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Possibilities for the Enhancement of Biological Nitrogen Fixation

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Possibilities for the enhancement of biological nitrogen fixation

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Modern developments in plant science and knowledge of nitrogen fixation have opened exciting medium and long term possibilities:

(1) Extended exploitation of existing systems such as exotic legumes; non-leguminous shrubs; the newly discussed grass associations; wider use of 'green manures'.

(2) Augmentation of the effectiveness of existing systems by altering the complement of nitrogen fixation (*nif*) genes; altering the genetic control systems to avoid repression by fixed nitrogen; avoiding wastage of nitrogen fixing capacity in hydrogen evolution; altering the efficiency of uptake of essential metals such as Mo and Fe; altering the character of the plant partner in symbioses.

(3) Constructing new nitrogen fixing systems by preparing somatic hybrids of desirable crop plants with natural nitrogen fixing plants; introducing *nif* into new commensal or symbiotic microbes.

(4) Transfer of nitrogen fixing ability to plants by (*a*) constructing DNA viruses or transferable plasmids carrying *nif* genes as possible vectors; (*b*) associating *nif* genes with plant mitochondrial or chloroplast DNA; (*c*) seeking uptake of nitrogen fixing microbes as potential organelles.

(5) Introducing *nif* genes into rumen microbes; use of nitrogen fixing microbes as animal fodder.

All these projects require extensive basic research; the fact that evolution has not so far provided such systems may mean that unforeseen obstacles to their construction may now exist.

For this contribution I have been asked to review the more long term, and sometimes exotic, possibilities for the enhancement of biological nitrogen fixation. Such possibilities arise mainly from recent developments in plant physiology, in somatic hybridization of plant cells, and in understanding of the biochemistry and genetics of nitrogen fixation. Since this is largely new research at the frontiers of knowledge, it can be difficult to decide to what extent various bright ideas which arise can ever achieve reality. It is therefore imperative to start with a word of caution: the most immediately important practicality is to exploit existing knowledge of microbial nitrogen fixation. This means taking such action as increasing the area devoted to leguminous crops and returning to rotation involving legumes, particularly where fertilizer might be undesirable or environmentally deleterious. It means investigating and adopting new types of legume crop, such as the temperate soya beans or the protein rich fava beans (though both of these, I believe, have disadvantages in the British climate). The use of non-legumes in forestry, the testing of green manures, the introduction of nitrogen fixation steps into the recycling of farm and domestic wastes are largely self-evident ways in which our present farming techniques could be modified without drastic change. It will become evident that most of the possibilities to be discussed in this contribution are long term ones, which require considerable technical innovation at all levels from the laboratory to the field. Hardy (1976)

† Elected F.R.S. 17 March 1977.

recognized the time lag which occurs between laboratory science and its application and suggested 10 years as the minimum time for any agronomic effect to be felt from a seemingly simple theoretical advance such as the extension of associative symbiosis to cereal crops. He offered 25 years as the minimum for exploitation of a complex scientific development such as successful transfer of nitrogen fixation genes to crop plants. Implicit in Hardy's estimates is the fact that, even if a nitrogen fixing cereal became available in the laboratory tomorrow, several seasons of testing and checking would be needed before it could be used in normal agricultural practice.

If one bears that warning in mind, it is reasonable to consider what present day research at the strategic level indicates might be in store for British agriculture over the next few decades. Much of what I shall discuss applies beyond these shores, of course.

Extending existing nitrogen fixing systems

The development of new strains of legumes scarcely needs further comment: it must proceed, and one hopes that forage, grain and green manure legumes appropriate to our eccentric climate will become available. It is important not to neglect the non-leguminous systems: the alder tree, which harbours a nitrogen fixing endosymbiont in root nodules, can contribute 100 kg N/ha from leaf fall alone and scrub alders have been used in Asia to upgrade exhausted soil. Bond (1977) has advocated the deliberate use of alders and similar plants in forestry. Blue-green algae are established nitrogen fixing microbes which are of great importance in arctic and tropical areas (Stewart 1974); they may have a rôle in temperate agriculture as a green manure. But perhaps the most exciting development of recent years has been the evidence from Dr Johanna Döbereiner's group (see Burris 1977) for associations between nitrogen fixing bacteria and grasses. The principal associations are between the sand grass *Paspalum notatum* and the bacterium *Azotobacter paspali*, and between the grass *Digitaria decumbens* and *Spirillum lipoferum*. In neither association are root nodules formed, but there is a considerable degree of interdependence between plant and microbe: in the *Paspalum* system the microbes form a sheath round the root and in the *Digitaria* case the microbes penetrate the root, with seemingly beneficial effect. These grasses are tropical, but instances in which temperate grasses form comparable associations are now known (see table 1).

Of particular interest in an agricultural context is the fact that temperate cultivars of maize have been found capable of associating with *S. lipoferum*. Unfortunately, some popular reports of the effectiveness of these associations in cereal crops were exaggerated; sober accounts of the situation were given by Burris (1977) and Döbereiner (1977). There is no doubt that the associations are real, and a net conversion of N_2 to plant N has been shown using $^{15}N_2$. The use of these 'associative symbioses', as they are termed, in a tropical context is now being evaluated. In temperate situations, demonstration of an association between nitrogen fixing bacteria and maize pre-dated the work with *S. lipoferum* (Raju, Evans & Seidler 1972) and evidence for comparable associations in temperate weeds was presented by Harris & Dart (1973, see also table 2). Any of these systems, given genetically appropriate strains of plant, might provide the key to a new and valuable nitrogen fixing agricultural crop, but considerable effort in physiological and ecological research is still needed.

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Table 1. Nitrogen fixation associated with the roots of some grasses and cereals

(Nitrogen fixing activity expressed as nmol acetylene reduced g^{-1} root h^{-1}; data by courtesy of Rothamsted Experimental Station.)

plant and origin	common name	activity
Brazil		
Brachiara mutica	para grass	150–750
Cynodon dactylon	bermuda grass	20–270
Digitaria decumbens	pangola grass	20–400
Paspalum notatum	bahia grass	2–300
Pennisetum purpureum	elephant grass	5–1000
Saccharum officinarum	sugar cane	5–20
Nigeria		
Andropogon gayanus	gamba grass	15–270
Cynodon dactylon	star grass	10–150
Panicum maximum	guinea grass	75
Pennisetum typhoides	millet	3–195
France		
Zea mays	maize seedlings	1000–3000
U.S.A.		
Zea mays	maize	14–16
Philippines		
Oryza sativa	rice	8–80
Australia		
Pennisetum clandestinum	kikuyu grass	21–140
(Active leguminous plant		1000–3000)

Table 2. Plants on Broadbalk Wilderness, Rothamsted, which have nitrogen fixing bacteria closely associated with their roots

(Data by courtesy of Rothamsted Experimental Station.)

systematic name	common name
Stachys sylvatica	hedge woundwort
Heracleum sphondylium	hogweed
Anthriscus sylvestris	cow parsley
Mercurialis perennis	dog's mercury
Rumex acetosa	sorrel
Convolvulus arvensis	bindweed
Viola canina	dog's violet
Nepita glechoma	ground ivy
Hedera helix	ivy

Augmenting existing systems

Plants and higher organisms, even fungi and yeasts, do not fix nitrogen alone. All 'nitrogen fixing plants' are actually conventional plants with nitrogen fixing microbes in association. The group of genes which confer ability to fix nitrogen are called *nif* genes by geneticists, and they are restricted to bacteria or blue-green algae. In the past half dozen years the understanding of the genetics of *nif* has advanced and in principle one can already see possibilities for making use of this knowledge. If every symbiotic microbe has double its quota of genes, would it fix twice as much nitrogen? The short answer is probably 'no', because many enzymes and materials other than just the products of nitrogen fixation genes are needed for efficient nitrogen fixation (for specialists, materials such as ATP, reductant, electron donor or

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physiological regulator might separately or together become limiting), but experiments on gene dosage effects of this kind are now feasible and desirable.

Ammonium ions are the primary product of the fixation of nitrogen. If the microbe can obtain sufficient of these from its environment, it does not make nitrogenase (the nitrogen fixing enzyme). This situation is illustrated in figure 1, where a continuous culture of a nitrogen fixing organism was grown with stepwise increases in concentration of an ammonium salt in its culture medium and the nitrogenase activity of the population measured. The enzyme activity declined in proportion to the amount of ammonium ion available until, when there was sufficient to satisfy completely the requirement of the whole population for fixed nitrogen (demonstrated by the appearance of residual ammonia in the spent culture fluid), no nitrogenase activity remained. This principle applies to all natural nitrogen fixing organisms studied so far; expressed in a more sophisticated way, it means that expression of *nif* genes is regulated by ammonium ions. Scientists now know in some detail the molecular mechanism of ammonium regulation (Magasanik *et al.* 1974) but a detailed discussion is not appropriate here. An essential

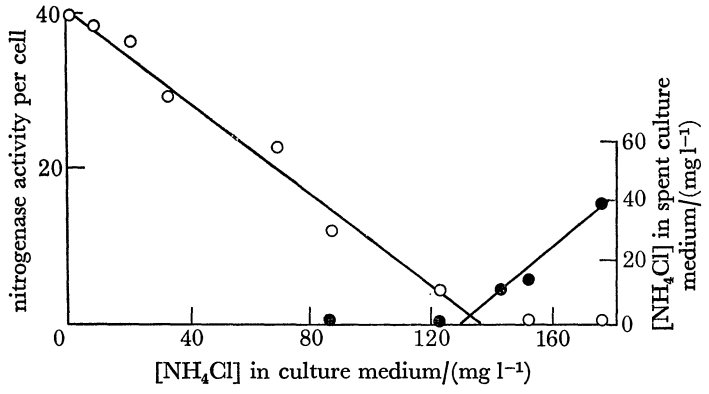

FIGURE 1. Regulation of nitrogenase activity by an exogenous ammonium salt. A continuous culture of *Azotobacter chroococcum* was grown with stepwise increase in the amounts of NH_4Cl in the medium and, at each level, the maximum nitrogen fixing (acetylene reducing) activity of the population was measured [○] as well as the residual ammonia in the culture fluid [●]. Nitrogen fixation declined in proportion to the availability of ammonia and reached zero as residual ammonia became detectable in the culture fluid. (After Drozd, Tubb & Postgate 1972.)

point, however, is that scarcity of ammonia promotes formation of an important component of the regulatory system, the enzyme glutamine synthetase. This enzyme is very unusual: it is involved in the biosynthesis of glutamine, but it not only acts biosynthetically, it also acts as a regulator of *nif*, promoting expression of the *nif* genes. The genes determining glutamine synthetase formation are not among the *nif* cluster, they are remote therefrom on the bacterial chromosome. It is possible genetically to manipulate these genes (called *gln* by geneticists) and obtain organisms which make glutamine synthetase constitutively (i.e. they make it even when ammonia is present). Such organisms, if they have *nif* genes, then fix nitrogen even when they have ammonia. In all nitrogen fixing bacteria so far studied, ammonia resulting from fixation is incorporated as the amino acid glutamate, by way of an enzyme called glutamate synthase. The genes determining this enzyme can be mutated too, and, by blocking this enzyme genetically in an organism with a constitutive glutamine synthetase, one constructs a microbe which cannot incorporate the nitrogen it fixes. If it is grown with a little glutamate, it then fixes nitrogen and excretes ammonia. Professor Valentine's group (Shanmugam & Valentine 1975)

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have prepared such mutants of *Klebsiella pneumoniae* and pointed out that comparable mutations in blue-green algae could be valuable 'factories' for making atmospheric nitrogen into ammonia at the expense of solar energy. (Their present mutants do it at the expense of sugar.) Symbiotic nitrogen fixing bacteria in some ways resemble these mutants: they excrete a lot of the nitrogen they fix and the plant takes it up; the possibility obviously arises of making new types of symbiont by manipulating the regulatory processes of conventional organisms.

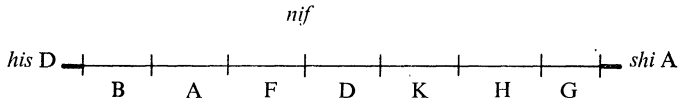

FIGURE 2. A provisional map of the *nif* gene cluster. B is concerned with incorporation of Mo into nitrogenase. F is concerned with electron transfer into nitrogenase. A is concerned with regulation of synthesis of nitrogenase. D, K and H are concerned with synthesis of the enzyme protein. G is concerned with regulation of synthesis of nitrogenase. *his*D is part of a gene cluster specifying biosynthesis of the amino-acid histidine; *shi*A is a determinant concerned with the uptake of shikimic acid. Neither is relevant to the *nif* cluster.

A third consideration arises from the fact that nitrogenase contains atoms of molybdenum, and that a biochemical apparatus is required to absorb molybdenum and incorporate it into the enzyme. Figure 2 is a provisional sketch of the *nif* gene cluster as far as it is understood at the time of writing. Neither the number nor the order of determinants is wholly established at present, but already we realize that the *nif* cluster specifies more than just the nitrogenase proteins. These, in fact, are synthesized in response to the information in the regions termed D, K and H; of interest in the present context is another gene, *nif*B, which has something to do with getting molybdenum into the appropriate peptide of the enzyme (Brill, Steiner & Shah 1974). However, *nif*B does not carry all the necessary information. There are other molybdenum-containing enzymes in bacteria and the genetic information relevant to building molybdenum into one of these, nitrate reductase, is also used to mobilize molybdenum for incorporation into nitrogenase (Kennedy & Postgate 1977). One thing is certain: genetically improved nitrogen-fixing microbes will require adequate Mo uptake systems. One approach to manipulating such systems might be to exploit the tungsten atom, for this can compete with molybdenum and prevent it getting into the enzyme protein: a tungsten resistant strain of microbe might well have improved its ability to take up and utilize molybdate.

A fourth prospect for augmenting existing systems arises from a special property of nitrogenase. This enzyme, though it normally reduces nitrogen to ammonia, is able to reduce a number of other small, triply-bonded molecules such as hydrogen cyanide, nitrous oxide, acetylene and so on (see Burris (1971); its ability to reduce acetylene is exploited in the acetylene test, which has revolutionized the understanding of biological nitrogen fixation). If no such reducible substrate is available, the enzyme reacts with the hydrogen ion of water, forming gaseous hydrogen. This reaction was discovered in 1965 (Bulen, Burns & Le Comte 1965) and it occurs to some extent even when nitrogen is available and is being fixed. It took 10 years for its relevance to productivity to be properly realized: Schubert & Evans (1976) showed that field grown soya beans may actually evolve hydrogen to the atmosphere, wasting a considerable portion of their nitrogen fixing ability as it becomes sidetracked into evolution of hydrogen gas. Free living nitrogen fixing bacteria also have this problem, and we now know that they, as well as the root nodule bacteria, possess an enzyme (hydrogenase) which 'picks up' the hydrogen formed by this side reaction of nitrogenase and re-uses it for nitrogen fixation. (See

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Yates (1977) for details and documentation.) Table 3 shows that the hydrogen-recycling apparatus of some symbiotic systems are less than perfect; a genetic approach to 'tightening up' such systems may well be possible.

These are four examples of areas in which present knowledge of the biochemistry of nitrogenase and the genetics of *nif* enable us to see ways in which the efficiency of existing systems might be augmented. Nitrogenase also contains atoms of iron; it uses up biological energy as ATP and it requires specialized reductants (ferredoxins or flavodoxins). These aspects will require attention in genetically or physiologically improved systems, but more basic research is needed before plausible ways of influencing them can be proposed. The physiology and genetics of the plant partner in symbiotic systems can be of crucial importance too (Holl & La Rue 1976). Havelka & Hardy (1976) showed that late developing strains of soya beans fixed more nitrogen than early maturing plants, and that photosynthetic CO_2 assimilation could be a limiting factor in prolonging the nitrogen fixation phase during the growth of the plant and maturation of the crop. Lowering photorespiration, either genetically or artificially with inhibitors, is one promising way of increasing the amount of CO_2 converted to photosynthate by the host plant.

TABLE 3 HYDROGEN EVOLUTION BY SYMBIOTIC NITROGEN FIXING SYSTEMS

	nitrogenase activity diverted to H_2 evolution (%)
leguminous plants	
lucerne	48
white clover	50
subterranean clover	60
lupin	42
soya bean	55
garden pea	39
cowpea†	< 1
non-legumes	
alder	6
russian olive	13
californian myrtle	2

† Inoculated with *Rhizobium* strain 32H1.

Nodules cut from wild or pot grown plants were assayed for hydrogen evolution with or without N_2 using an amperometric technique. Data modified from Schubert & Evans (1976) who presented an extensive survey including many more plants, direct assay of nitrogen fixing ability by the acetylene test and some analogous experiments with whole plants.

CONSTRUCTION OF NEW SYMBIOSES

The development of somatic hybridization as a tool in plant breeding and genetics (Cocking 1975) opens possibilities for the construction of new hybrids which fix nitrogen. Essentially, somatic hybridization involves the removal of the walls of isolated plant cells with the aid of enzymes and placement of the 'protoplasts' so formed in an environment (Ca salts; dilute $NaNO_3$; polyethylene glycol) which induces some of the protoplasts to fuse. If protoplasts of different origins are mixed, a proportion of heterologous fusions may occur and, since protoplasts can often be regenerated into whole plants in special circumstances, new, asexually generated, hybrids can in theory be produced. This is not an area in which I have specialized knowledge, but I understand that somatic hybrids of closely related plants have been prepared successfully in this way (Gamborg 1975; Power *et al.* 1976). With more distantly separated

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plants, fusion definitely occurs but selection of the hybrid fused protoplasts and their regeneration is a problem. Constabel, Dudits, Gamborg & Kao (1975) have obtained somatic hybrids of nitrogen fixing legumes: protoplasts of peas and soya beans have fused and yielded heterokaryons but these have not been regenerated to give complete plants. Attempts have been made to obtain hybrids of barley and soya beans but these have foundered at the regeneration stage. Nevertheless, the approach has obvious promise as a means of spreading the ability to fix nitrogen to new types of plant.

Rhizobia normally form associations with leguminous plants, but an instance is known (Trinick 1975) in which *Trema cannabina*, a tropical non-legume from New Guinea, is the natural host to a rhizobium. Tissue cultures of many plant cells can form nitrogen fixing associations with rhizobium and, in particular, a culture of plant cells from a non-legume such as tobacco is as effective in forming an association as a culture from a legume such as soya bean (Scowcroft & Gibson 1975). The types of rhizobium able to form these 'exotic' associations are now known to be capable of fixing nitrogen in the total absence of plant material; they are in fact free living nitrogen fixing bacteria of exceptional oxygen sensitivity (see Bergersen (1977) for documentation and earlier references). The possibility obviously arises of setting up associations between rhizobia, or other aerobic nitrogen fixing bacteria, and new plants; essentially, some physiological inter-dependence would have to be devised. An example in which a mutualistic association between carrot callus and a mutant of azotobacter was set up was described by Carlson & Chaleff (1974); unfortunately quantitative data on N_2 incorporated into carrot tissue were not reported.

Setting up mutualistic associations between plant and microbe might be avoided if natural rhizosphere commensals, which presumably have some dependence on their host plants already, could be made to fix nitrogen. Many of them do so naturally; others might do so if provided with *nif* genes. In recent years, means have been discovered to transfer *nif* genes to a variety of new bacterial hosts. Most such transfers have made use of *nif* genes from *K. pneumoniae* but *nif* from *Rhizobium trifolii* has been transferred to *K. aerogenes* (Dunican, Tierney & O'Yara 1976) and preliminary reports suggesting *nif* gene transfer in photosynthetic bacteria have been published. Figure 3 summarizes the transfers of *K. pneumoniae nif* to date.

Three comments are relevant in the present context.

(1) Most transfers of *nif* have made use of extrachromosomal genetical elements (plasmids). Instances have been reported of chromosomal integration of *nif* in a new host (see, for example, Cannon, Dixon, Postgate & Primrose 1974).

(2) Plasmids carrying *Klebsiella nif* in new genera (e.g. *Salmonella*) may readily lose the *Klebsiella* DNA in unselective conditions (Krishnapillai & Postgate 1977).

(3) Not all hosts fix nitrogen when provided with *nif* genes. *Agrobacterium tumefaciens*, for example, appears to make nitrogenase peptides which are inactive (Dixon, Cannon & Kondorosi 1976).

Numerous problems obviously exist in ensuring complete expression of *nif* even in new bacterial hosts, yet some of the most attractive root commensals for this purpose are fungi such as mycorrhiza. These are eukaryotes (nucleate organisms), whose genetic apparatus is very different from that of bacteria. The transfer of *nif* to such hosts may present little difficulty, but ensuring its expression may present grave obstacles. A report (Giles & Whitehead 1975) of transfer of *nif* genes from *Azotobacter* to the mycorrhizal fungus *Rhizopogon* can be criticized on the grounds that microbiological checks for persistence of live *Azotobacter* alongside or

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within the 'transformed' fungus were not adequate. At present we know of no obstacle in principle to *nif* expression in nucleate cells, but we still need more information on the obstacles which already exist in bacteria such as *Agrobacterium* before we can seek expressions of *nif* in nucleate cells with any prospect of success.

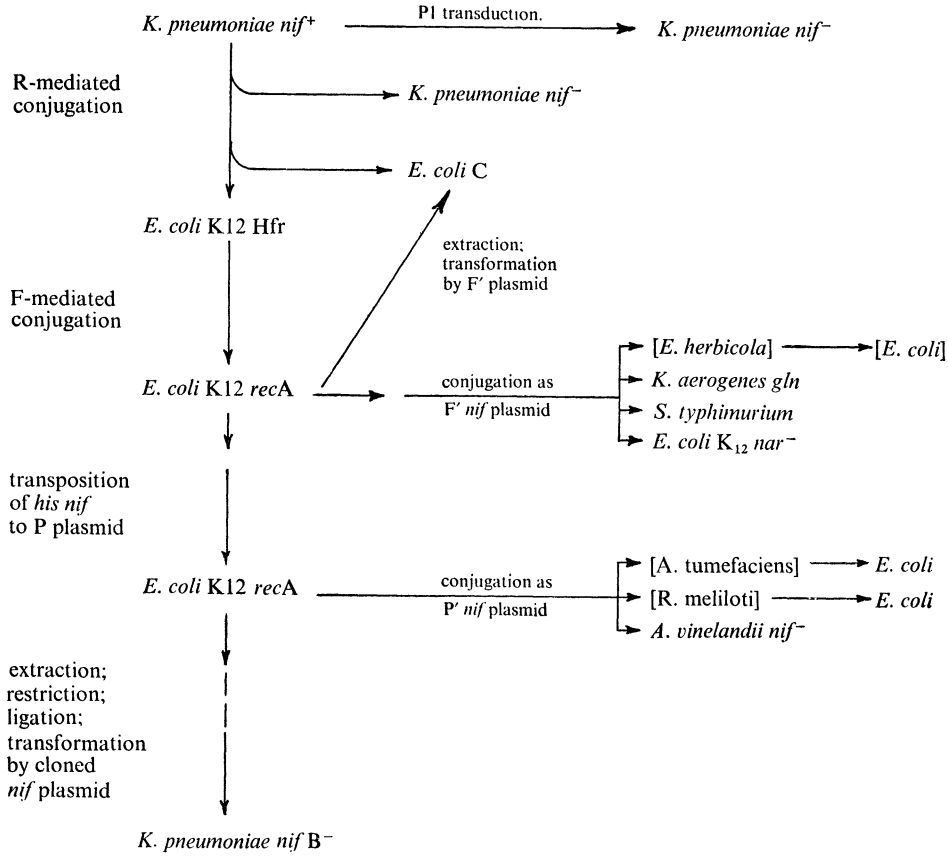

FIGURE 3. Some genetic manipulations of nitrogen fixation genes (*nif*) originating in *Klebsiella pneumoniae* strain M5a1.

CONSTRUCTION OF NITROGEN FIXING PLANTS

The problem just cited – that of promoting expressions of bacterial *nif* genes in eukaryotic fungi – applies with equal force to what is perhaps the least probable of the possibilities I shall discuss: that of constructing plants which use *nif* genes themselves, without need of associated bacteria. The idea is particularly attractive because conversion of N_2 to plant products ought to be very efficient as well as immune to the strain variability in effectiveness which characterizes the microbial partners in symbioses. Only 2 years ago it seemed to be a very promising prospect, for several lines of evidence suggested that microbial DNA could be taken up by plant cells and that the information contained therein could be transcribed, translated and expressed as an alteration of the plant's phenotype. Evidence seemed to be available for the replication of microbial DNA in plant cells, for the 'correction' with bacterial DNA of mutant plants (*Arabidopsis*) which required thiamine, for the expression of bacterial lactose genes in plant cell tissue cultures. These topics are presented in a volume edited by Ledoux

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(1975). Disappointingly, each of the aspects of DNA transfer to plants mentioned above has, more recently, proved either difficult to repeat or has been subject to fundamental criticism of its experimental basis. A critique is inappropriate to this article, but the conclusion is inescapable that plant cells now seem to be less readily transformable by microbial DNA than many scientists thought a year or two ago. Yet even if the pessimistic view is sustained by further experimentation, which one hopes it will not be, some feasible approaches to transfer of expressible *nif* into the plant genome remain. Plant viruses, which cause plant diseases, are packages of bacteria-like genetic information which the plant's genetic apparatus recognizes, transcribes and translates, usually to its own detriment. Some, such as cauliflower mosaic virus, are DNA viruses, and the technology now exists for introducing *nif* genes artificially into such a virus and of discovering whether the plant then reads *nif* genes along with the viral genes. Another possible vector for introducing *nif* genes into a plant is the plant pathogenic bacterium *Agrobacterium tumefaciens*. This is the 'crown gall' organism, and evidence is accumulating that pathogenicity results from transfer of microbial DNA from *A. tumefaciens* as a plasmid into the plant cells (see Schilperoot & Bomhoff 1975; Schell 1975). Presumably information on this plasmid is read by the plant's genetic apparatus and tumour formation follows; *nif* genes carried on such a plasmid ought also to be read. The success of Ausubel, Cannon & Reidel (1977) in transferring parts of Klebsiella *nif* to a small amplifiable plasmid shows that construction *in vitro* of such a plasmid, or of a virus carrying *nif*, is feasible. Apart from the plant cell nucleus, possible 'host' entities for *nif* DNA in a plant might be a chloroplast or a mitochondrion; both organelles have their own DNA, separate from that in the plant cell nucleus, and both apparently have more bacteria-like transcription and translation mechanisms. Yet whatever methods are ultimately used to transfer *nif* to plants, it must be obvious that much subsidiary information beyond that on a *nif* cluster such as that of Klebsiella will be necessary to enable the plant actually to use the genes and fix nitrogen.

Many authorities believe that the chloroplasts and mitochondria of plants evolved originally from endophytic microbes – possibly a blue-green alga and a bacterium respectively – which are now totally part of the organism and replicate along with it. True or not, this concept raises the possibility of an operation which might be simpler than splicing bacterial *nif* DNA (and other necessary genetic information) directly into the plant's genome or those of its organelles. One could imagine the construction of benign endophytic bacteria (or blue-green algae) which would behave like nitrogen fixing organelles; one would make use of the ability of plant protoplasts to take up small particles as a means of introducing such constructs. Problems such as localization, avoiding pathogenicity, preventing repression by endocellular nitrogen compounds, synchronizing multiplication and many others arise, but again, none seems insuperable in principle.

By-passing plants

Much of agriculture is directed to animal production. If the bacteria of the rumen fixed nitrogen, then a cow or a sheep could be reared on cellulose exclusively (e.g. waste paper) with consequent advantages in recycling of fixed carbon and direct conversion of N_2 to high grade protein. In fact, the rumina of animals do contain such bacteria, but they are not permanent residents and the nature of ruminant physiology is such that there is probably always sufficient ammonia free in the rumen fluid to repress nitrogenase synthesis (see figure 1). Transfer of a constitutive *nif* gene cluster to a normal rumen inhabitant might allow one to

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construct a nitrogen fixing ruminant, assuming that ammonia toxicity did not become a problem to the animal. But at present the only available *nif* constitutive microbes are not mutated in the *nif* cluster but in the remote *gln* genes (see above).

As a less spectacular prospect, the deliberate culture of nitrogen fixing microbes as animal feeds might hasten conversion of residues or photosynthate into protein. Examples are the numerous strains of nitrogen fixing methane oxidizing bacteria (Dalton 1974), a group of microbes which is already taken seriously as a potential source of single cell protein, and the blue-green algae which, in reindeer moss, already form an important part of the diet of some sub-arctic mammals.

Discussion

Exciting vistas for the exploitation of biological fixation have opened in the past decade and have become increasingly cogent in view of the rapid rise in costs of manufacture and transport of nitrogenous fertilizer, as well as the deleterious environmental effects which may result from its intensive use. Yet excitement must not blind us to reality, even though the world's press may consider itself immune from this precept. If the construction of plants which fix nitrogen without bacteria, or of nitrogen fixing ruminants, were today an easy matter, evolution would surely have provided such biotypes already. New nitrogen fixing plants may one day be constructed successfully, and problems of stability of the *nif* genes (or new symbionts) in their new hosts may be overcome, together with physiological problems, such as exclusion of oxygen (Postgate 1974), which have not been discussed here. But a price will have to be paid: nitrogenase consumes, in the test tube, some 50 kcal mol^{-1} NH_3 formed, which is better than the 150 kcal mol^{-1} NH_3 of the Haber process (see Bergersen (1971) for these data) but is still a considerable consumption of energy in biological terms. Some living cells such as *Azotobacter* (Hill, Drozd & Postgate 1972) seem to be more efficient, but they still consume some biological energy in order to fix nitrogen. That energy must come from photosynthesis, which means, for example, that a nitrogen fixing cereal plant would have a lower northern or southern geographic limit than its ammonia fertilized cousin. Whether this difference would be swallowed up in the Earth's climatic variation is impossible to calculate at present, but it means that, in any locality, a crop based on nitrogen fixation would necessarily be somewhat less productive in conversion of photosynthate to harvestable product than a similar crop using ammonia fertilizer. In practice, of course, even legume crops are never totally dependent on fixation and the property would still be of enormous agronomic advantage. With such a crop, productivity would soon become limited by other nutrients – P, K, S, etc. according to geographical location – and deficiencies in soil molybdenum might be more common, because of the enzyme's need for this element. However, given wise husbandry and careful use of minor fertilizers, the ultimate limiting nutrient in fertile, reasonably insolated areas would be CO_2, as it is today with well fertilized soya beans (Havelka & Hardy 1976). No global catastrophe need be expected; nitrogen fixing weeds would not spread rampant over the world's arable land. On the contrary, some of the more negative aspects of intensive agriculture, such as the nitrate pollution problems resulting from run-off, would be alleviated (though not eliminated: 'natural' fertilizers can also cause run-off). Genetic and somatic manipulation, like many other kinds of research into breeding, can entrain biological hazards and, recently, some intemperate views of these have been expressed. In the context of agriculture, modern genetical developments, applied intelligently, seem to show a highly favourable ratio of benefit to risk.

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Conclusions

The global prospects over the next two or three decades for the enhancement of biological nitrogen fixation are good but still vague in detail. Many directions must be explored, ranging from the extension of known nitrogen fixing systems to the creation of entirely new ones. For a temperate island such as Britain, with highly technological agriculture and still wealthy by world standards, there is time for an orderly progress from exploitation of existing systems to the development of new and imaginative ones. But mounting energy costs, land shortages, environmental problems and changed patterns of plant disease tell us that agriculture in the twenty-first century will have to be very different from today. Some of the ideas in this paper will be forgotten; others may prove to have been of critical importance.

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