reactions, $[Fe_4S_4(SPh)_4]^{4-}$ and $[Mo_2Fe_6S_8(SPh)_9]^{5-}$ are the suggested catalytic species in controlled potential electrolysis experiments in water or 1:1 THF:methanol. The last result particularly is surprising as these clusters do not appear to have the ligand-binding properties necessary for activation of small molecules, particularly N_2. However, the monocubanes, $[MoFe_3S_4(SR)_3\text{-}(cat)X]^{2-/3-}$ (X = displaceable solvent), form carbonyl, phosphine, cyanide and thiolate complexes (Mascharak *et al.* 1983; Palermo & Holm 1983) suggesting that, under the appropriate conditions, N_2 activation might be possible.

6. RECENT INFORMATION FROM FEMOCO STUDIES

Here, only recent findings arising mainly from our continuing investigations will be highlighted as they affect the development of improved N_2-fixing systems for the future.

For any material to act catalytically in a redox process, it must carry out the basic functions of accepting and donating reducing equivalents. With FeMoco, because it shows no catalytic properties after removal from the protein, the constraints imposed by the protein matrix must also be investigated and understood. In addition, for FeMoco to become the basis of a catalytic process, a procedure for its synthesis is needed; its structure is a third area of interest.

Oxidation–reduction properties

In the protein, FeMoco exists in three one-electron interconvertible redox states (Orme-Johnson 1985; Watt *et al.* 1981).

$$\begin{array}{ccccc} [\text{FeMoco(ox)}] & \leftrightarrow & [\text{FeMoco(sr)}] & \leftrightarrow & [\text{FeMoco(red)}] \\ S = 0 & & S = \tfrac{3}{2} & & S = \text{integer} \\ \text{EPR-silent} & & \text{EPR-active} & & \text{EPR-silent} \end{array}$$

The 'oxidized' (ox) state is produced by dye-oxidation of the 'semi-reduced' (sr) state, which can be re-formed with excess dithionite. These changes, also elicited electrochemically (Watt *et al.* 1981), have been characterized in detail. The 'reduced' (red) state occurs transiently only in the presence of the Fe protein, Mg–ATP and reductant and then effects substrate reduction. Only [FeMoco(sr)] is detectable by electron paramagnetic resonance (EPR) spectroscopy,

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exhibiting the $S = \frac{3}{2}$ signal. After extrusion, FeMoco shows similar features: dyes produced FeMoco(ox) from FeMoco(sr) in a reaction reversible by excess dithionite (Burgess *et al.* 1980), but no information was then available on the potential of this change or the number of electrons involved. No success has occurred in producing FeMoco(red), despite a claim to the contrary (Rawlings *et al.* 1978). Not even $[Fe_4S_4(SEt)_4]^{3-}$, which has a redox potential of −1.3 V, is effective (Newton *et al.* 1984).

Electrochemical investigations (Schultz *et al.* 1985) show FeMoco to be electroactive in *N*-methylformamide (NMF) at a glassy carbon electrode. Cyclic voltammetry (CV) of approx. 1 mM solutions showed two redox processes, at −0.32 V (against NHE) and −1.00 V (against NHE). Chemical redox titrations with voltammetric, potentiometric and EPR monitoring, showed the redox process at −0.32 V to correspond to $\text{FeMoco(ox)} + 1e^- \rightarrow \text{FeMoco(sr)}$. These studies at a reticulated vitreous carbon electrode (RVC), where FeMoco solutions of *ca.* 0.1 mM can be studied easily, now include coulometry and differential pulse voltammetry (DPV) (W. E. Newton, S. F. Gheller & F. A. Schultz, unpublished results). Coulometry has confirmed the one-electron nature of the −0.32 V couple and indicates a catalytic nature for the −1.00 V wave. The sensitivity achieved at RVC, plus improved solvent background, allows detection of a third redox process at *ca.* −1.25 V. DPV also shows clearly the third process. On diluting (1:15) *ca.* 2 mM FeMoco in NMF into DMF, the two, more positive couples in the CV shift to *ca.* −0.10 and −0.65 V, respectively; positive shifts of *ca.* 300 mV. DPV confirms these shifts, but shows that the −1.25 V couple does not shift appreciably, and detects a fourth process at *ca.* −1.65 V. The solvent-independent nature of the −1.25 V couple may indicate that it is not FeMoco-related but rather due to a contaminant.

Protein-induced constraints on FeMoco

Because (i) amide-type solvents are the means of extracting FeMoco from the denatured MoFe protein pellet, (ii) it is very difficult to remove NMF from isolated FeMoco (Yang *et al.* 1982), and (iii) dried FeMoco films exhibit an FT–IR spectrum indicative of ligated, N-deprotonated NMF (Walters *et al.* 1985), the mode of binding of FeMoco into the MoFe protein is likely to be largely via amide-type amino acid residues. Further, because only one thiol is bound per FeMoco, a conserved cysteinyl residue may also be involved. Although deprotonated, backbone peptide nitrogen atoms are stated to bind FeMoco to the MoFe protein (Walters *et al.* 1985), our preference is for the amide function of several of the conserved asparagine residues around Cys 275 in the α-subunit (Brigle *et al.* 1985). This conclusion comes from a detailed comparison of homology in amino acid sequences of seven α and four β subunits and of homology among the products of *nifD* and *nifE* plus those of *nifK* and *nifN* (K. E. Brigle, M. C. Weiss, W. E. Newton and D. R. Dean, unpublished). These comparisons form the basis of our investigations of how FeMoco is bound to, and possibly constrained by, the protein matrix, such that it is active in catalysing N_2 reduction.

Our initial targets of site-directed mutagenesis were therefore the strictly conserved residues, Cys 183, Cys 154, Cys 275, and Asn 280, in the α-subunit of the MoFe protein. The resulting mutants, with Ser 154, Ser 275 and Asp 280, cannot grow in the absence of fixed nitrogen, while the Ser 183 mutant grows slowly on N_2 (W. E. Newton, M. C. Weiss, J. C. Cantwell, K E. Brigle & D. R. Dean, unpublished). All four mutants have normal levels of Fe protein activity in crude extracts after derepression. We believe that these results substantiate our premise of important functional roles for these residues. We are now in the process of investigating the nature of the catalytic defect caused by each mutation.

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Structure and composition

The empirical composition of FeMoco appears to be $Mo_1Fe_{7\pm1}S_{9\pm1}$. How these elements are bonded to each other is much less clear. No X-ray diffraction data are available because crystals of the appropriate quality have yet to be produced. No recognizable Fe–S clusters can be extruded from FeMoco (Kurtz *et al.* 1979), but its degradation by oxygen produces $[MoOS_3]^{2-}$ and $[MoS_4]^{2-}$ (Newton *et al.* 1985, 1986). The latter results are consistent with our X-ray absorption spectroscopic results, both XANES (Conradson *et al.* 1985) and EXAFS (extended X-ray absorption fine structure) (Conradson *et al.* 1987), at the Mo K-edge. These results indicate that the environment of Mo in isolated FeMoco is best simulated by a MoS_3O_3 core. Observable differences at Mo occur in the protein and on treatment with thiol.

We have recently extended our XAS studies to the S K-edge and the Mo L-edges of isolated FeMoco (B. Hedman, K. O. Hodgson, S. F. Gheller & W. E. Newton, unpublished). It turns out that the S K-edge is exquisitely sensitive to the sulphur's oxidation state (Hedman *et al.* 1986). The S K-edge spectrum of FeMoco(ox), produced by 'self-oxidation' (Schultz *et al.* 1985), showed a clear S-edge at *ca.* 2469 eV, attributable to sulphide, at lower energy than those due to the 'self-oxidized' dithionite products. Absorptions attributable to thiolate-type sulphur were absent as expected. These investigations are part of our probes into the nature and origin of the extra low-Z donors to Mo in isolated FeMoco compared with protein-bound FeMoco. The investigation of S-containing species may well be very timely with the recent report that dithionite (although postulated mainly as a reductant) is necessary for extraction of FeMoco into DMF (Lough *et al.* 1986).

The Mo L_2- and L_3-edge spectra (Hedman *et al.* 1984) are also available from the same XAS run. We have investigated these spectra in isolated FeMoco(ox), using a variety of Mo complexes, to extract the oxidation state and ligation information contained therein. The Mo L_2-edge at *ca.* 2630 eV and the L_3-edge at *ca.* 2525 eV, both of which have 'white lines' corresponding to allowed p → d transitions, contain identical information. By using the L-edges of Mo complexes, several trends can be deduced from shifts and splittings of these white lines and applied to the FeMoco(ox) spectrum. Its gross features, a split white line with a shoulder on the low-energy side and a poorly defined 'p → continuum' band, resemble those of $[Mo_2Fe_6S_8(SEt)_9]^{3-}$ more than $[MoS_4(FeCl_2)_2]^{2-}$. These last two complexes, however, have the same white line peak energy of 2629 eV, a coincidence due to cancellation of opposite shifts. The white line maximum of FeMoco(ox) is 2930 eV, 1 eV higher than these models. This result could indicate an oxidation state higher than +IV (as occurs in the dicubane) or could result from a mixed ligand coordination sphere. The very poorly defined 'p → continuum' band is likely support for the latter suggestion. This interpretation is consistent with the MoO_3S_3 core structure deduced from our Mo K-edge studies.

7. FUTURE PROSPECTS FOR N_2-REDUCING SYSTEMS

Haber technology is mature and as such is unlikely to change substantially in the future. Gradual shifts in the centres of industrial ammonia production would not offset the problems of high transport and storage costs involved in ammonia production and distribution. Other solutions must be sought, of which chemical N_2 fixation under ambient conditions is one. Because it is more compatible with a simple, low-temperature and low-pressure system, it could form the basis of a complementary process. Continued efforts at elucidating the structure and

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mode of functioning of FeMoco should lead to the design and synthesis of materials with improved N_2-reducing capabilities. Success here will also affect efforts to improve the efficiency of the biological process and attempts to extend the capability to non-fixing cereal crops. If the responsible agents can be made more robust, particularly toward O_2, and impregnated on an appropriate surface to overcome the cycling requirements of current laboratory-scale systems, reductive or oxidative processes could be developed. These might be operated at an electrode and driven by a renewable resource, such as solar, wind, or water power, and be located in or near irrigation streams. These systems might produce and apply fixed nitrogen as required or store it for later application.

Maybe the semiconductor materials described above could form the basis of a light-driven nitrogen-fixation process. If the positive 'hole' remaining after electron photoexcitation to reduce N_2 could also be used to draw electrons from N_2 to give NO_2 (rather than from water to give O_2), then a process producing NH_4NO_3 becomes feasible. However, much more research is needed. In contrast, the electric arc system is being researched now in a very similar context, particularly for remote areas, where fertilizer production of a few tonnes per year can have a significant impact on agricultural production.

Simple, inexpensive, small-scale systems may have a place in the future in areas where cheap hydroelectric or solar power is available and where the high capital investment requirements for a large-scale manufacturing system cannot be justified. Developing countries with very limited fossil fuel supplies and limited means to purchase fertilizer are the likely first beneficiaries of such systems. Although the progress made since the 1960s has been very substantial, the same cannot be said for the conversion of the accrued information into useful agricultural products and processes. It remains to be seen if greater advantage can be taken of the increased capacity for research into nitrogen fixation which has been developed.

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REFERENCES

Allen, A. D. & Senoff, C. V. 1965 Nitrogenopentammineruthenium(II) complexes. *Chem. Commun.*, pp. 621–622.

Armor, J. A. & Taube, H. J. 1970 Linkage isomerization in nitrogen-labeled $[Ru(NH_3)_5N_2]Br_2$. *J. Am. chem. Soc.* **92**, 2560–2565.

Baumann, J. A. & George, T. A. 1980 Reactions of coordinated dinitrogen. 8. Formation of ammonia by protonation. *J. Am. chem. Soc.* **102**, 6153–6154.

Baumann, J. A., Bossard, G. E., George, T. A., Howell, D. B., Koczon, L. M., Lester, R. K. & Nodding, C. M. 1985 Formation of ammonia and hydrazine from the reactions of acids with bis(dinitrogen) complexes of molybdenum. *Inorg. Chem.* **24**, 3568–3578.

Bercaw, J. E., Marvich, R. H., Bell, L. G. & Brintzinger, H. H. 1972 Titanocene as an intermediate in reactions involving molecular hydrogen and nitrogen. *J. Am. chem. Soc.* **94**, 1219–1238.

Bickley, R. I. & Vishwanathan, V. 1979 Photocatalytically induced fixation of molecular nitrogen by near UV radiation. *Nature, Lond.* **280**, 306–308.

Borod'ko, Yu. G., Ivleva, I. N., Kachapina, L. M., Salienko, S. I., Shilova, A. K. & Shilov, A. E. 1972 Isolation of a new intermediate complex in the nitrogen-fixing system. *J. chem. Soc. chem. Commun.*, p. 1178.

Borod'ko, Yu. G., Ivleva, I. N., Kachapina, L. M., Kvashina, E. F., Shilova, A. K. & Shilov, A. E. 1973 Hydrazine-producing intermediate in the nitrogen-fixing system. *J. chem. Soc. chem. Commun.*, pp. 169–170.

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Bortels, H. 1930 Molybdenum as a catalyst in the biological fixation of nitrogen. *Arch. Mikrobiol.* **1**, 333–342.

Brigle, K. E., Newton, W. E. & Dean, D. R. 1985 Complete nucleotide sequence of the *Azotobacter vinelandii* nitrogenase structural gene cluster. *Gene* **37**, 37–44.

Brulet, C. R. & van Tamelen, E. E. 1975 Ammonia formation from molybdenum-bound dinitrogen under weakly reducing protic conditions. *J. Am. chem. Soc.* **97**, 911–912.

Burbo, E. M., Denisov, N. T., Shestakov, A. F. & Shilov, A. E. 1982 Reduction of nitrogen by chromium(II) hydroxide. *Kinet. Katal.* **23**, 191–193.

Burgess, B. K., Stiefel, E. I. & Newton, W. E. 1980 Oxidation–reduction properties and complexation reactions of the iron–molybdenum cofactor of nitrogenase. *J. biol. Chem.* **255**, 353–356.

Chatt, J., Richards, R. L., Fergusson, J. E. & Love, J. L. 1968 The reduction of nitrogen complexes. *Chem. Commun.*, pp. 1522–1523.

Chatt, J., Heath, G. A. & Richards, R. L. 1974 Diazene-*N*-(di-imide) and hyrazido-(2-)*N*-(aminoimido) complexes: The addition of acids to dinitrogen complexes. *J. chem. Soc. Dalton Trans.*, pp. 2074–2082.

Chatt, J., Diamantis, A. A., Heath, G. A., Hooper, N. E. & Leigh, G. J. 1977*b* Reactions of ligating dinitrogen to form carbon-nitrogen bonds. *J. chem. Soc. Dalton Trans.*, pp. 688–697.

Chatt, J., Pearman, A. J. & Richards, R. L. 1977*a* Conversion of dinitrogen in its molybdenum and tungsten complexes into ammonia and possible relevance to the nitrogenase reaction. *J. chem. Soc. Dalton Trans.*, pp. 1852–1860.

Chatt, J., Dilworth, J. R. & Richards, R. L. 1978 Recent advances in the chemistry of nitrogen fixation. *Chem. Rev.* **78**, 589–625.

Christou, G., Garner, C. D., Mabbs, F. E. & King, T. J. 1978 Crystal structure of a Fe_3MoS_4 cubic cluster dimer. *J. chem. Soc. chem. Commun.*, pp. 740–741.

Churchill, M. R. & Wasserman, H. J. 1982*a* The Ta(u–N_2)Ta system. 2. A binuclear dimido complex of octahedral tantalum(V). *Inorg. Chem.* **21**, 218–222.

Churchill, M. R. & Wasserman, H. J. 1982*b* The Ta(u–N_2)Ta system. 3. Crystal structure of an octahedral tantalum(V) imide and comparisons with related Ta(u–N_2)Ta systems. *Inorg. Chem.* **21**, 223–226.

Churchill, M. R., Li, Y.-L., Blum, L. & Schrock, R. R. 1984 Synthesis and crystallographic characterization of a dinuclear 1,2-hydrazido^{2-} complex of tungsten(VI). *Organometallics* **3**, 109–113.

Coucouvanis, D. 1984 The synthesis, structures and electronic properties of Fe–Mo–S polynuclear aggregates. In *Advances in nitrogen fixation research* (ed. C. Veeger & W. E. Newton), pp. 81–91. Dordrecht, Boston and Lancaster: Martinus Nijhoff.

Conradson, S. D., Burgess, B. K., Newton, W. E., Hodgson, K. O., McDonald, J. W., Rubinson, J. F., Gheller, S. F., Mortenson, L. E., Adams, M. W. W., Mascharak, P. K., Armstrong, W. H. & Holm, R. H. 1985 Structural insights from the Mo K-edge XANES of the iron–molybdenum protein of nitrogenase and its iron–molybdenum cofactor. *J. Am. chem. Soc.* **107**, 7935–7940.

Conradson, S. D., Burgess, B. K., Newton, W. E., Mortenson, L. E. & Hodgson, K. O. 1987 Structural studies of the molybdenum site in the MoFe protein and its FeMo cofactor by EXAFS. *J. Am. chem. Soc.* (In the press.)

Day, V. W., George, T. A. & Iske, S. D. A. 1975 Photochemical reactions of alkyl halides with coordinated dinitrogen. *J. Am. chem. Soc.* **97**, 4127–4228.

Denisov, N. T., Shuvalova, N. I. & Shilov, A. E. 1973 Inhibiting action of trivalent vanadium on the reduction of nitrogen by divalent vanadium. *Kinet. Katal.* **14**, 1325–1326.

Dickson, C. R. & Nozik, A. J. 1978 Nitrogen fixation in photoenhanced reduction on p-GaP electrodes. *J. Am. chem. Soc.* **100**, 8007–8009.

Didenko, L. P., Gavrilina, O. K., Yablonskaya, E. E., Shilova, A. K. & Shilov, A. E. 1983 Phospholipid-dependent catalytic dinitrogen reduction in the presence of molybdenum complexes. *Nouv. J. Chim.* **7**, 605–611.

Dilworth, M. J. 1966 Acetylene reduction by nitrogen-fixing preparations from *Clostridium pasteurianum*. *Biochim. biophys. Acta* **127**, 285–294.

Dilworth, M. J. & Thorneley, R. N. F. 1981 Nitrogenase of *Klebsiella pneumoniae*. *Biochem. J.* **193**, 971–983.

Evans, H. J., Bottomley, P. J. & Newton, W. E. (eds) 1985 *Nitrogen fixation research progress*. Dordrecht, Boston, and Lancaster: Martinus Nijhoff.

Friesen, G. D., McDonald, J. W., Newton, W. E., Euler, W. B. & Hoffman, B. M. 1983 Synthesis, characterization and EPR spectral studies of the multimetal species. *Inorg. Chem.* **22**, 2202–2205.

George, T. A. 1983 Catalysis of nitrogen-fixing model studies. In *Homogeneous catalysis with metal–phosphine complexes* (ed. L. H. Pignolet), pp. 405–441. New York: Plenum Press.

George, T. A. & Tisdale, R. C. 1985 New mono-dinitrogen complexes of molybdenum that produce ammonia and hydrazine. *J. Am. chem. Soc.* **107**, 5157–5159.

Hardy, R. W. F. & Knight, E. Jr. 1967 ATP-dependent reduction of azide and HCN by N_2-fixing enzymes of *Azotobacter vinelandii* and *Clostridium pasteurianum*. *Biochim. biophys. Acta* **139**, 69–76.

Harrison, D. F., Weissberger, E. & Taube, H. 1968 Binuclear ion containing nitrogen as a bridging group. *Science, Wash.* **159**, 320–322.

Hawkes, T. R., McLean, P. A. & Smith, B. E. 1984 Nitrogenase from *nifV* mutants of *Klebsiella pneumoniae* contains an altered form of the iron–molybdenum cofactor. *Biochem. J.* **217**, 317–321.

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Hedman, B., Penner-Hahn, J. E. & Hodgson, K. O. 1984 Molybdenum LII, III edge studies. In *Proceedings in physics*, vol. 2 (*EXAFS and near edge structure*, III), pp. 64–66. Berlin, New York and Tokyo: Springer-Verlag.

Hedman, B., Frank, P., Penner-Hahn, J. E., Roe, L. E., Hodgson, K. O., Carlson, R. M. K., Brown, G., Cerino, J., Hettel, R., Troxel, T., Winick, H. & Yang, J. 1986 Sulfur K edge x-ray absorption studies using the 54-pole wiggler at SSRL in undulator mode. *Nucl. Instrum. Meth.* A246, 797–800.

Henderson, R. A. 1982 Mechanism of formation of hydrazido(2-). *J. chem. Soc. Dalton Trans.*, pp. 917–925.

Henderson, R. A. 1984 Reactions between $Mo(N_2)_2$ and HCl. *J. chem. Soc. Dalton Trans.*, pp. 2259–2263.

Henderson, R. A., Leigh, G. J. & Pickett, C. J. 1983 The chemistry of nitrogen fixation and models for the reactions of nitrogenase. *Adv. inorg. Chem. Radiochem.* **27**, 197–292.

Hidai, M. 1985 Reactions of dinitrogen complexes: dinitrogen-reducing model systems. In *Metal ions in biology* (ed. T. G. Spiro), vol. 7 (*Molybdenum enzymes*), pp. 285–341. New York: Wiley–Interscience.

Hidai, M., Tominari, K. & Uchida, Y. 1972 Preparation and properties of dinitrogen–molybdenum complexes. *J. Am. chem. Soc.* **94**, 110–114.

Hidai, M., Mizobe, Y. & Uchida, Y. 1976 Preparation and properties of dinitrogen–molybdenum complexes. 5. Conversion of N_2 into N_2CRR'. *J. Am. chem. Soc.* **98**, 7824–7825.

Holm, R. H. & Simhon, E. D. 1985 Molybdenum/tungsten–iron–sulfur chemistry: current status and relevance to the native cluster of nitrogenase. In *Metal ions in biology* (ed. T. G. Spiro), vol. 7 (*Molybdenum enzymes*), pp. 1–87. New York: Wiley–Interscience.

Jeffery, J., Lappert, M. F. & Riley, P. I. 1979 Organozirconium dinitrogen complexes. *J. Organometal. Chem.* **181**, 25–36.

Jonas, K., Bauer, D. J., Kruger, C., Roberts, P. J. & Tsay, Y.-H. 1976 'Side-on' dinitrogen–transition metal complexes. *J. Am. chem. Soc.* **98**, 74–79.

Kanatzidis, M. G. & Coucouvanis, D. 1986 New $[Fe_6S_6Cl_6\{Mo(CO)_3\}_2]^{n-}$ clusters with relevance to nitrogenase. *J. Am. chem. Soc.* **108**, 337–338.

Kelly, M., Postgate, J. R. & Richards, R. L. 1967 Reduction of cyanide and isocyanide by nitrogenase of *Azotobacter chroococcum*. *Biochem. J.* **102**, 1c–3c.

Khrushch, A. P., Shilov, A. E. & Vorontsova, T. A. 1974 Role of acids in reduction of acetylene catalyzed by molybdenum–thiol complexes. *J. Am. chem. Soc.* **96**, 4987.

Kruger, C. & Tsay, Y.-H. 1973 Molecular structure of a dinitrogen–nickel–lithium complex. *Angew. Chem. Int. Ed. Engl.* **12**, 998–999.

Kurtz, D. M. Jr., McMillan, R. S., Burgess, B. K., Mortenson, L. E. & Holm, R. H. 1979 Identification of iron–sulfur centers in the iron-molybdenum proteins of nitrogenase. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4986–4989.

Li, J.-G., Burgess, B. K. & Corbin, J. L. 1982 Nitrogenase reactivity: cyanide as a substrate and inhibitor. *Biochemistry, Wash.* **21**, 4393–4397.

Liu, N.-H., Strampach, N., Palmer, J. G. & Schrauzer, G. N. 1984 Reduction of molecular nitrogen in molybdenum(III–V) hydroxide/titanium(III) hydroxide systems. *Inorg. Chem.* **23**, 2772–2777.

Lockshin, A. & Burris, R. H. 1965 Inhibitor of nitrogen fixation in extracts from *Clostridium pasteurianum*. *Biochim. biophys. Acta* **111**, 1–8.

Lough, S., Jacobs, D. L., Lyons, D. M., Watt, G. D. & McDonald, J. W. 1986 Solubilization of the iron–molybdenum cofactor of *Azotobacter vinelandii* nitrogenase in dimethylformamide and acetonitrile. *Biochem. Biophys. Res. Commun.* **139**, 740–746.

Manriquez, J. M., Sanner, R. D., Marsh, R. E. & Bercaw, J. E. 1976 Reduction of molecular nitrogen to hydrazine. Structure of a dinitrogen complex of bis(pentamethylcyclopentadienyl)zirconium(II) and an ^{15}N labeling study of its reaction with hydrogen chloride. *J. Am. chem. Soc.* **98**, 3042–3044.

Manriquez, J. M., McAllister, D. R., Rosenberg, E., Shiller, A. M., Williamson, K. L., Chan, S. L. & Bercaw, J. E. 1978 Solution structure and dynamics of binuclear dinitrogen complexes of bis(pentamethylcyclopentadienyl)titanium(II) and bis(pentamethylcyclopentadienyl)zirconium(II). *J. Am. chem. Soc.* **100**, 3078–3083.

Marvich, R. H. & Brintzinger, H. H. 1971 A metastable form of titanocene. *J. Am. chem. Soc.* **93**, 2046–2048.

Mascharak, P. K., Papaefthymiou, G. C., Armstrong, W. H., Foner, S. Frankel, R. B. & Holm, R. H. 1983 Electronic properties of single- and double-$MoFe_3S_4$ cubane-type clusters. *Inorg. Chem.* **22**, 2851–2858.

McKenna, C. E., McKenna, M.-C. & Higa, M. T. 1976 Chemical probes of nitrogenase. 1. Cyclopropene. *J. Am. chem. Soc.* **98**, 4657–4659.

McMillan, R. S., Renaud, J., Reynolds, J. G. & Holm, R. H. 1979 Biologically related iron–sulfur clusters as reaction centers. *J. inorg. Biochem.* **11**, 213–227.

Moorehead, E. L., Robinson, P. R., Vickrey, T. M. & Schrauzer, G. N. 1976 The chemical evolution of a nitrogenase model. 12. Stoichiometric reduction of acetylene and of molecular nitrogen by mononuclear cyano complexes of oxomolybdate(IV). *J. Am. chem. Soc.* **98**, 6555–6562.

Mulliken, R. S. 1959 The lower excited states of some simple molecules. *Can. J. Chem.* **36**, 10–23.

Newton, W. E. 1981 Nitrogen fixation. In *Kirk-Othmer*: *Encyclopedia of chemical technology*, vol. 15 (3rd edn), pp. 942–968. New York: Wiley and Sons, Inc.

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Newton, W. E., Burgess, B. K., Cummings, S. C., Lough, S., McDonald, J. W., Rubinson, J. F., Conradson, S. D. & Hodgson, K. O. 1984 Structural aspects and reactivity of the iron-molybdenum cofactor from nitrogenase. In *Advances in nitrogen fixation research* (ed. C. Veeger & W. E. Newton), p. 160. Dordrecht, Boston and Lancaster: Martinus Nijhoff.

Newton, W. E., Gheller, S. F., Schultz, F. A., Burgess, B. K., Conradson, S. D., McDonald, J. W., Hedman, B. & Hodgson, K. O. 1985 Redox and compositional insights into the iron-molybdenum cofactor of *Azotobacter vinelandii* nitrogenase as a guide to synthesis of new Mo–Fe–S clusters. In *Nitrogen fixation research progress* (ed. H. J. Evans, P. J. Bottomley and W. E. Newton), pp. 605–610. Dordrecht, Boston and Lancaster: Martinus Nijhoff.

Newton, W. E., Gheller, S. F., Hedman, B., Hodgson, K. O., Lough, S. & McDonald, J. W. 1986 Elicitation of thiomolybdates from the iron–molybdenum cofactor of nitrogenase. *Eur. J. Biochem.* **159**, 111–116.

Nikonova, L. A., Efimov, O. N., Ovcharenko, A. G. & Shilov, A. E. 1972 Reduction of acetylene in aqueous solutions of divalent vanadium complexes. *Kinet. Katal.* **13**, 249.

Nikonova, L. A., Isaeva, S. A., Pershikova, N. I. & Shilov, A. E. 1975/6 A comparison of the reduction of dinitrogen by a vanadium(II)–catechol system with nitrogenase. *J. molec. Catal.* **1**, 367–374.

Nikonova, L. A., Rummel, S., Shilov, A. E. & Wahren, M. 1980 Is dimide an intermediate in dinitrogen reduction by vanadium(II) complexes in protic media? *Nouv. J. Chim.* **4**, 427–430.

Orme-Johnson, W. H. 1985 Molecular basis of biological nitrogen fixation. *A. Rev. Biophys. biophys. Chem.* **14**, 419–459.

Palermo, R. E. & Holm, R. H. 1983 Reactivity properties of single $MoFe_3S_4$ cubane-type clusters. *J. Am. chem. Soc.* **105**, 4310–4318.

Pez, G. P., Apgar, P. & Crissey, R. K. 1982 Reactivity of $(u–C_5H_4)(C_5H_5)_3Ti_2$ with dinitrogen. Structure of a titanium complex with a triply-coordinated N_2 ligand. *J. Am. chem. Soc.* **104**, 482–490.

Pickett, C. J. & Talarmin, J. 1985 Electrosynthesis of ammonia. *Nature, Lond.* **317**, 652–653.

Rawlings, J., Shah, V. K., Chisnell, J. R., Brill, W. J., Zimmermann, R., Munck, E. & Orme-Johnson, W. H. 1978 Novel metal cluster in the iron–molybdenum cofactor of nitrogenase. *J. biol. Chem.* **253**, 1001–1004.

Rocklage, S. M. & Schrock, R. R. 1982 Preparation of organoimide and u-dinitrogen complexes of tantalum and niobium from neopentylidene complexes. *J. Am. chem. Soc.* **104**, 3077–3081.

Rocklage, S. M., Turner, H. W., Fellman, J. D. & Schrock, R. R. 1982 Preparation of tantalum u–dinitrogen complexes from molecular nitrogen and reduced tantalum complexes. *Organometallics* **1**, 703–707.

Sacco, A. & Rossi, M. 1967 Hydride and nitrogen complexes of cobalt. *Chem. Commun.*, p. 316.

Safrany, D. R. 1974 Nitrogen fixation. *Scient. Am.* **231**, 64–80.

Sanner, R. D., Manriquez, J. M., Marsh, R. E. & Bercaw, J. E. 1976*a* Structure of u-dinitrogen-bis[bis(pentamethylcyclo-pentadienyl)-dinitrogenzirconium(II)]. *J. Am. chem. Soc.* **98**, 8351–8357.

Sanner, R. D., Duggan, D. M., McKenzie, T. C., Marsh, R. E. & Bercaw, J. E. 1976*b* Structure and magnetism of u-dinitrogen-bis[bis(pentamethylcyclopentadienyl)-titanium(II)]. *J. Am. chem. Soc.* **98**, 8358–8365.

Schollhorn, R. & Burris, R. H. 1967 Reduction of azide by the N_2-fixing enzyme system. *Proc. natn. Acad. Sci. U.S.A.* **57**, 1317–1323.

Schonherr, O. 1909 Manufacture of nitric acid from air. *Elektrotech. Z.* **30**, 366–402.

Schrauzer, G. N., Kiefer, G. W., Tano, K. & Doemeny, P. A. 1974 Chemical evolution of nitrogenase model. VII. Reduction of nitrogen. *J. Am. chem. Soc.* **96**, 641–647.

Schrauzer, G. N. 1975 Nonenzymatic simulation of nitrogenase reactions. *Angew. Chem. Int. Ed.* **14**, 514–521.

Schrauzer, G. N. & Guth, T. D. 1977 Photolysis of water and nitrogen on titanium dioxide. *J. Am. chem. Soc.* **99**, 7189–7193.

Schrauzer, G. N., Strampach, N., Liu, N.-H., Palmer, M. R. & Salehi, J. 1983 Nitrogen photoreduction on desert sands. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3873–3876.

Schrock, R. R., Blum, L. & Theopold, K. H. 1984 Hydrazido(1-), 1,2-hydrazido(2-) and hydrazido(4-) complexes of tungsten(VI). In *Advances in nitrogen fixation research* (ed. C. Veeger & W. E. Newton), pp. 75–80. Dordrecht, Boston and Lancaster: Martinus Nijhoff.

Schultz, F. A., Gheller, S. F., Burgess, B. K., Lough, S. & Newton, W. E. 1985 Electrochemical characterization of the iron–molybdenum cofactor from nitrogenase. *J. Am. chem. Soc.* **107**, 5364–5368.

Sellman, D. & Weiss, W. 1977 First reaction of the N_2 ligand with bases. *Angew. Chem. Int. Ed. Engl.* **16**, 880–881.

Sellman, D. & Weiss, W. 1978 Consecutive nucleophilic and electrophilic attack on N_2 ligands. *Angew. Chem. Int. Ed. Engl.* **17**, 269–270.

Shah, V. K. & Brill, W. J. 1977 Isolation of an iron–molybdenum cofactor from nitrogenase. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3249–3253.

Shilov, A., Denisov, N., Efimov, O., Shuvalov, N., Shuvalova, N. & Shilova, A. 1971 New nitrogenase model for nitrogen reduction in protic media. *Nature, Lond.* **231**, 460–461.

Smith, B. E. 1977 Structure and function of nitrogenase. *J. less-common Metals* **54**, 465–475.

Sobota, P. & Jezowska-Trzebiatowska, B. 1978 Properties of the system: transition metal compound–magnesium–N_2, CO_2, CO, H_2. *Coord. Chem. Rev.* **26**, 71–104.

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Tanaka, K., Tanaka, M. & Tanaka, T. 1981 Reduction of acetylene to ethylene in a model reaction to nitrogenase. *Chem. Lett.*, pp. 895–898.

Tanaka, K., Imasaka, Y., Tanaka, M., Honjo, M. & Tanaka, T. 1982*a* Reduction of CH_3NC and CH_3CN in model reactions to nitrogenase. *J. Am. chem. Soc.* **104**, 4258–4260.

Tanaka, K., Hozumi, Y. & Tanaka, T. 1982*b* Dinitrogen fixation catalyzed by reduced clusters. *Chem. Lett.*, pp. 1203–1206.

Teuben, J. H. 1973 Binuclear dinitrogen complexes of titanium(III). *J. organometal. Chem.* **57**, 159–167.

Treharne, R. W., Moles, D. R., Bruce, M. R. & McKibben, C. K. 1978 Nitrogen fertilizer generator for farm use. *Proc. Second Review Meeting of I.N.P.U.T.S. Project, East–West Center, Honolulu, Hawaii*, pp. 279–287.

van der Weij, F. W. & Teuben, J. H. 1976 On the mechanism of the reduction of molecular nitrogen. *J. organometal. Chem.* **102**, 223–228.

van der Weij, F. W., Scholtens, H. & Teuben, J. H. 1977 Synthesis and properties of dicyclopentadienyltitanium(III) neo-pentyl. *J. Organometal. Chem.* **127**, 299–304.

van Tamelen, E. E., Boche, G. & Greeley, R. 1968 An organic–inorganic system for reaction with nitrogen of the air. *J. Am. chem. Soc.* **90**, 1677–1679.

van Tamelen, E. E., Fechter, F. R., Schneller, S. W., Boche, G., Greeley, R. H. & Akermark, B. 1969 Titanium(II) in the fixation-reduction of molecular nitrogen. *J. Am. chem. Soc.* **91**, 1551–1552.

van Tamelen, E. E. & Seely, D. A. 1969 The catalytic fixation of molecular nitrogen by electrolytic and chemical reduction. *J. Am. chem. Soc.* **91**, 5194–5195.

van Tamelen, E. E. & Rudler, H. 1970 Utilization of molecular nitrogen in the synthesis of organic amines and nitriles. *J. Am. chem. Soc.* **92**, 5253–5254.

Vol'pin, M. E. & Shur, V. P. 1964 Fixation of nitrogen on complex catalysts. *Dokl. Akad. Nauk. SSSR* **156**, 1102–1104.

Vol'pin, M. E. & Shur, V. B. 1966 Nitrogen fixation by transition metal complexes. *Nature, Lond.* **209**, 1236.

Vol'pin, M. E., Ilatovskaya, M. A., Kosyakova, L. V. & Shur, V. B. 1968*a* Catalytic fixation of nitrogen. *J. chem. Soc. chem. Commun.*, pp. 1074.

Vol'pin, M. E., Shur, V. B., Kudryavstev, R. V. & Prodayko, L. A. 1968*b* Amine formation in molecular nitrogen fixation. *J. chem. Soc. chem. Commun.*, pp. 1038.

Vol'pin, M. E., Belyi, A. A., Shur, V. B., Katkov, N. A., Nekaeva, I. M. & Kudryavtsev, R. V. 1971 Formation of amines from aromatic hydrocarbons in molecular nitrogen fixation reactions. *Chem. Commun.*, pp. 246–247.

Watt, G. D., Burns, A. & Tennent, D. L., 1981 Stoichiometry and spectral properties of the MoFe cofactor and noncofactor redox centers in the MoFe protein of nitrogenase. *Biochemistry, Wash.* **20**, 7272–7277.

Walters, M. A., Chapman, S. K. & Orme-Johnson, W. H. 1985 The nature of amide ligation to the metal sites of FeMoco. *Polyhedron* **5**, 561–565.

Weathers, B. J., Grate, J. H., Strampach, N. A. & Schrauzer, G. N. 1979 Chemical evolution of a nitrogenase model. 18. Reduction of molecular nitrogen with molybdoinsulin catalysts. *J. Am. chem. Soc.* **101**, 925–928.

Wolff, T. E., Berg, J. M., Warrick, C., Hodgson, K. O., Holm, R. H. & Frankel, R. B. 1978 The molybdenum–iron–sulfur cluster complex. *J. Am. chem. Soc.* **100**, 4630–4631.

Yamamoto, A., Kitazume, S., Pu, L. S. & Ikeda, S. 1971 Reversible combination of molecular nitrogen with a cobalt complex. *J. Am. chem. Soc.* **93**, 3071.

Yang, S.-S., Pan, W.-H., Friesen, G. D., Burgess, B. K., Corbin, J. L., Stiefel, E. I. & Newton, W. E. 1982 Iron–molybdenum cofactor from nitrogenase. *J. biol. Chem.* **257**, 8042–8048.

Zones, S. I., Palmer, M. R., Palmer, J. G., Doemeny, J. M. & Schrauzer, G. N. 1978 Hydrogen evolving systems. 3. Reduction of molecular nitrogen. *J. Am. chem. Soc.* **100**, 2113–2121.

Discussion

G. D. WATT (*Batelle-Kettering Laboratory, Yellow Springs, Ohio, U.S.A.*). Could Dr Newton explain the nature of the FeMoco mutants mentioned in his paper and explain the purpose of their use in his experiments?

W. E. NEWTON. We have two series of mutants: firstly, those in *nifE*, *N*, *B* and *V*, which Dr Dean and I are using to probe the biosynthesis of FeMoco. For example, we believe that mutations in *nifE* or *nifN* alter the template on which FeMoco is assembled. All such mutants have a Nif^- phenotype. The second series is produced by site-directed mutagenesis, in which a single amino acid residue in one or other of the MoFe protein polypeptides is changed. These mutants are being used to probe where the cluster-binding domains of the protein are located

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and to assign responsibilities in the catalytic process. All those studied so far have lowered or zero C_2H_2-reducing activity.

J. Chatt, F.R.S. (*University of Sussex, Brighton, U.K.*). How does the energy consumed in producing fertilizer nitrogen by the small electric arc production units, such as Dr Newton has established in Nepal, compare with that consumed, for example, by the Haber process?

W. E. Newton. Energy use is equivalent to about 50000 kW h t^{-1}, or several times larger than that of the Haber process. However, there are significant advantages in the small scale of the apparatus, its simplicity and low cost and particularly its ability to utilize otherwise wasted mechanical energy to generate power for the arc.

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