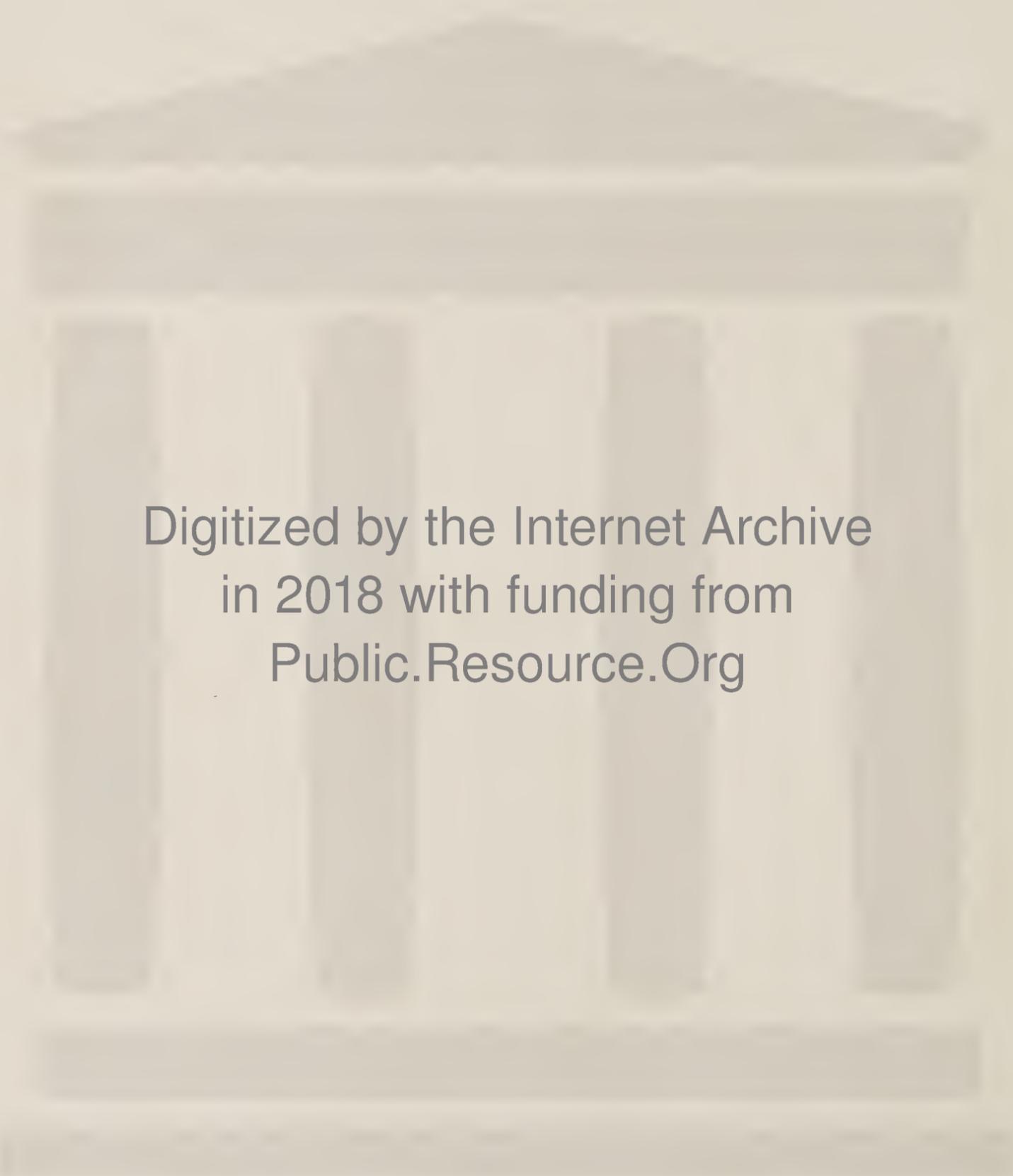


SPECIAL PUBLICATION 1

LECTURES ON CATALYSIS



**INDIAN ACADEMY OF SCIENCES
BANGALORE 560 006**



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INDIAN ACADEMY OF SCIENCES
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**LECTURES
ON
CATALYSIS**

Supplied Free of Cost
On the eve of
Raman's Centenary-1988

41st Annual Meeting, Nagpur, November 1975

General Editor
S. RAMASESHAN

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FOREWORD

During the last sixty years, from small beginnings the study of catalysts and catalytic transformations has enlarged in many different ways involving several scientific disciplines and numerous techniques of analysis and observations. Heterogeneous catalysis has been a field of intense experimental and theoretical explorations leading not only to a vastly superior understanding of the underlying phenomena but also to several hundreds of industrial applications on a vast scale for the production on the one hand, of basic inorganic chemicals such as sulphuric acid, nitric acid and ammonia and nitrogenous fertilisers and on the other, to a myriad of coal and petroleum-based organic chemicals and polymers which have found applications to every basic human need and welfare. Homogeneous catalytic changes have also acquired importance in a similar way. Scientific excitement and output have been of high order offering scope for logic as well as serendipity. Unusual catalyst supports, multimetal and metal organic chemical combinations, novel catalyst preparative and assessment techniques, innovation in methodology for assessing short life intermediates as well as the nature of catalyst surfaces have all been characteristic of this excitement. The search continues for catalysts and conditions for further specified and directed transformations under milder conditions of pressure and temperature.

The study of related areas of biological catalysis, involving biochemical enzymological studies has also increased even more rapidly in the last three decades. Micro-organisms have served as major tools in providing a fertile field for investigations on such enzyme catalysed transformations. Mutational studies and molecular biology have begun to unravel whole new rich areas for research and application. Thus microbes are now the basis of production of many new life-saving drugs, nutrient chemicals and vitamins and even primary food materials such as proteins. To achieve further refinements in economic directed synthesis and industrial production, it has now become possible to use purified enzymes on suitable inert supports in much the same way as catalysts are employed. Simultaneously a high degree of understanding of many basic biological processes at cellular, enzymic and molecular level in plant and animal life has also been achieved and attempts are afoot to harness this new knowledge for beneficial purposes. Such fundamental phenomena as photosynthesis of carbon compounds and biological fixation of nitrogen are receiving attention with this end in view. Speculations and

hypotheses on the mechanisms by which enzyme proteins are able to effect intricate, specified chemical transformations at high speed, under isothermal conditions are constantly being put forward.

With these rapid advances and high level of activity, the similarities and contrasts between biological and non-biological catalysis need greater attention. The Indian Academy of Sciences with its Fellowship drawn from many different scientific areas decided to organise a symposium on the subject of catalysis in its widest sense, as a part of its 41st Annual Meetings at Nagpur on 7-10 November 1975. The papers presented by invited speakers covered a wide range of interests in catalysis and in biological and enzymic functions. Each author has attempted to outline briefly for the benefit of a multi-disciplinary audience, the background current scientific knowledge in the area before placing his own specific new contribution in that area. The symposium provided an unusual opportunity for scientists of diverse disciplines to come together on a theme of common interest. The five papers presented have been compiled together in the present publication. It is hoped that this will serve in some small measure to focus attention on the very large potential that exists for mutual assistance and scientific collaboration for future work and to give a glimpse of the many applications that can follow.

I wish to thank all the authors for their considerable efforts in presenting these papers at the symposium and for their co-operation in preparing these for publication.

S. VARADARAJAN,
Convener

Biocatalysis—unique design of enzyme-proteins

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1. INTRODUCTION

Catalysis in living cells must occur in a medium of water and at ambient temperature. Water is the smallest and most abundant molecule in living cells—it is the very matrix of life. It had been aptly stated by Gutfreund¹ that “All biological processes are either directly or indirectly under the influence of some of the characteristic properties of water.” It exhibits anomalous properties and should have been a gas at the restrictive temperature range employed for life processes but for the extensive ordered structure imposed by hydrogen bonding between oxygen atoms of water molecules. Its large specific heat can also make it a suitable medium to keep constant temperature—a veritable thermostat. At the body temperature of 37°C chosen in higher animals, the chemical reactions are slow and the rates cannot sustain the energy requirements of the living cells. By designing proteins as catalysts the rates have been accelerated by 10^4 - 10^{14} times—an awesome potential gained by proteins that is unequalled by any model system of organic or inorganic catalysis. The rate acceleration is of the relative speed advantage of “Aryabhata” over an ant. For example, solutions of starch or dipeptides, stable at room temperature for several days, would be hydrolyzed in minutes in the presence of tiny amounts of specific enzymes. What information do we obtain from comparative study of enzymes and other catalysts? Do the physical and chemical principles apply equally to biocatalysis? Or must we invoke the enigmatic vital force, overwhelmed by ignorance of the mysteries of life processes? What structural features of protein impart the functional capability of catalysis? In essence, what makes a “protein” an “enzyme” is one of the unsolved riddles in biology.

2. ENZYMES IN CELL PROCESSES

Only a small group of compounds are permitted to enter the living cells. With these raw materials a host of compounds that constitute the structure

of the cells are built. The living cells employ a set of reactions for each process and an enzyme for each reaction. Oxidation-reduction, hydrolysis, dehydration, aldol condensation, alkylation, ammonolysis and acylation are among the wide selection of reactions known to occur. Implicated in these is the making or breaking of a chemical bond between two atoms of the most commonly occurring elements in cell material: C, O, N, H, P and S. The numerous reactions are classified into six groups by the Enzyme Commission of the International Union of Biochemistry based on the type of reaction catalyzed (figure 1). There are very few reactions in the cells which are non-enzymatic with rates sufficient to participate in the overall processes. Even a simple reaction such as hydration of carbon dioxide to carbonic acid is catalyzed by an enzyme which speeds the rate several-thousand fold.

CLASSIFICATION	BONDS AFFECTED
1. OXIDOREDUCTASES	
2. TRANSFERASES	
3. HYDROLASES	
4. LYASES	
5. ISOMERASES	
6. LIGASES	

FIGURE 1. CLASSIFICATION OF ENZYMES AND BONDS AFFECTED

3. ENZYMES ARE PROTEINS

The first isolation of a cell-free enzyme was done by Buchner² in 1897 who demonstrated fermentation of sugar by yeast extracts. By the turn of the 20th century a number of enzymes were recognized and serious attempts were started for purification of these from cell extracts notably by Willstater³, an authority in those days, who considered biocatalysts are also likely to be small molecular weight substances. In 1926, Sumner⁴ isolated a protein from jack bean possessing catalytic properties of hydrolysis of urea to ammonia and carbon dioxide. It required the tenaciousness of the remarkable man, Sumner, to stand up to the overriding opposition of Willstater and to prove that the isolated protein was indeed the active catalyst and not a mere carrier.

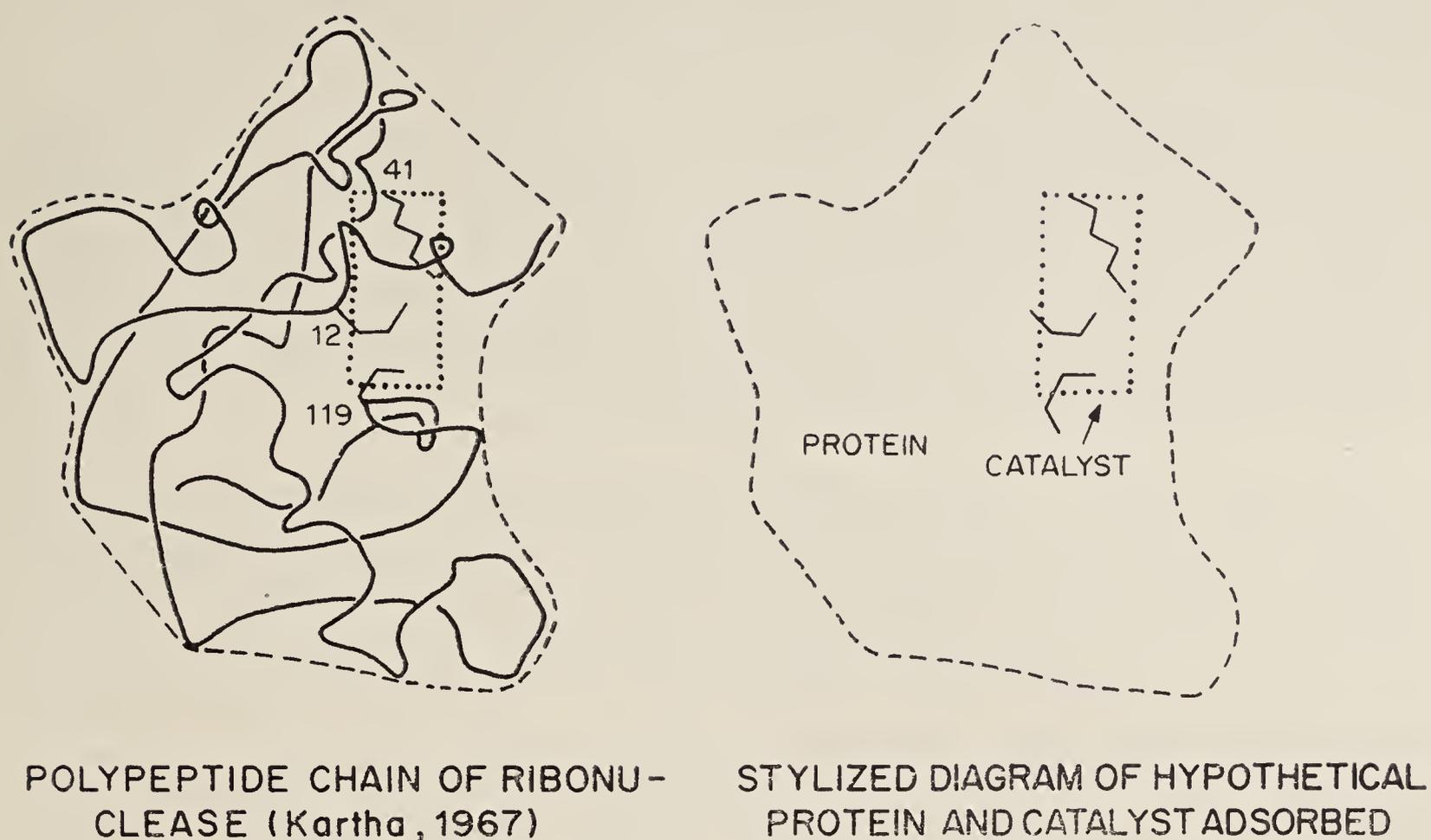


FIGURE 2. STYLIZED DIAGRAM OF ACTIVE SITE IN ENZYME POSITION

In a stylized diagram (figure 2), the protein as a carrier of a purported catalyst adsorbed on its surface is shown on the right and the same from the currently known structure of enzyme protein, ribonuclease, with its active site region determined by Kantha and coworkers is depicted on the left⁵. The spatial disposition of the amino acids in a relatively small region of the active site easily lends to the misconception of an extraneous catalyst adsorbed on the disproportionately large surface of the protein. Subsequently, through the efforts of Northrop and coworkers⁶ a number of evidences had been obtained to prove that the enzyme proteins are the true biocatalysts. A large number of enzymes have since been crystallized and their structures fully elucidated. The evidence on the protein nature of enzymes is so incontrovertible that if there were to be a discovery of a non-protein biocatalyst, one has to face greater opposition than even Sumner had to, to prove the point.

4. SPECIAL FEATURES OF ENZYMES

The special features of enzymes are briefly described below :

1. **RATE ENHANCEMENT:** Many concepts of heterogeneous catalysis are applicable to enzyme reactions. Enzyme protein in solution offers a pseudo-phase providing an interface. The adsorption of the substrate on the protein surface may involve considerable change in electron distribution as in a chemical reaction. Rate enhancement of several orders of magnitude have

been obtained for a variety of chemical reactions involving the bonds as shown in figure 1—all at ambient temperature and pH of living cells.

2. LOWERING OF ACTIVATION ENERGY: The rate constant of a reaction depends on the energy of activation. A catalyzed reaction achieves lower energy of activation by utilizing different activated species. For example, the energy of activation (K cal/mole) for decomposition of hydrogen peroxide decreases from 18.0 in uncatalyzed reaction to 11.7 and 5.5 with catalysts of platinum and the enzyme, catalase, respectively⁷.

3. SPECIFICITY: Most enzymes have remarkable specificity of the type of reaction in terms of bonds broken or made. A good number of them have a narrow requirement of the type of substrate molecule on which they act. Even a small difference of position of a single hydrogen atom can be discerned. Only one of the isomers is handled by enzymes. Some enzymes can process apparently symmetric substrate molecules in an asymmetric fashion. These considerations have led to the dictum of Fischer⁸ of “lock and key” and to the theory of Ogston⁹ of three-point attachment.

4. VERSATILITY: Enzymes are now discovered for a wide variety of reactions involving bonds of all known elements occurring in living cells. The list of known enzymes is over a thousand and is growing. It is axiomatic that an enzyme will surely be detected for each reaction found.

5. REGULATORY POTENTIAL: The activity of some key enzymes can be modulated by metabolites. This gives a regulatory potential to these enzymes to provide reaction rates commensurate with demands of metabolic activity.

6. UNIVERSAL STRUCTURAL STRATEGY: All enzymes are proteins of globular nature—an “oil-drop” model with hydrophobic residues turned inside and polar groups on the surface exposed to the medium.

7. FORMATION *de novo*: They are formed *de novo* within each cell by polymerization of the constituent amino acids of varying chain lengths. The linkage between amino acids is a peptide bond formed by condensation of α -carboxyl group of one amino acid with the α -amino group of the following. The linear polymer never uses the side-chain amino or carboxyl groups.

8. BIODEGRADABILITY *in situ*: After a certain life time—half-life ranging from hours to days—the enzyme molecule is degraded *in situ* to amino acids which are recycled. Thus, biocatalysts offer no residue problems.

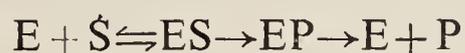
5. ACTIVE SITE

The first step in an enzyme reaction is binding of the substrate to the enzyme protein. Chemical approaches showed that this is achieved through the amino acid residues. The most important of these are histidine, serine, lysine, cysteine and aspartic acid. These provide groups of carboxyl, amino, thiol or imidazole juxtaposed in a small localized area of about 5 Å which is but a tiny fraction of the large surface area of the enzyme protein. The recent solutions of 3-dimensional structures of a number of enzymes obtained by the powerful technique of X-ray crystallography¹⁰ had further aided the identification of the active site regions. The small region of active site is appropriate in the sense that all enzyme reactions deal with either making or breaking a set of chemical bonds normally of the order of 0.9-1.5 Å in distance. But the events occurring after the complex of enzyme-substrate is formed and before the product is released, however, baffle understanding.

Model compounds with appropriately located amino acids of the active site *sans* protein do increase the rates but short of several orders of magnitude. Imidazole catalysis of hydrolysis of esters is a typical example¹¹. Rate enhancement of glycosidase type activity was obtained by suitably embedding γ -carboxyl group of glutamate in a hydrophobic peptide¹². But there appears something more intrinsic in the architecture of the bulk of protein which is ignored while the spot-light is turned on the trifle little of the active site. Can the competence of a gifted sculptor be delineated by merely describing the two hands that worked on the stone ?

6. MECHANISMS OF ENZYME-CATALYZED REACTIONS

The mechanisms proposed for explaining the rate enhancement by enzymes are based on the knowledge of catalysis in general and dealt with either the substrate (S) or the enzyme (E). The most widely used concept is developed by Michaelis and Menten¹³ who assumed the formation of an intermediate enzyme-substrate complex before the product (P) is formed :



The importance of ES complex is well recognized as the cornerstone in the understanding of the mechanisms. The following six are currently in vogue¹⁴ :

1. PROXIMITY : By collecting the substrate on a defined and specific complex on the protein surface, random collisions needed for reactions will be obviated resulting in decreased activation energy.

2. ACID-BASE CATALYSIS : A combination of nucleophilic and electrophilic species together on one molecule can produce catalytic rates larger than simple summation. This will result in "push-pull" situations with concerted catalysis on several positions of substrate. The classic example is provided by Swain and Brown¹⁵ who showed that 2-hydroxypyridine was 7000 times more effective than an equivalent mixture of pyridine and phenol in catalyzing mutarotation of α - to β -form of tetramethyl glucose. This remarkable effect is considered to be due to possession of both acidic and basic groups sterically arranged in the molecule to carry out concerted acid-base catalysis.

3. INTERMEDIATES : By designing covalent metastable intermediates such as Schiff's base, acyl- or phospho-serine, phosphoryl imidazole, the enzyme may very well obtain a pathway with overall lower activation energy.

4. ORIENTATION : It has been suggested that the atoms of the susceptible bond are aligned at the active site. This perfect orientation during anchoring of the substrate to the enzyme may lead to rate enhancement. An orbital steering model had been proposed¹⁶.

5. ENVIRONMENT : The globular proteins provide a hydrophobic interior which may aid the process of binding and formation and stabilization of transient states of the relatively apolar organic substrates. This theory is the only one which, even if remotely, makes use of the invariant feature of hydrophobic interior of the globular proteins. It is instructive to quote the remarks of Gutfreund and Knowles¹⁴ in this context : ".....the influence of solvent polarity on the strengths of hydrogen bonds and electrostatic interactions may be significant enough to provide enzymes with reaction pathways which are not feasible in aqueous solution".

6. STRAIN : The proximity and orientation themselves do not explain completely the catalytic phenomenon. If the substrate molecule has a strain which is released or is bent in the process of binding to the enzyme, the energy of the ground state and the rate of reaction may be enhanced. Strain and conformation change in enzyme catalysis had been discussed by Jencks¹⁷.

These are no more than six bland manoeuvres to seize an elephantine problem. The solution lies probably in all these put together and more.

7. ARCHITECTURE OF GLOBULAR PROTEINS

It is appropriate to recapitulate the basic structural features of globular proteins for comprehension of any cryptic significance. Enzymes are polypeptides arranged in specified sequences of amino acids, called the "primary

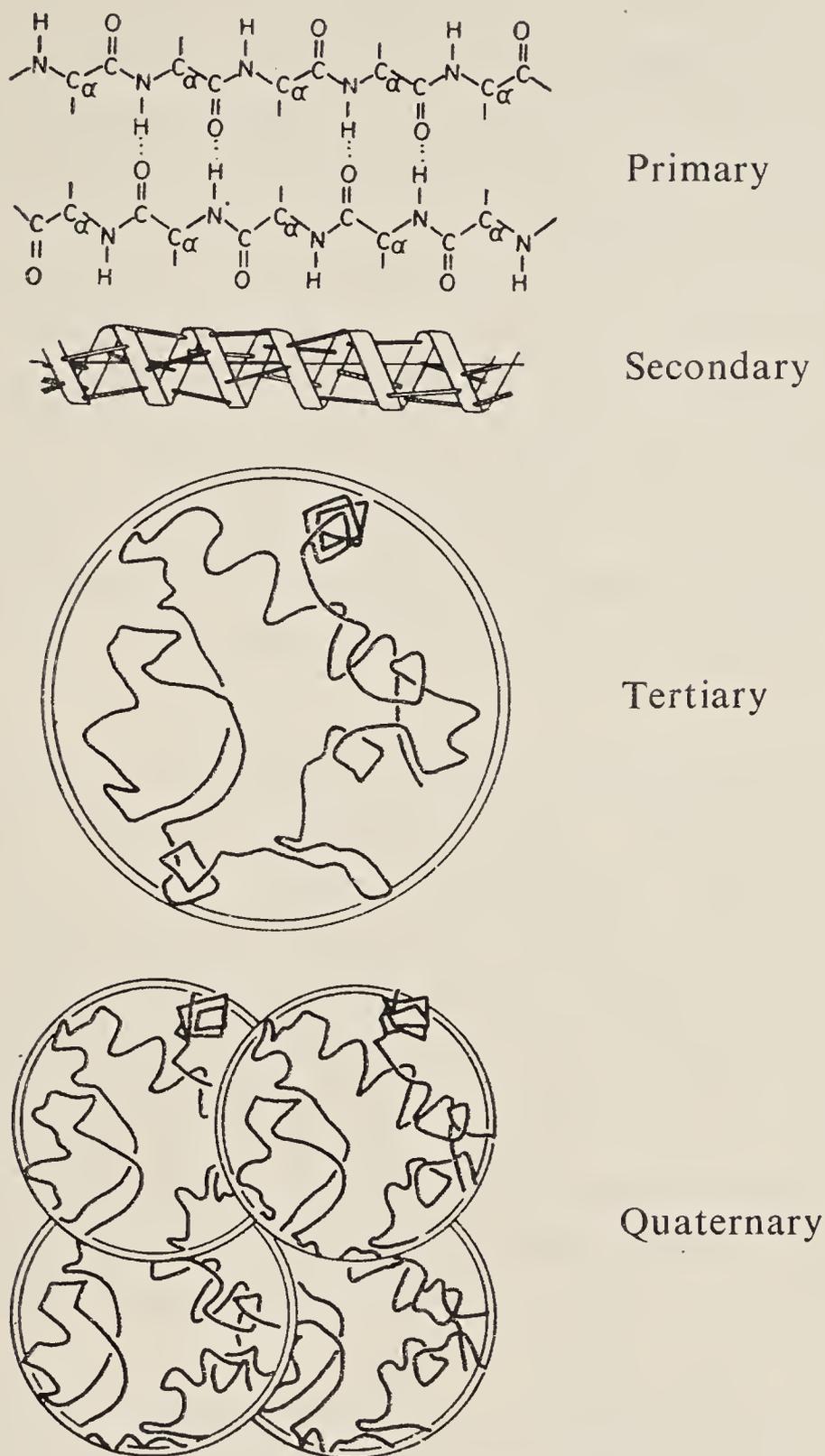


FIGURE 3. STRUCTURAL LEVELS OF PROTEINS

structure” (figure 3). Their size varies in the range of 124-10,000 amino acids units. Given 20 different amino acids and the size of 150 chain length, the number of different proteins that can be formed are astronomical and these can provide infinite possible conformations of active sites. Linear polymers take the forms of α -helix and β -sheets utilizing the capacity of hydrogen-bond formation between carbonyl and imino groups of peptide bonds, called the “secondary structure” (figure 3). Pauling^{18,19} proposed a molecular structure with a winding of, the then unconventional, non-integer helix of 3.6 amino acids per turn—the α -helix. This was found to be the principal structural feature in haemoglobin by Perutz^{19,20} and in myoglobin by Kendrew²¹.

α -helix is known to occur extensively, but not exclusively, in globular proteins. The pleated sheets, known as β -structure, are formed by hydrogen bonding between two chains of polypeptide running either parallel or anti-parallel to each other. The dispersal of the secondary structure throughout the protein becomes possible on account of the tendency of folding of the polypeptide over itself. The "tertiary structure" is a consequence of such foldings and has now become a structural dogma²² in globular proteins. The tertiary structure is stabilized by a variety of weak bonds and, in some cases, with covalent disulphide bridges, and is considered thermodynamically the most stable form to which the polypeptide will return to if uncoiled²³. A great number of enzymes has several identical, or non-identical, polypeptide subunits aggregated together, called the "quarternary structure" (figure 3) and these are active only in the multimeric form.

All the enzymes have globular architecture with hydrophobic interior and hydrophilic exterior. So far no catalytic activity comparable to enzymes is observed with any non-globular protein. The 20 amino acids provide side-chains with alluring properties—neutral, acidic and basic groups; polar and apolar chains; reactive groups such as C=O, N-H, S-H, O-H; hydrogen bonding capabilities; aromatic rings with π -electron clouds; different lengths in the range 1–12 Å from the α -carbon with varied rotational and translational possibilities. Thus, they can provide contours of variegated shapes and charges. These attractions of the functional groups at the active site on the surface had overshadowed the major portion of the protein molecule and had possibly diverted away attention from the unexplored depths of the protein.

No simple correlation exists between the presence and quantity of secondary structure and the nature and function of proteins. Both α - and β -forms occur in all enzyme proteins in the range of 6–42% individually and together in the range of 35–70% of the total amino acids (figure 4). No enzyme so far discovered is devoid of secondary structure, or has this as the sole structural feature. The folding in α -helix is always right-handed although left-handedness has no apparent disadvantage and occurs naturally in the structural protein, collagen²⁴. A considerable percentage of amino acids having the potential groups do form hydrogen bonding either with the main chain or side-chains. Is this the consequence or the purpose of tertiary folding? In this context it is interesting to cite the hypothesis proposed by Rao and Rossmann²⁵ that while amino acid sequences are variant, secondary structure in two related enzymes—dehydrogenases of lactate and glyceraldehyde-3-phosphate—was conserved as a common "super-secondary structure".

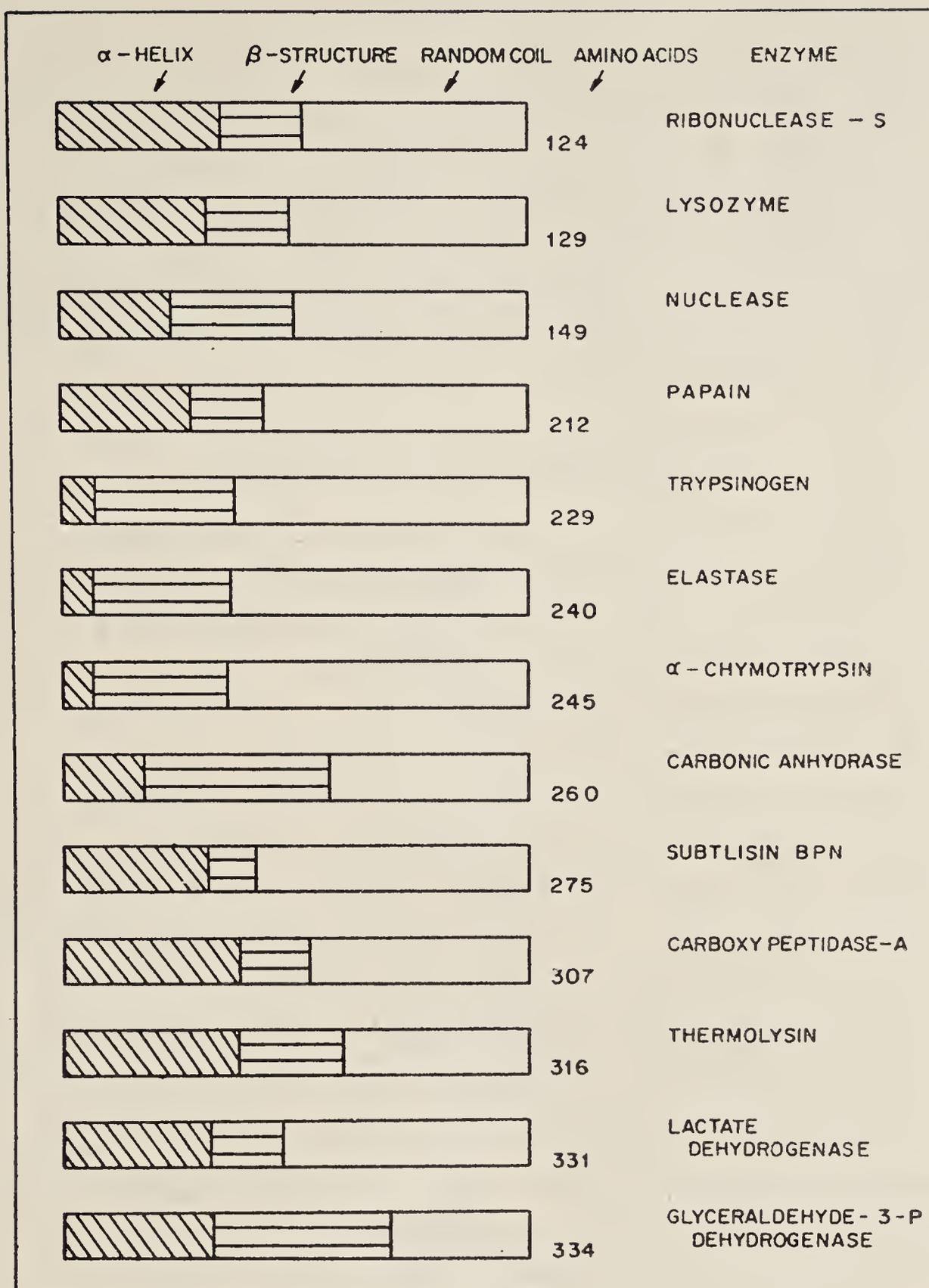


FIGURE 4. DISTRIBUTION OF SECONDARY STRUCTURE IN SOME ENZYMES

The other core structure of proteins is the peptide bond used for polymerization. The N-C=O bonds possess characters less than double bond at C=O and greater than single bond at N-C. As a result both bonds gain partial double bond character, and the electron is delocalized and occupies the entire structure, H-N=C=O. The resultant restriction of the rotation of N-C bond makes the peptide unit nearly planar, thus giving a plate-like structure between pairs of α -carbons carrying the side-chains. These have rotation possibilities, which are defined by the ϕ and ψ angles for the C_{α} -N and C_{α} -C respectively, described by Ramakrishnan and Ramachandran²⁶.

The rotation and flexibility of the polymer is not the requirement as a pseudo-polypeptide *sans* peptide linkages, using all-carbon polymer holding the side-chains cannot have even remotely the properties of proteins. The peptide bond alone is not the essential feature as nylon, with 4-6 carbons between peptide units, shows excellent structural but no catalytic properties²⁷. Nylon also possesses extensive hydrogen bonding similar to β -structure but is bereft of side-chains. Thus, for biocatalysis all the features seem to be required in one molecule: peptide bonds, hydrogen bonds, side-chain amino acids and the vital globular fold.

8. SEMICONDUCTION IN PROTEINS

Szent-Gyorgyi²⁸ brought to focus the need for the interactions at sub-molecular level in biology where the sum of parts falls short of the properties exhibited by the whole. He suggested a new hypothesis that proteins may behave as organic semiconductors by forming a network of peptide and hydrogen bonds and transfer electrons and energy. Measurements of electrical conductivity showed values in the range of about 2.8 eV for several proteins²⁹. In the theoretical calculations of delocalization of π -electron of peptide bond across a hydrogen bond, the energy gap of about 3 eV was obtained between highest filled and lowest empty bands³⁰. More refined calculations yielded figures upto 5eV³¹. It is now quite clear that delocalization of π -electrons in the system of ... HN..C..O...HN..C..O...is not excessive even after taking explicit account of the 2 P π -orbital of the bridge hydrogen since the electron population on various atoms is only slightly modified from that in the absence of hydrogen bond³². Various calculations on the 4- π -electrons in -NCO-system revealed a distinguishing feature that the energy of its lowest anti-bonding molecular orbital reasonably matches with that of a 2p- or sometimes 3d-orbital of the hydrogen atom. However, the electron of this system to be raised to the lowest empty orbital requires energy inputs higher than available in biological systems. It is instructive to note the life cycle of migrating electron in biology—it originates from oxygen of water during photolysis in chloroplasts, travels through a variety of cell components and structures and returns to oxygen during respiration in mitochondria. The electron, therefore, is extraneous to the protein structure. Using an extraneous electron, calculations of molecular orbitals showed that the entering electron is delocalized in the hydrogen-bonded peptide units with individuality of each unit being lost³³. Derivative to this is the possibility of electronic conductivities being higher in localized regions than the whole protein as now measured. Should this prove feasible, an *extraneous electron* can migrate within sections of polypeptide structures where such interlinked π -electron systems and hydrogen bonds exist.

9. SUPRAHELIX

A close examination of α -helix reveals that the intrachain hydrogen bonds themselves form interesting repeating sequences superimposed on the peptide helix. Each peptide group in the helix can be viewed as being held between two α -carbons and linked to another through a hydrogen bond. This structural feature provides continuous helical sequence of alternating peptide groups and hydrogen bonds (...HN..C..O...HN..C..O...) with the sense of winding opposite to that of peptide helix. There are three such sequences in α -helix. This intrinsic structural feature is called "suprahelix"³⁴ (figure 5). Each suprahelix provides such repeat sequences covering a linear distance of 27 Å for a full turn using a chain length of 18 amino acids.

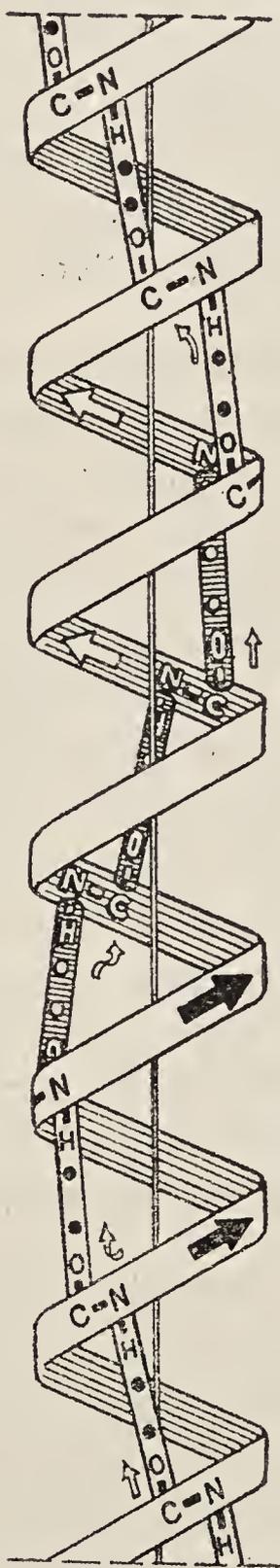


FIGURE 5. SUPRAHELIX

Is it possible that the invariant super-secondary structure conserved in evolution of dehydrogenases²⁵ utilizes in some manner this intrinsic structural feature ?

10. π -H PATHWAYS

In addition to the peptide units, delocalized π -electron systems are present in some side-chains of proteins—rings of phenylalanine, tyrosine, tryptophan and histidine, acid groups of aspartate and glutamate, amide groups of asparagine and glutamine and guanido group of arginine. Except for phenylalanine, others are involved in hydrogen bonding. In addition, the nitrogens of amino groups of lysine, glutamine and asparagine, and the oxygens of hydroxyl groups of serine and threonine form hydrogen bonds serving both as donors and acceptors. The hydrophobic interior seems to protect and aid formation of significant hydrogen bonding with side-chains besides the secondary structure. The combined networks of these interconnected hydrogen bonds and π -electron clouds obtained in proteins are referred, for operational convenience, as “ π -H pathways”.

π -H pathways are built of and are dependent upon the four principal structural features of enzyme proteins—peptide bonds, hydrogen bonds, side-chains and globular fold—an extraordinary coincidence. Spanning the entire sub-structure of each protein there are several of these pathways. If these π -H pathways gain a functional role the whole volume of the macromolecule becomes alive in the action. There has been persistent complacency that once the active site is defined we have explained all. The bulk of the protein is inconsequential except for sustaining the amino acids in proper orientation. This view had been forcefully stated by Monod³⁵ thus: “The catalytic step itself,.....need not detain us for long.....The belief today is that enzymic catalysis results from the inductive and polarizing action of certain chemical groupings present in the protein’s specific receptor”. Is the unfathomable design of the indispensable bulk of protein in enzymes meant for generating and sustaining π -H pathways ?

11. A MECHANISM OF CATALYSIS BY PROTEINS

All enzyme reactions are principally transfer reactions. They transfer one group to another, including hydrolytic reactions which transfer groups to elements of water. Essentially this means a redistribution of electrons from one atom to another. Implicit in this theme is the discharging or generating of an electron. An uncatalyzed reaction will be slow because the interactions are diffusion dependent and the active species may be dissipated in the aqueous medium. The primary function of the active site, no doubt, is to provide a

location to bind the substrate taking advantage of proximity, orientation, steric and electronic strain and potential for specific recognition. It is conjectured that the active site may also serve an additional purpose of extracting efficiently an electron from the donor atom of the bond to be split. This electron may then be held within the polypeptide framework and be transferred to another site with little loss of energy. Such inter- or intramolecular rearrangement of electrons between the concerned atoms may be achieved by using suitable π -H pathways characteristic to each protein (figure 6).

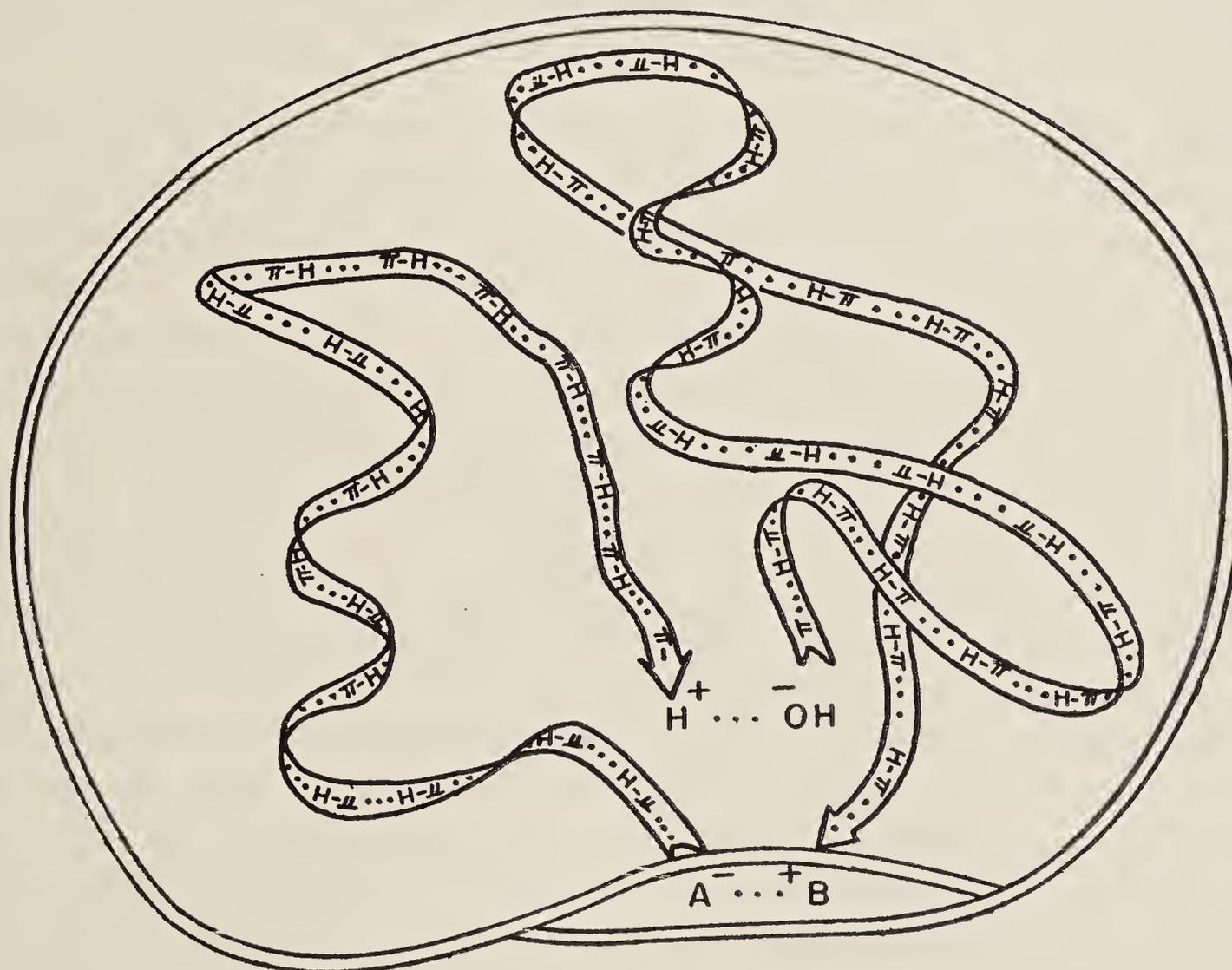


FIGURE 6. MODEL FOR ELECTRON REARRANGEMENT THROUGH π -H PATHWAYS

An analogy of machines is appropriate at this point. Each machine carries out specific jobs turning out different products. But the primary operation is carried out in each machine by the motor whose core function of circular motion drives other parts. It is conceivable that the π -H pathway may be the "motor" in the actions of proteins.

Several features of enzyme catalysis become self-evident on assuming the core function of π -H pathways: proteins gain catalytic power by their capability for extraction of electrons from the donor atoms, their mobilization within polypeptide framework and delivery to the acceptor atoms using π -H pathways; lowering of activation energy and rate enhancement and reactions at low ambient temperature are achieved because of retention of the electrons

within the protein with limited randomization: the pH effects can be explained as variations of charges in π -H pathways; loss of activity at higher temperature—an universal phenomenon with enzymes—may be due to break of hydrogen bonds in π -H pathways; the allostery is an alteration of π -H pathway at a distal location; subunit structure provides an intercalating π -H pathway; metals and coenzymes (with conjugated structures) activate by bridging the gaps in π -H pathways. Application of this hypothesis can hopefully provide an universal mechanism for biocatalysis and open new vistas in our understanding of functional proteins. Admittedly, all these are speculative but the theory which can explain the unique design of enzyme proteins and their diverse features is worthy of further consideration.

While proposing this model, I am fully aware that the acceptability will largely depend on the conduction of an electron by hydrogen bond. The present mood of hope and disappointment on this aspect had been eloquently presented by Cope³⁶: "Sceptics will say that this evidence is inadequate to prove the hypothesis of electron-conduction enzyme, and that direct measurements should be made before indulging in such excessive speculation. Unfortunately, the prospect of direct experimental measurement of resistivity within enzyme molecules seem dim for the immediate future.....So let us not become discouraged if direct evidence for electron conduction in enzymes is not immediately at hand. The concept and theory may nevertheless prove useful." Hydrogen bonds form the basic foundations of life processes, in water structure and in keeping fidelity, storage, and transfer of genetic information. Will this innate structural feature of hydrogen bonding have no role in functional proteins? It is appropriate to conclude this article with the prophetic words of Linus Pauling³⁷: "It has been recognized that hydrogen bonds restrain protein molecules to their native configurations, and I believe that as the methods of structural chemistry are further applied to physiological problems it will be found that the significance of hydrogen bond for physiology is greater than that of any other single structural feature".

ACKNOWLEDGEMENTS

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Biological nitrogen fixation

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I. INTRODUCTION

In view of its importance in agriculture, research work in the field of biological nitrogen fixation has been in progress since the isolation of nitrogen fixing bacteria in the late nineteenth century.

In the period after 1960 important strides have been made in the study of the actual enzyme systems involved in the fixation of atmospheric nitrogen to cellular constituents by various procaryotic cells. So far there is no conclusive evidence of eucaryotes possessing the above property.

The knowledge of the biochemical mechanism of nitrogen fixation would be of value in the development of inorganic catalysts capable of reducing nitrogen to ammonia (fertilizer) at ambient temperature and atmospheric pressure.

Several specialised reviews¹⁻¹⁷ and books¹⁸⁻²⁰ have appeared on the subject during the last 7 years. The subject matter for this review has been mostly drawn from the above. Few references have been quoted. It is hoped that the following pages would serve as an introduction to those who are unfamiliar with the literature on the subject. Research work appearing after 1970 has been summarised in more detail.

A good amount of pioneering work in the field was done at the University of Wisconsin by Professors P. W. Wilson, R. H. Burris and their colleagues during 1930-60 including the demonstration of first products of nitrogen fixation as ammonia, the development of the sensitive N^{15} incorporation technique, and the non-specific nature of nitrogen-fixing enzymes. These are some of the studies which have laid the foundations for the recent developments.

Biological nitrogen fixation is defined as the reduction of atmospheric N_2 to ammonia by microorganisms or cell-free extracts. This reaction is

performed by several species of living bacteria in soil as well as in the roots of leguminous plants in association with the rhizobia.

Till 1960, scores of unsuccessful attempts were made to obtain consistent nitrogen fixation in cell-free extracts. The main organisms used for study at that time were the aerobe *Azotobacter vinelandii* and the anaerobe *Clostridium pasteurianum*. It was only in 1961 that Mortensen and coworkers at Du Pont laboratories succeeded in obtaining consistent nitrogen fixation in crude extracts of *Clostridium pasteurianum* by adding large quantities of pyruvate to the extract. When this crude cell-free extract was passed through a DEAE-cellulose column a dark green-brown coloured protein was firmly absorbed on the column, allowing its easy purification. This protein contained 7-8 atoms of iron and equivalent number of labile sulphide atoms per molecular weight of 6000 and was named ferredoxin. The protein could be separated from the rest of the extract and was found to be essential for reduction of nitrogen with pyruvate. This breakthrough marked the beginning of detailed studies on the characterization of the enzymes and electron transfer proteins involved in the reduction of molecular nitrogen to ammonia.

In a subsequent development the extract was further fractionated and reduction of nitrogen to ammonia obtained with hydrogen and hydrogenase fraction, extract (minus ferredoxin), ferredoxin, ATP and Mg^{2+} . Thus, it was shown that once ferredoxin was reduced it could transfer electrons to nitrogenase with ATP and Mg^{2+} as cofactors.

The next finding of major importance was the fact that the electron donor system $H_2 +$ Hydrogenase from *Cl. pasteurianum* could donate electrons for nitrogen reduction with *Azotobacter* crude extract, ATP and Mg^{2+} . Even though *Azotobacter* was a strictly aerobic organism, no oxygen was required for nitrogen reduction. For the first time the complete anaerobic nature of the nitrogen fixation reaction was established.

As the techniques of enzyme purification under completely anaerobic conditions were perfected, the nitrogen fixing enzymes viz., the nitrogenases were purified as two components, Nitrogenase I and II. To simplify the nitrogen fixation assay work, the whole electron donor system was substituted by an inorganic reducing agent sodium hydrosulphite. The introduction of the acetylene reduction technique as an assay for nitrogenase made fractionation, purification and kinetic studies on nitrogenase much easier³. By 1968 it was definitely established that the nitrogen fixation reaction needed an electron donor, Nitrogenase I and II, ATP, Mg^{2+} and substrate which could be N_2 or any of the non-specific substrates like C_2H_2 , N_3^- , CN^- , N_2O or CH_3-NC . These developments have been tabulated in Table 1.

Table 1 DEVELOPMENTS SINCE 1960 WITH N₂ AS SUBSTRATE

In *Cl. pasteurianum*

Pyruvate + Crude Extract ————— NH₃ 1961

Pyr. + (Extract Minus Ferredoxin + Fd ————— NH₃ 1962
(Discovery of Ferredoxin)

(H₂ + Hydrogenase + Fd_{ox}) + (Ext - Fd) + ATP + Mg²⁺ — NH₃

In *Azotobacter vinelandii*

H₂ + Hydrogenase } + AZO. EXT. + ATP + Mg²⁺ — NH₃ 1965
from *Cl. past*

Nitrogen Fixation was completely anaerobic process

Na₂S₂O₄ + Azo. or Clos. Ext. + ATP + Mg²⁺ ————— NH₃ 1966

Na₂S₂O₄ + Nitrogenase I + II + ATP + Mg²⁺ ————— NH₃ 1967-1968
/ \
C₂H₂ C₂H₄

Various techniques developed during the period

- | | |
|--------------------------------|------------------------|
| (1) Anaerobic enzyme isolation | (2) Ammonia estimation |
| (3) Acetylene reduction | (4) ESR spectroscopy |
| (5) Iron sulphur proteins | |
-

2. THE ELECTRON DONATING SYSTEM

The electron donating system represents the electron transport chain leading to reduction of ferredoxin or flavodoxin. These two proteins are the final donors of electrons to the nitrogenases.

The properties of the electron carriers and ancillary proteins :

THE IRON-SULPHUR PROTEINS

These proteins were unknown till 1958. Earlier studies had indicated the presence of excessive quantities of iron other than accounted by heme containing proteins in preparations of succinic and NADH dehydrogenases from mitochondria and cytochrome fractions of *Azotobacter*. Subsequently pioneering work at The Institute for Enzyme Research, Madison by Professor H. Beinert and colleagues resulted in the discovery of an asymmetric Electron Spin Resonance (ESR) signal at $g=1.94$ in pure preparations of succinic and NADH dehydrogenases (when reduced with substrate) from

beef heart mitochondria. These preparations were free of heme content. The role of iron in the production of the ESR signal was proved by the demonstration of significant broadening (due to nuclear hyperfine interaction) of the ESR signal when the predominant Fe^{56} was substituted by Fe^{57} in the *Azotobacter* cells and the non-heme iron fraction isolated and examined. Using a similar isotopic substitution method sulphur was shown to be a part of the structure responsible for the ESR signal at $g=1.94$. In view of the presence of sulphur as ligand of iron, these proteins have been designated iron-sulphur proteins. The main reason for their not being discovered earlier was their small and featureless absorption in the visible and uv regions compared to the high and sharp extinctions seen with the hemoproteins. Simultaneously, the discovery of ferredoxin caused a spurt of activity in the field of biological nitrogen fixation and iron-sulphur and related proteins.

PROPERTIES COMMON TO IRON-SULPHUR PROTEINS

The active center which reduces and oxidises consists of Fe and S^- known as labile sulphide. These sulphur atoms are different from the SH group of cysteines which are also present in positions adjacent to Fe. In all the iron-sulphur proteins the iron and labile sulphide are liberated upon acidification of the protein. An interesting property of these proteins is that if the apoprotein was kept anaerobic with mercaptoethanol the iron and labile sulphide could be reincorporated upon adjusting the pH to neutral. This reconstituted protein also regains the original biological activity. All the iron-sulphur proteins have low oxidation reduction potentials and are characterised by different ESR signals which have helped in their isolation and identification.

IRON SULPHUR PROTEIN TYPE I

This type refers to the protein having 2 Fe : 2 S^- per molecular weight of about 21,000 and showing an ESR signal at $g = 1.94$ on reduction. These have been isolated and characterised from *Azotobacter vinelandii*, *Cl. pasteurianum*, *Pseudomonas putida* and adrenal glands.

The $g = 1.94$ signal is also shown by several flavin enzymes including succinic and NADH dehydrogenases, xanthine oxidase, dihydroorotic dehydrogenase in the presence of their respective substrates. The optical and ESR spectrum of this type of iron-sulphur protein can be traced from review¹⁴.

The biological function of the isolated iron-sulphur proteins from *A. vinelandii* and *Cl. pasteurianum* is not known. The putidaredoxin and

adrenodoxin function in the hydroxylation of camphor and steroid respectively. Both the Type I and Type II (below) iron-sulphur proteins accept one electron on reduction.

IRON-SULPHUR PROTEIN TYPE II

This also contained $2\text{Fe} : 2\text{S}^-$ per molecule and the oxidised optical and reduced ESR spectrum was similar to the Type I. The main differences are in the absorption maxima of the oxidised protein and the higher temperature sensitivity⁶ and shape of the ESR spectrum around the $g = 2.0$ region. This type of iron-sulphur protein is represented by the spinach or chloroplast ferredoxin which functions in photosynthesis and iron-sulphur protein II of *Azotobacter*. The above two types of iron-sulphur proteins do not directly play a role in biological nitrogen fixation.

IRON-SULPHUR PROTEIN TYPE III

This is represented by the bacterial ferredoxins. These have a molecular weight of approximately 6,000 daltons with 7-8 atoms of iron and equivalent S^- per molecule. Ferredoxins have been isolated from several *clostridia*, facultative aerobic bacteria and all nitrogen fixing organisms. The strict aerobe *Azotobacter vinelandii* has two such ferredoxins (I and II) with a molecular weight of 14,500 and 6,000 respectively. The facultative aerobe *Bacillus polymyxa* has a ferredoxin with 4Fe and 4 S^- atoms in a molecular weight of 9,400. The optical absorption spectra of these ferredoxins are featureless showing a shoulder at 400 $m\mu$ wavelength. On reduction two electrons are accepted. The ESR spectra are similar in shape to the Type II iron-sulphur proteins but much more temperature sensitive. Liquid helium temperatures of -260°C were required to show the ESR spectra of these proteins. The ferredoxins are characterised by a low oxidation-reduction potential of -420 mv and function as final electron donors to nitrogenase in the nitrogen fixation reaction, and also in CO_2 fixation cycles in photosynthetic anaerobes.

The amino-acid sequence of several ferredoxins (6,000 mw) have been determined.

The 1.5 Å resolution X-ray crystallographic structure of a ferredoxin from *Micrococcus aerogenes* has been determined. Two cubical clusters containing $4\text{Fe} : 4\text{S}^-$ have been found in the molecule. The properties of these iron-sulphur proteins have been discussed in detail in references 6 and 14.

The reduced form of the *Cl. pasteurianum* ferredoxin showed 3 polarised Raman lines using the helium-neon laser beam as the incident light. These lines disappeared on reoxidation of the protein²¹.

NITROGENASE I AND II

These are the iron-sulphur proteins responsible for the reduction of molecular nitrogen to ammonia in presence of an electron donor, ATP and Mg^{2+} .

Nitrogenase I was an $nFe : 2Mo : nS^-$ containing protein whose molecular weight ranges around 200,000 according to the species. Nitrogenase II contained 2Fe and 2S⁻ with a molecular weight around 50,000. Similar nitrogenases have been isolated and characterised from all nitrogen-fixing bacteria so far studied. Given an electron donor and cofactors the nitrogenases I and II can be interchanged (with a few exceptions) from various species for the reduction of nitrogen to ammonia.

These nitrogenases are extremely sensitive to oxygen. Upon exposure to air, in one or two minutes 50 percent of the activity was lost. This was the main reason for the delay of several years in accurately characterizing the proteins. The isolation and handling requires completely anaerobic conditions including DEAE-cellulose chromatography and sephadex gel filtration. On contact with air these proteins are irreversibly denatured. Reconstitution has not been possible. ESR signals have been shown for the nitrogenases at $g = 1.94, 3.67$ and 4.3 . The increase in signal $g = 3.67$ has been used to monitor derepression of nitrogenase in intact cells of *Azotobacter* when combined nitrogen was exhausted in the medium. Detailed properties of nitrogenases could be found in the reviews mentioned ^{15,16}.

There are two more non-heme iron proteins, Rubredoxin (two types) and High Potential Iron Protein (HIPIP) isolated from several aerobic and anaerobic bacteria and the photosynthetic organism *Chromatium* respectively. These proteins have only Fe but no labile sulphide. Their biological function was unknown.

FLAVODOXINS

These refer to flavoproteins which substitute for ferredoxin in transferring electrons to nitrogenase. They are FMN containing proteins and are present in all nitrogen-fixing microorganisms. The average molecular weight in all species was around 23,000. The flavoprotein from *Azotobacter vinelandii* which has been shown to have flavodoxin function has unique properties. On reduction with sodium dithionite or NADH or NADPH with a reductase fraction from the same organism, this flavoprotein accepts one electron and forms quantitatively a stable anion type blue semiquinone. This unique property has been of immense value in studying the flavin-protein interactions.

It has been shown conclusively by Yates²² that this flavoprotein transfers electrons to nitrogenase during the oxidation of completely reduced form of this flavoprotein to the semiquinone form. However the mechanism of complete reduction of this flavoprotein in the *A. vinelandii* cell is yet to be elucidated.

3. THE PATHWAYS OF ELECTRON TRANSPORT TO NITROGENASE IN AEROBIC AND ANAEROBIC BACTERIA

In *Clostridium pasteurianum* the phosphoroclastic split of pyruvate provides the reduced ferredoxin and ATP required for the reduction of nitrogen. This is well established.

In the strict aerobes, however, it is surmised from indirect evidences that a combined action of several dehydrogenases, particularly isocitrate dehydrogenase generates high concentrations of NADH or NADPH which causes reduction of ferredoxin or flavodoxin. It should be pointed out that the oxidation-reduction potential of reduced pyridine nucleotides was higher than that of reduced ferredoxin. Hence electron flow is envisaged against the potential gradient. It is suggested that a very high ratio of reduced versus oxidised pyridine nucleotides can cause such a reversal of electron flow. There have been suggestions of the presence of phosphoroclastic enzymes in *Azotobacter*. However, these reports have yet to be confirmed^{9,20}.

4. THE MECHANISM OF NITROGEN-FIXATION

The ideas concerning the mechanism of nitrogen reduction have been derived from studies on the properties of nitrogenases, kinetic data on the reduction of nitrogen and nonspecific substrates and analogies drawn from studies on inorganic compounds. The main hypothesis is that there are two sites on the nitrogenase enzyme. On site one electron activation takes place and on site two step by step reduction of nitrogen takes place. In the absence of substrate, nitrogenase shows the ATP-dependent hydrogen evolution which is not inhibited by carbon monoxide. Whereas the actual reduction of nitrogen was inhibited by CO. This differential inhibition gave rise to the two site hypothesis. The reduction pattern of all the non-specific substrates indicated transfer of electrons in pairs. An inorganic complex of platinum showed the deuterium exchange reaction similar to that shown by the nitrogenase. This platinum complex was also reduced by addition of two electrons at every step. Using these facts and analogies it has been suggested that nitrogen is reduced to ammonia *via* diimide and hydrazine. However, these intermediates have not been detected so far and are probably enzyme bound¹².

5. INORGANIC ASPECTS OF NITROGEN-FIXATION

The knowledge gained from the enzymes involved in biological nitrogen-fixation has stimulated several groups of inorganic chemists to attempt making exotic catalysts which could fix nitrogen at atmospheric pressure and ambient temperature. The literature on this aspect can be found in ref. 4. Several metal complexes have been synthesised which can bind molecular nitrogen. However the catalyst itself gets decomposed when reduced to form ammonia. Van Temelen and collaborators²³ have obtained very low yields of ammonia from molecular nitrogen by using a molybdenum coordination compound and an iron complex having the cubicle iron configuration similar to ferredoxin from bacteria (iron-sulphur protein Type III). Joseph Chatt and collaborators believe that only one metal was involved in the reduction of nitrogen. Recently²⁴ they have synthesised several complexes with tungsten and iron and obtained high yields of ammonia and hydrazine from nitrogen. However, the catalyst was not recoverable after the reduction.

6. GENETICS AND REGULATION OF NITROGENASE

The genetics and regulation of nitrogen fixation has been the focus of interest of many workers in recent years. The knowledge in the field is of particular significance especially with the objective of expressing *nif* genes (genes which are responsible for the synthesis of nitrogenase components) in non-leguminous plants such as cereals. Though this is a far reaching aim, considerable progress has been made in recent years on the charactersation and regulation of *nif* genes. The following account summarises some of the ideas which have been discussed in the reviews published recently.²⁵⁻²⁷

Nif GENES

The crucial genes responsible for nitrogen fixation have been mapped in *Klebsiella pneumoniae*, (a closely related genus to the well studied *E.coli*) as clustered on a small segment of chromosome²⁸⁻³⁰. Though a definite number of structural genes cannot be specified, a minimum of three genes are obvious for *C. pasteurianum* and *K. pneumoniae*, two for *R. lupini* and *R. japonicum*, one for Rjl and Rlj. The number of regulatory genes are not well defined³¹. A common genetic determinant for nitrate reductase and nitrogenase has been suggested³².

Genetic analysis has been carried out by transduction and conjugation. Bacteriophage P1 can act as a generalised transducing phage for nitrogen fixing strain of *K. pneumoniae*. The transductional analysis revealed that several *nif* genes are located on the genetic linkage map of *Klebsiella* near histidine

(*his* - genes responsible for enzymes for histidine biosynthesis) operon²⁸. Simultaneously it has been reported that the chromosome transfer mediated by a derepressed *R* factor in the nitrogen fixing strain of *K. pneumoniae* M5a1 through conjugation from wild type *K. pneumoniae* (*nif*⁺) to mutants defective in nitrogen fixation (*nif*⁻)²⁹. The intergeneric transfer of *nif* from *K. pneumoniae* to *E. coli* has been demonstrated³⁰. From the above studies it has been concluded that *his* is a marker close to *nif* genes of *K. pneumoniae*²⁸⁻³⁰. The expression of the transferred genes are repressed by ammonia, showing that the regulatory genes are also being transferred.

Further studies using cotransductional mapping revealed that *nif* genes are located between histidine and shikimate permease (Shi. A) genes in *Klebsiella* though the exact location has yet to be worked out³³. Plasmid (extrachromosomal DNA) that codes for nitrogenase has been reported³⁴. *E. coli* hybrids with *nif* integrated into the *E. coli* chromosomes or in the form of plasmids have been obtained^{34,35}.

From the genetic map of *nif* mutation it seems that there are at least 4 *nif* genes linked to *his* D and two *nif* genes unlinked. At least 3 or 4 structural genes and 2 or 3 regulatory genes are required for expression of nitrogenase. For an optimum control one operon should code for both subunits of a single component³⁶.

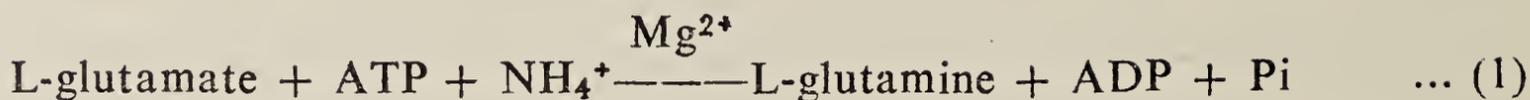
REGULATION

Ammonium ion NH_4^+ , the product of the nitrogenase reaction represses the synthesis of nitrogenase³⁷. On the basis of kinetic studies in *Azotobacter vinelandii* a coordinated repression and derepression of both components of nitrogenase have been reported³⁸. In *Cl. pasteurianum* the situation is different. Both by activity measurement and serological tests it is shown that the synthesis of nitrogenase component I precedes that of component II³⁹. Evidences in *A. vinelandii* suggest that structural genes for both I and II are located in a cluster on the chromosome and they are under the control of one common operator gene and thus respond coordinately to the same regulatory elements. But evidence in *Clostridium* indicates that genes for I and II may not be contiguous, may not be linked and may not have a similar operator,³⁹ In *Klebsiella* also, ammonia repression occurs at the transcription level, rather than the translational level⁴⁰.

RELATION BETWEEN GLUTAMINE SYNTHETASE (GS) AND NITROGEN FIXATION

Recent studies have shown that Glutamine synthetase plays a role in the regulation of the synthesis of nitrogenase.

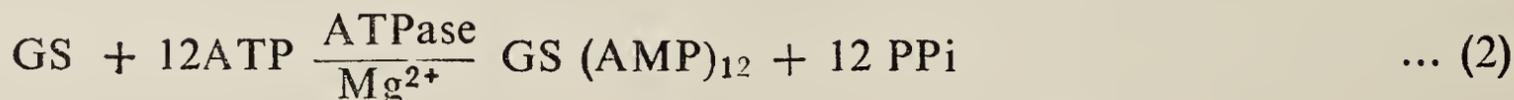
GS catalyses the following reaction :



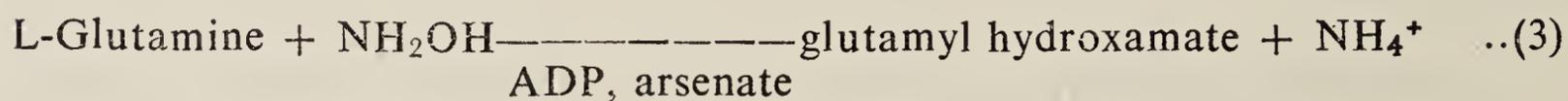
This enzyme plays a role in the nitrogen metabolism of several microorganisms. The enzyme has a mol.wt. of 600,000 daltons consisting of 12 identical subunits, each of molecular weight 50,000. A specific tyrosyl residue of each of the twelve subunits can be adenylylated enzymatically to form a 5-adenylyl O-tyrosyl derivative. Adenylylation and deadenylylation are catalysed by a cascade type of enzyme system.

There are four different mechanisms of cellular control of GS viz. (1) repression (2) Divalent cation specificity (Mg or Mn) (3) feed back inhibition (4) Adenylylation - Deadenylylation. These have been discussed in detail⁴¹. Here we shall focus on the last aspect.

Both adenylylation and deadenylylation reactions are catalysed by adenylyltransferase in the presence of Mg^{2+} or Mn^{2+}



Apart from the reaction (1) the enzyme catalyses the γ -glutamyl transfer reaction -



Using the difference in the divalent cation specificity in the biosynthetic reaction and that in the γ -glutamyl transfer reaction, a relationship for measuring the average state of adenylylation of GS has been derived⁴¹.

$$n = 12 - 12(b)/(a) \quad \dots (4)$$

n varies from 0-12 moles 5'-adenylyl groups/mole of active enzyme.

(a) is the measure of total transferase activity of both adenylylated and unadenylylated subunit in the presence of 0.3 mM Mn^{2+} at pH 7.15.

(b) is the measure of the transferase activity of only unadenylylated subunits in the presence of 0.3 mM Mn^{2+} + 60 mM Mg^{2+} at pH 7.15.

In the transferase activity assay the presence of 60 mM Mg^{2+} completely adenylylated subunits from expressing this activity. This has been used to find out the extent of adenylylation.

When intact *E. coli* cells grown on glutamate were suddenly confronted with ammonium, glutamine synthetase activity fell down to about 25% of the initial activity within 2 minutes. This is due to the inactivation of GS by adenylation⁴². The role of GS as a regulatory protein (apart from its own catalytic function) was shown by Boris Magasanik and his group from MIT. In *K. aerogenes*, degradative enzymes like β -galactosidase, histidase, and proline oxidase are subject to catabolite repression by glucose in the presence of an additional nitrogen supply and this repression is relieved by cyclic AMP. But under the conditions of nitrogen limitation the catabolite sensitive enzyme that degrade histidine and L-proline are relieved in c.AMP-strains while β -galactosidase remains repressed unless cyclic AMP is given, suggesting a cytoplasmic factor other than cyclic AMP can stimulate transcription of histidine and proline utilization genes in *K. aerogenes*⁴³. The cytoplasmic factor was later shown to be GS⁴⁴. In the *in vitro* transcription system, the ability of deadenylylated GS to activate the transcription of *his* (histidine utilization) DNA has been demonstrated, which is similar to the positive control system that has been ascribed to cyclic-AMP and cyclic-AMP binding protein⁴⁵.

Similar to the above system, GS appears to act as a positive control element for nitrogenase synthesis from the following evidences :^{46,47}. (1) Glutamine requiring mutant that lack catalytically active glutamine synthetase, cannot synthesise nitrogenase. A mutant in which there is an alteration in the regulation of GS had lost its ability to grow on a large number of compounds as sole nitrogen source and failed to synthesise nitrogenase components. (2) *nif* genes can be transferred to GS constitutive mutant of *K. aerogenes* resulting in constitutive expression of *nif*. (3) When an episome F:133 carrying GS genes are introduced into Gln A⁻ mutants they can synthesise nitrogenase components. Glutamine auxotrophs of *K. aerogenes* have lesions which map at two separate loci. Gln A and Gln B. Most revertants of GlnB strain produce glutamine synthetase constitutively (Gln C⁻) which have a second mutation at a site cotransducible with Gln A⁴⁴. (4) Partial derepression of nitrogenase was obtained by the introduction of a F'*nif* DNA into Gln C⁻ (constitutive) mutant of *K. aerogenes*, in a medium containing NH₄⁺.⁴⁸

A working model of the role of GS as a positive control has been proposed⁴⁹. Adenylation blocks the binding of GS to the *nif* promoter. Deadenylylation leads to the binding of GS and subsequent activation of *nif* transcription. The presence of NH₄⁺ ions may cause the adenylation of GS and thus blocking the activation of *nif* transcription.

Recently, it has been shown that NH₄⁺ is not the actual repressor of the nitrogenase, because nitrogenase synthesis occurs even in the presence of excess

NH_4^+ if L-methionine sulfone (MS) or methionine sulfoximine (MSX) are present in the medium⁵⁰. MS and MSX are glutamate analogues which have been shown to inhibit glutamine synthetase and glutamate synthetase. From the above experiments, it has been concluded that the actual effector of the repression is either glutamate or glutamine. Mutants that fix nitrogen in presence of NH_4^+ have been reported⁵¹. It has been suggested that the mutation is at operator - promotor regions²⁶.

A strain of *Klebsiella* (*K. pneumoniae*, M524) synthesises nitrogenase, even in the presence of NH_4^+ to 65% level compared to the activity in the absence of NH_4^+ . In this strain GS is constitutively synthesised, but it is glutamic dehydrogenase negative. This strain excretes large amount of fixed nitrogen as NH_4^+ .⁴⁹ The authors speculate the use of such a strain for the microbial production of NH_4^+ , at the expense of cheaply available energy sources, such as cellulose or molasses.

Evidence indicates the presence of a cofactor common to all molybdo-proteins including nitrogenase I, with a molecular weight of less than 100. This factor is not detected in NH_4^+ grown cells unless the glutamine synthetase inhibitor, methionine sulfone is added. The role of this factor is not clearly understood^{27,52}.

7. IMMEDIATE INHIBITORY EFFECT OF NH_4^+ ON NITROGENASE

Apart from the repression of nitrogenase biosynthesis, NH_4^+ has an immediate inhibitory effect on nitrogenase activity in aerobes like *Azotobacter*^{37,53,54} and *Anabaena cylindrica*⁵⁵. This is not observed in the anaerobic organism *Klebsiella pneumoniae*. Several possibilities like rapid synthesis and turnover of the enzyme⁵⁴, destruction⁵⁶ and feed back inhibition⁵⁷ have been suggested.

We have reported⁵⁸ that this inhibition is a reversible phenomenon in case of *Azotobacter vinelandii*. Even though excess of ammonium acetate inhibits nitrogenase activity immediately, the *Azotobacter* cells continue to reduce acetylene for more than two hours in the presence of chloramphenicol and rifampicin at the concentrations at which they inhibit protein synthesis and RNA synthesis respectively. Cells grown in nitrogen free medium and treated with ammonium acetate (20 mM) inhibited nitrogenase activity. When these cells were washed free of ammonia, the acetylene reduction was observed even in the absence of new protein synthesis. A short term controlling mechanism including substantial alteration of the paramagnetic centre of nitrogenase component I without the elimination of antigenic moiety has been observed⁵⁹. Ammonia inhibition on nitrogenase function in subcellular

preparation of *A. vinelandii* has been shown⁶⁰. Further disruption of the preparation by freeze thawing abolished the effect. But the extent of inhibition is not more than about 30%. Since the reversible inhibition that occurs *in vivo* does not occur in the cell free extracts, it has been suggested that the inhibition is due to the competition for reductants and (or) ATP between nitrogenase activity and assimilation of ammonia⁶¹. In *E. coli* it has been reported that NH_4^+ causes a burst of glutamine synthesis imposing a drastic drain of ATP⁶². The studies on *Anabaena cylindrica* shows that oxygen is essential for NH_4^+ to exert this activity⁵⁵. In *Azotobacter* this inhibition is not observed when glutamine analogues are present in the medium. Whether the inhibition is due to the rapid drain of ATP and (or) reducing power, or ammonia causing a change (as detected in EPR) is yet to be answered. If the first idea is correct, mutants that fix nitrogen in presence of NH_4^+ must lack glutamine synthetase as suggested²⁶. The other suggested regulatory mechanisms for nitrogenase include (1) control by ATP/ADP ratio⁶³⁻⁶⁵; (2) respiratory control in *Azotobacter*^{65,66}, where respiratory protection occurs against oxygen inactivation; (3) conformational protection of nitrogenase due to a subcellular compartmentation of nitrogenase. These have been discussed in detail⁶⁷. Evidences indicate that conformationally unprotected enzyme is both active and damaged by oxygen though the possibility of uncompetitive interference by oxygen in electron transport to nitrogenase is not excluded⁶⁸.

8. NITROGEN FIXATION BY FREE LIVING RHIZOBIA

For a long time it was thought that rhizobia cannot fix atmospheric nitrogen independently. Several workers have failed to demonstrate the ability of cultured Rhizobia to fix nitrogen either freely or with plant extracts.

Dilworth and Parker⁶⁹ have suggested that some genes determining *Rhizobium's* ability to fix nitrogen were coded by the plant host. But the first evidence to show that *Rhizobium* possesses complete *nif* genes came from the work of Duncan and Tierney⁷⁰ who have transferred *nif* genes from *R. trifoli* to a *nif*⁻ strain of *K. aerogenes*.

Bishop *et al*⁷¹ have detected Mo-Fe component of nitrogenase from free living *Rhizobium* by immunological cross reactivity against Mo-Fe component prepared from soybean root nodule bacteroids. Trinick⁷² has reported a nitrogen fixing association between a slow growing *Rhizobium* and a nonleguminous plant of the genus *Trema*. Strains of cowpea type of *Rhizobium* fixed nitrogen in the presence of callus of nonleguminous plants, and also continue to fix nitrogen up to 12 hr after callus was removed^{73,74}.

Thus it has been concluded that the callus was providing a diffusible substance which permitted nitrogen fixation by rhizobia and all genes for nitrogen fixation were in the free living rhizobia.

Recently it has been reported that rhizobia can fix nitrogen when grown in defined media^{75,79}. A pentose sugar like arabinose or xylose, a dicarboxylic acid such as succinate and a fixed nitrogen source (*e.g.* glutamine) are essential for the reduction of nitrogen. But the most important factor as rightly pointed out by Postgate⁸⁰ is the maintenance of correct oxygen tension. This suggests that legume *Rhizobium* symbiosis gives the bacteroid the correct environment for maintaining the required oxygen tension for nitrogen fixation. In contrast to the other nitrogen fixing bacteria and bacteroids, nitrogen fixation is not repressed by ammonia in cultured rhizobia.

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Biological aspects of nitrogen fixation*

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1. INTRODUCTION

The combination of atmospheric nitrogen with hydrogen to form ammonia at ordinary temperature and pressure mediated by the enzyme nitrogenase is known as biological N₂ fixation. The known microorganisms and biological systems capable of fixing atmospheric N₂ are listed in table 1. The atmospheric N₂ is approximately 75% by weight (3800 billion tons) and 78% by volume of air. Nearly 170 million tons of N₂ are fixed by

Table 1. BIOLOGICAL SYSTEMS CAPABLE OF FIXING ATMOSPHERIC NITROGEN

Type of system	Type of microorganism involved
Asymbiotic, free living	
1) Bacteria	<i>Azotobacter, Bacillus, Beijerinckia, Chlorobium, Chromatium, Clostridium, Derxia, Desulphovibrio, Methanobacterium, Pseudomonas, Rhodomicrobium, Rhodopseudomonas, Rhodospirillum, Spirillum</i>
2) Blue-green algae	<i>Anabaena, Anabaenopsis, Aulosira, Calothrix, Chlorogloea, Cylindrospermum, Fischerella, Haplosiphon, Mastigocladus, Nostoe, Scytonema, Stigonema, Tolypothrix, Westiellopsis,</i>
3) Yeasts	<i>Rhodotorula</i>
Symbiotic	
4) Root nodules of legumes	<i>Rhizobium</i>
5) Root nodules of non-legumes	Probably Actinomycetes
6) Leaf nodules Psychotria	<i>Klebsiella</i>

biological systems whereas only 30 million tons are fixed annually by industrial means¹. These figures, although approximate, indicate that biologically fixed nitrogen is far greater in magnitude and importance than that of industrially fixed nitrogen, in the nitrogen economy of the biosphere.

It is said that there has been no major breakthrough either in the chemistry or biology during the last six decades to provide more nitrogen to crops. Added to this, the shortage and high price of oil-based inorganic nitrogenous fertilizers have brought in a new dimension to the problem all of a sudden and created a sense of urgency to find a solution for the future.

Organic fertilization of soil and the maintenance of soil fertility by biological means appear to be an inescapable approach to solve the present problem. Even before the advent of inorganic fertilizers, it was known that soil microorganisms were responsible for nitrogen transformations in soil. During the last decade, when greater emphasis was laid on the utilization of inorganic fertilizers than on organic ones for crop growth, microbiologists were busy in isolating and characterizing the enzyme nitrogenase from many nitrogen fixing bacteria and blue-green algae such as *Clostridium pasteurianum*, *Azotobacter vinelandii*, *Rhizobium japonicum*, *Anabaena cylindrica*, *Bacillus polymyxa*, *Chromatium* sp. and *Desulfovibrio gigas*.

The enzyme catalyzes the reduction of C_2H_2 to C_2H_4 in the same way as it converts N_2 to NH_3 . This reaction has been utilized for the development of an assay method for detection of N_2 fixation in any biological system. Originally proposed by Hardy *et al*², the assay method is simple, sensitive and effective for *in situ* determinations. Ever since the method came into wide practice nitrogenase activity in different ecosystems has been quantified and even incipient fixation of N_2 in remote microhabitats demonstrated and highlighted in research journals and news media. Nevertheless, certain outstanding questions remain to be answered: 1) How far does the N_2 fixed in biological systems (especially those not involved in crop production) actually help in increased turnover of N_2 in the biosphere because biological denitrification processes are equally at work in any ecosystem? The validity of this question is more important in non-symbiotic systems (where it is not coupled with plants) than in symbiotic systems where the fixed nitrogen is not only protected from exogenous breakdown but also speedily translocated for storage within foliage or grains of plants as exemplified by root nodules of legumes and non-legumes. 2) Since recent reports of biological N_2 fixation are demonstrations of N_2 fixing potentialities of new microhabitats *in situ* and do not specify methods to improve the output of fixed N_2 into the biosphere, what are the possible implications of such findings beyond the fact that they

have helped in the elucidation of natural phenomena involved in recuperation and maintenance of soil nitrogen? and 3) If so, by what other methods can biological N_2 fixation be augmented in spite of denitrification processes and is there a possibility of the development of new technologies towards this end?

In recent years, certain leads have been obtained which may result in a breakthrough to augment biological N_2 fixation by unconventional as well as conventional means. They are listed in table 2 and a close examination of such possibilities will be attempted to understand their potentialities as well as their limitations.

Table 2. APPROACHES FOR AUGMENTING BIOLOGICAL N_2 FIXATION

-
1. Discovery of new associations between N_2 fixing bacteria and higher plants such as *Azotobacter paspali* in the rhizosphere of *Paspalum* and *Spirillum lipoferum* in roots maize.
 2. Protoplast fusion and callus cultures.
 3. N_2 fixation in cereal plants and root nodulation in cereals.
 4. Genetic recombinations between N_2 fixing and other bacteria and induction of N_2 fixation by rhizobia *in vitro*.
 5. Conservation of soil nitrogen and improvement of present day practices.
-

2. DISCOVERY OF NEW RHIZOSPHERE ASSOCIATIONS

There have been reports of fixation of N_2 in the rhizosphere of weeds and grasses³. More recently, Dobereiner has found an association between *Spirillum lipoferum* and maize roots which fixes as much nitrogen as *Rhizobium*-soybean association. Reports of N_2 fixation from bacteria isolated from the roots of *Cyperus* and from aerial roots of banyan were also published earlier^{4,5}. The rhizosphere of rice plants is known to fix abundant amounts of nitrogen through bacteria associated with roots^{6,7}. Many more reports are bound to come in future since acetylene reduction method can detect minute amounts of N_2 fixed in any microhabitat. At best, such reports indicate the elucidation of a natural phenomenon in nature at a given time or period of experimentation and may not be of immediate advantage to increased crop production, unless methods are found either to create conditions to improve such fixation or to apply artificially produced inoculants to soil.

3. PROTOPLAST FUSION AND CALLUS CULTURE

When explants from roots (of both legumes and non-legumes) are raised into callus cultures and inoculated with appropriate rhizobia, acetylene reduction activity can be observed indicating the establishment of symbiosis⁸⁻¹¹. Fusion between protoplasts of higher plant cells and those of N₂ fixing bacteria such as *Azotobacter* have also been attempted recently with some degree of success by Cocking at the University of Nottingham. The ultimate objective of such experiments is to generate plants which can fix nitrogen without having to symbiose with bacteria. Without denying the ultimate possibility, the achievements upto this day do not warrant us to conceive any breakthrough in the near future for increased productivity.

4. ROOT NODULATION IN CEREALS

The specificity of rhizobia to infect and produce N₂ fixing root nodules in legumes (that too in 10% of the members of the family leguminosae) and the inability of the bacteria to form nodules on other plants has baffled many investigators. One of the approaches to induce nodulation in cereals is to transfer nitrogen fixing 'nif' genes from N₂ fixing bacteria to cells of roots in cereals or to chloroplasts in leaves. The latter may hold promise since the presence of nuclear material in chloroplasts is known. A rather conventional approach is to hybridize a legume with a cereal with the object of producing a hybrid capable of producing nodules but this would involve intergeneric hybridization. Intergeneric fusions of plant protoplasts followed by raising of plants from fused protoplasts is one possible approach. In fact, such fusions have been attempted with success in barley-soybean, corn-soybean, and pea-soybean combinations where 40% of the heterokaryotes divided at least once¹². Assuming that the genetic determinants for nitrogenase synthesis (nif operon) are successfully transplanted into the protoplasm of a cereal cell, how will the aerobic cell of the higher plant protect the oxygen sensitive nitrogenase from being denatured? In the legume root nodule, the protection from the inhibitory effects of O₂ is provided by leghaemoglobin, a pigment containing protein and iron similar to the one in the blood of human beings.

The leghaemoglobin (the prefix 'leg' indicating its presence in leguminosae) envelopes the bacteroids in root nodules and functions as a biological valve in letting O₂ at sufficiently low levels to foster the nitrogenase reaction. The pink colour of haemoglobin in nodules (when the iron is in the ferrous state) turns dark brown (ferric state or methemoglobin) at which state it is no longer useful in the transport of O₂. Some nodules are green in colour due to the conversion of haemoglobin to choleglobin by the opening of the heme ring, at which stage the nodule tissue stops fixing N₂. As the leguminous plant grows

older the number of pink nodules gets reduced and often the brown and green coloured nodules predominate. The amount of haemoglobin in nodules is directly related to the amount of N_2 fixed but the role of the pigment in N_2 fixing activity of bacteroids is only indirect and in some way related to the capacity of the pigment to reduce O_2 levels at the site fixation.

Therefore, until we are able to implant a mechanism in the higher plant cell to scavenge O_2 at the site of N_2 fixation and also establish compatibility between the genomes of higher plant cells and N_2 fixing bacteria, the idea of making cereals fixing their own nitrogen by root nodulation is bound to remain highly conjectural.

5. GENETIC RECOMBINATIONS BETWEEN BACTERIA AND INDUCTION OF N_2 FIXATION IN FREE LIVING RHIZOBIA

The number of free-living N_2 fixing bacteria is small in relation to the total number of bacteria in soil. One way of solving the nitrogen problem is to render as many species of soil bacteria as possible into N_2 fixing ones by genetic manipulations. If this is accomplished, increased amounts of N_2 of the atmosphere can be fixed in the soil and the nitrogen requirements of plants solely met by nitrogenase activity.

In recent years, mutants of N_2 fixing bacteria lacking in one or both the proteins of nitrogenase have been produced artificially. Evidence is also available to indicate that each protein component is coded by a separate operon and that genes that control the synthesis of subunits of each protein exist. Nitrogenase activity from N_2 fixing *Klebsiella* has been transferred by conjugation or transduction to a non-nitrogen fixing mutant of *Klebsiella* and to a non-nitrogen fixing strain of *Escherichia coli*¹³.

It was believed earlier that the genetic complement for N_2 fixation was shared by the legume and the *Rhizobium* in root nodule symbiosis. Recently, a strain of *Rhizobium* sp. (cowpea group) has been induced to fix nitrogen in vitro in the absence of the legume partner when [arabinose, xylose succinate and a small amount of glutamate (fixed nitrogen) was added to the medium. These results indicate that *Rhizobium* has all the complement of genes for N_2 fixation but they become operative only under certain conditions¹⁴⁻¹⁶. In the next few years, we may have to reconsider the recognition of legume-*Rhizobium* association within the nodule as a true instance of symbiosis. Besides this implication, the finding that rhizobia can fix N_2 in the absence of the host may lead to further clues to the establishment of more free-living N_2 fixers in soil.

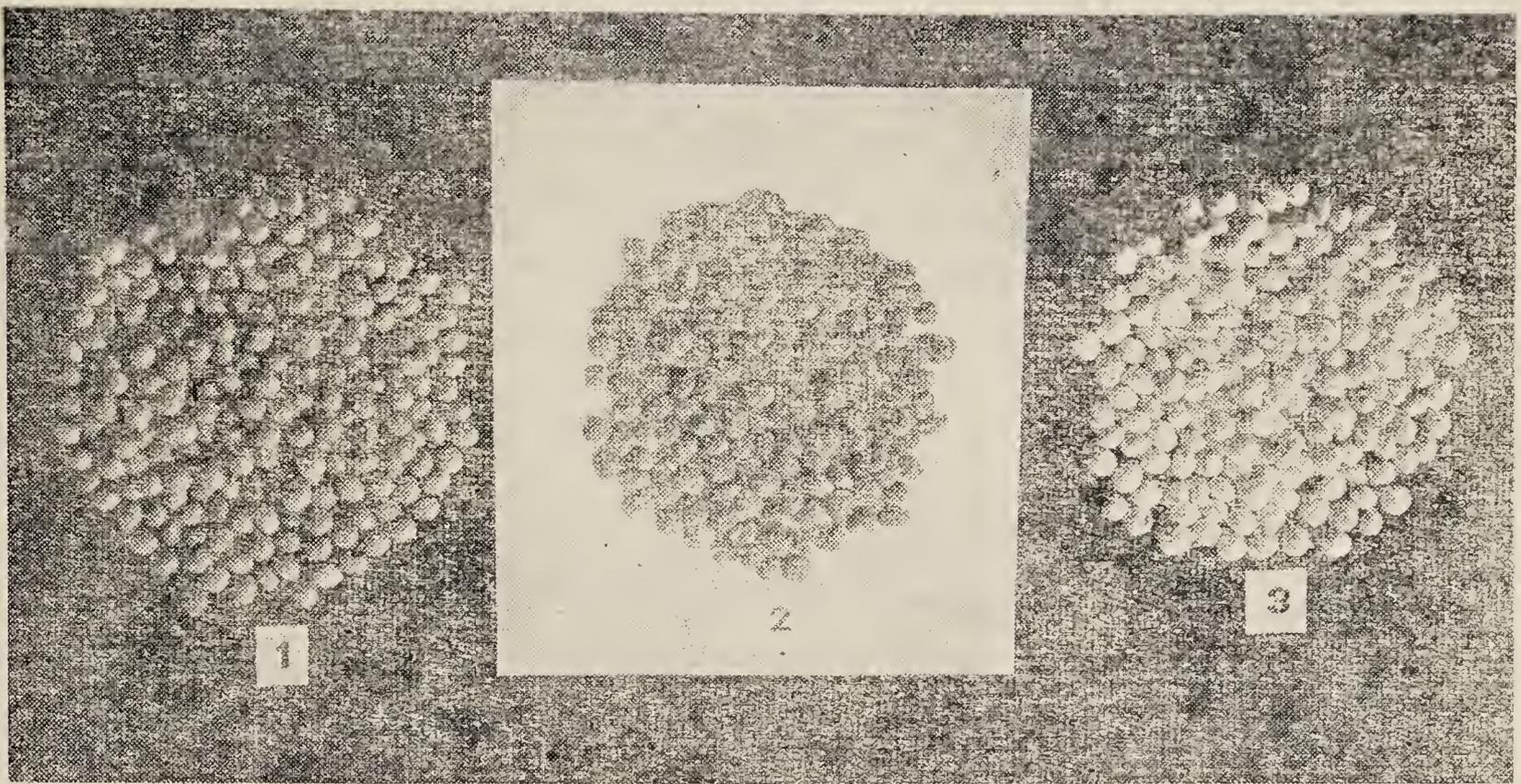
6. CONSERVATION OF SOIL NITROGEN AND IMPROVEMENT OF PRESENT DAY PRACTICES

This line of approach is more pragmatic and can be achieved by selection of N-responsive varieties of legumes, by devising novel forms of fertilizers that do not prevent biological fixation of N_2 and by the use of nitrification inhibitors and coated fertilizers so as to control the rate of nutrient release from nitrogenous fertilizers¹⁷. Examples of this kind of fertilizers are ureaform, isobutyledene diurea, Crotonilidene diurea and sulphur coated urea which are sparingly soluble in water. By virtue of this property, they can regulate the release of nitrogen from fertilizers. Nitrification inhibitors can increase nitrogen uptake by plants when added with ammonium sulphate. These chemicals are substituted pyridines, pyrimidines, acetanilides, anilines and isothiocyanates. Two major compounds which are commercially available are 'N-Serve' and 'AM' which are trade names. Seeds of neem tree (*Azadiracta indica*) contain certain lipid associates which act as nitrification inhibitors and thereby increase the efficiency of urea fertilizer. A search for similar inhibitors from other oil seeds may be vitally important.

Equally important is the selection of nitrate tolerant microorganisms capable of fixing N_2 so that both fertilizer N and biological fixation could work side by side since it is now known that high amounts of ammonia represses the activity of nitrogenase.

Carbon is essential for N_2 fixation and products of photosynthesis are translocated to the root nodule which is the site of fixation. By providing an artificial canopy of CO_2 , Hardy (personal communication) has been able to augment yields of soybean by increasing nitrogenase activity. It is difficult to foresee how we can enrich CO_2 in the vicinity of leaves of legumes by inexpensive means. If this can be done, we may have an alternate technology to augment biological N_2 fixation.

Artificial inoculation of seed by rhizobia have increased yields of certain grain legumes in India from 5 to 70% over control depending on the agro-climatic conditions and the variety planted (figure 1). If new and more vigorous strains could be mass multiplied and applied after suitable quality control, the nitrogen requirement for growing legumes could be solely met by rhizobial application¹⁸. Artificial addition of non-symbiotic microorganisms like *Azotobacter* and blue-green algae is another approach to improve the nitrogen fertility of soil and this could be explored with urgency to assess its impact on production. In short, application of microbial inoculants to seed and soil may partly alleviate the pressure on inorganic fertilizers.



A

B

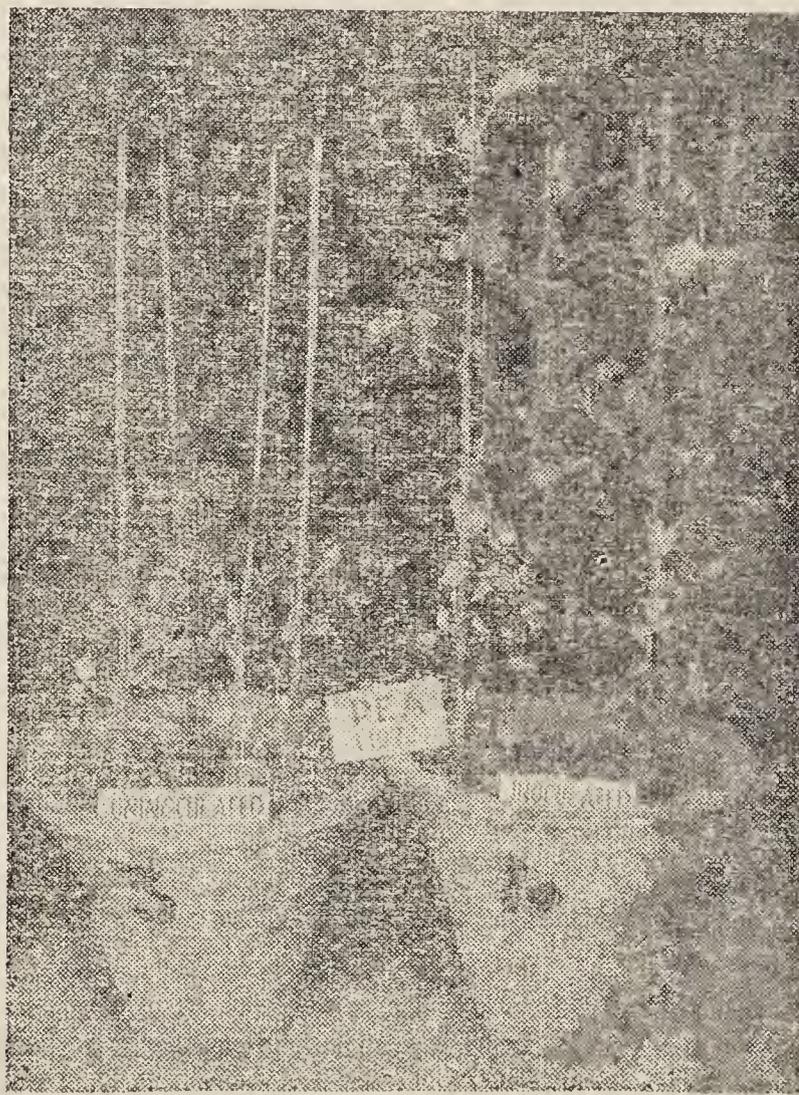


FIGURE 1

A — Method of application of *Rhizobium* inoculant ; 1) Soybean seeds before inoculation, 2) Soybean seeds inoculated by mixing the seed with a slurry of live cells of rhizobia cultured on a peat based carrier, and 3) In acid soils, the establishment of legumes is taken care of by pelleting inoculated seeds with lime so as to bring the rhizosphere to neutral pH.

B—The effect of *Rhizobium* inoculation of *Pisum sativum* (pea plants) on growth.

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Molecular sieves in heterogeneous catalysis

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1. INTRODUCTION

Molecular sieves are highly porous, crystalline aluminium silicates. In nature they occur as zeolite minerals. In 1930-32 McBain* studied the adsorption of gases on zeolitic minerals. He observed their capacity to adsorb smaller molecules but not larger ones and thus to separate smaller molecules from larger ones, just like a sieve—hence the name molecular sieves. In the years 1935-50, Barrer in England conducted pioneering and extensive studies on the synthesis, structure and properties of zeolites. He observed that some zeolites could separate straight-chain or normal paraffins from a mixture of normal and branched paraffins, and even oxygen from nitrogen in air. In the 1950's Union Carbide in the United States launched a massive research programme on synthesis and applications of molecular sieves, with Professor Barrer as a consultant and advisor. Very soon they developed molecular sieves as driers and desiccants, as adsorbents for gases and vapours, and as a catalyst for isomerization of normal paraffins to the higher-octane-number branched paraffins.

The introduction of molecular sieves (MS) as catalysts in catalytic cracking by Mobil Oil Company in 1962 was a major break-through in petroleum technology. Today 95% of the catalytic cracking capacity in the United States is based on MS-incorporated cracking catalysts. The scientific importance of MS can be noticed from the three international congresses on MS held at London (1967), Worcester, Mass. (1970) and Zurich (1973) and several recent reviews¹⁻⁶. This paper gives a very brief introduction to MS, emphasizing their unique characteristics and potentialities for some important applications in heterogeneous catalysis as in catalytic cracking, reforming, oxidations, etc.

* who later came over to India as the first Director of National Chemical Laboratory, Poona.

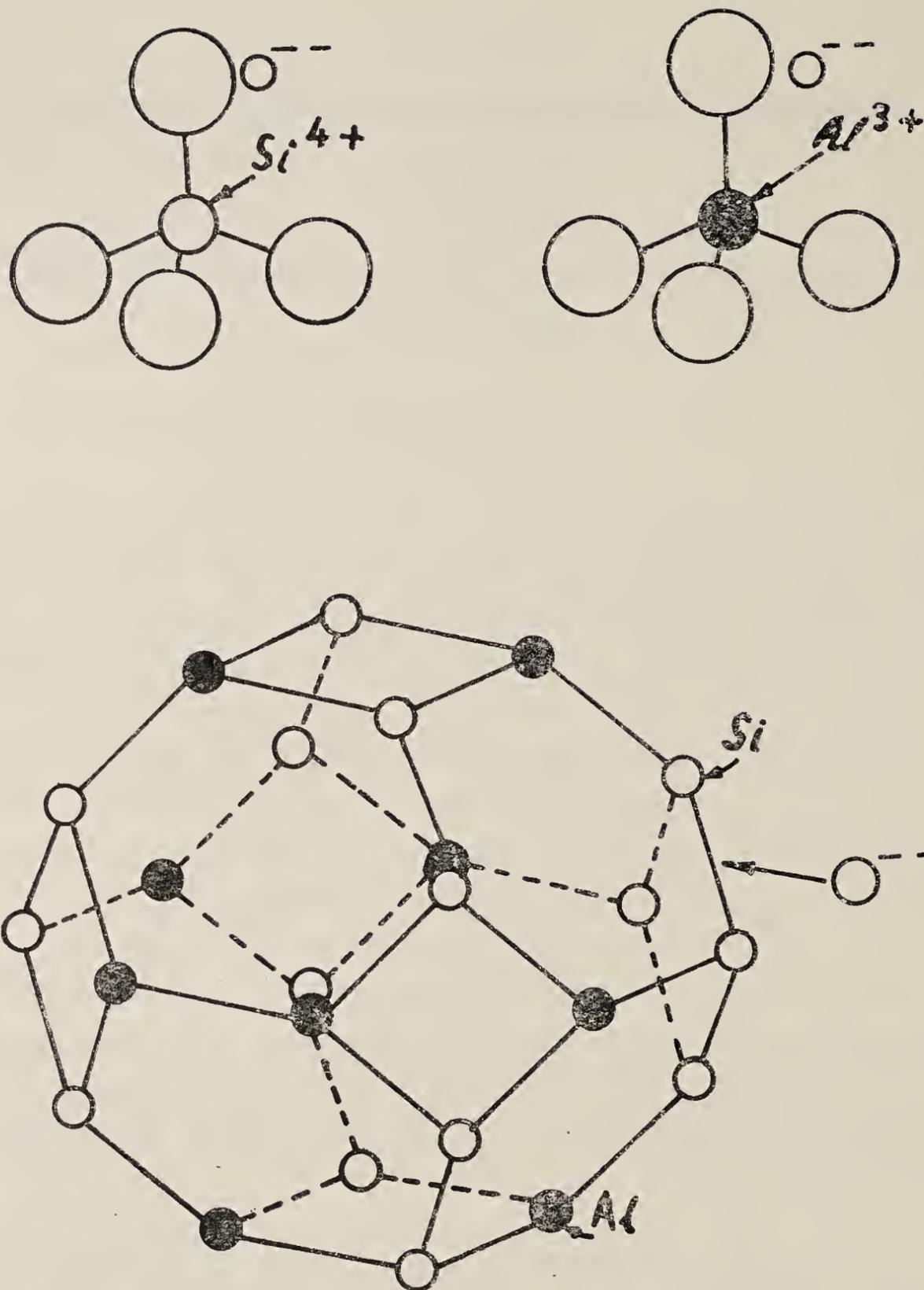


Figure 1. The sodalite unit is a cubo-octahedron formed from 24 tetrahedra of AlO_4 and SiO_4 , the Al and Si atoms sharing an O atom to form a bond between them. This sodalite unit is the basic building block for A, X and Y sieves.

2. DESCRIPTION AND APPLICATION

2.1 STRUCTURAL ASPECTS OF MOLECULAR SIEVES

Molecular Sieves are porous, crystalline three-dimensional aluminosilicates. In nature they occur as zeolite minerals. The zeolites are built from SiO_4 and AlO_4 tetrahedra, which share their corners. Their crystal skeleton consists of Si and Al atoms each surrounded by four oxygen atoms to form a small pyramid or tetrahedron. These tetrahedra can then be arranged in many ways to form different porous crystal structures, eg., 24 tetrahedra form a sodalite unit (figure 1), which is a cubo-octahedron consisting of 6

four-membered faces (the cube faces) and 8 six-membered rings (the octahedron faces). One condition here is that two Al atoms are never in adjoining oxygen tetrahedra but are separated by at least one silicon-oxygen tetrahedron. The sodalite cages formed in this way are the basic building blocks for A-, X- and Y-type zeolitic molecular sieves.

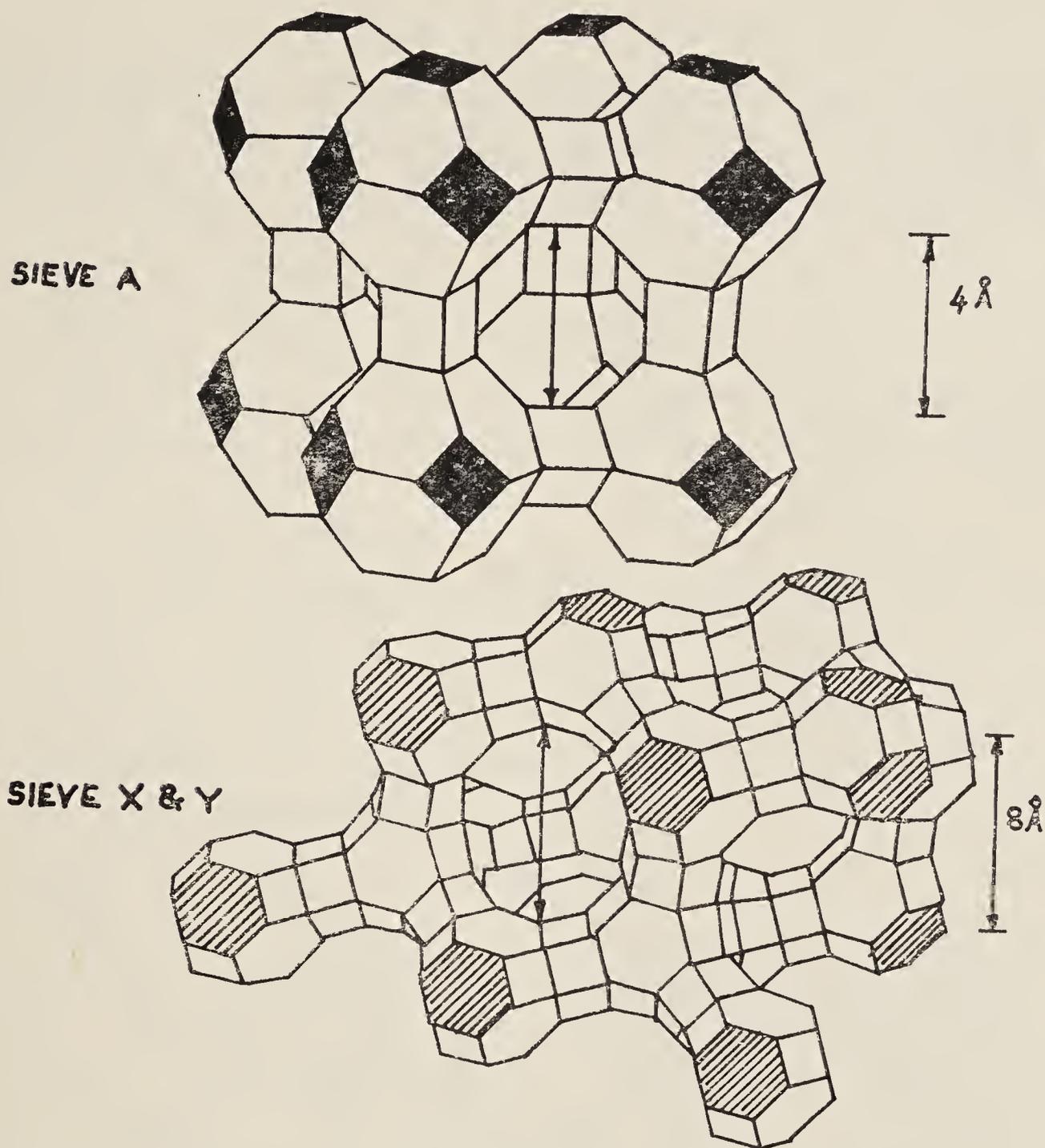


Figure 2. a) Sodalite cages linked on their square faces to form a cubical array. This is molecular sieve A.
 b) When the hexagonal faces are connected to one another, the structures of X and Y sieves result.

When the sodalite octahedra are linked in a cubic array by joining them with cubes on their square faces, the resulting structure is the A-type zeolite*. This structure (figure 2a) has two types of pores or cavities in it : the sodalite

* Over three centuries ago this type of structure was already being used in the floor plan of Moghul architecture, e.g. in Taj Mahal at Agra. (See figure 3)

cages enclose a super-cage or α -cage with a diameter of 11 \AA and a cage-mouth opening or aperture of about 4 \AA , while every sodalite cage itself is hollow and has smaller cavities or β -cages with a diameter of 6.6 \AA and aperture of 2.2 \AA . The pore volume of this extremely uniform three-dimensional pore system is about 0.3 cc/g .

If the sodalite cages are connected to one another at their hexagonal faces through hexagonal prisms of 6 oxygen atoms, the X and Y zeolite structures are formed (figure 2b). The structures of X and Y zeolites are identical,

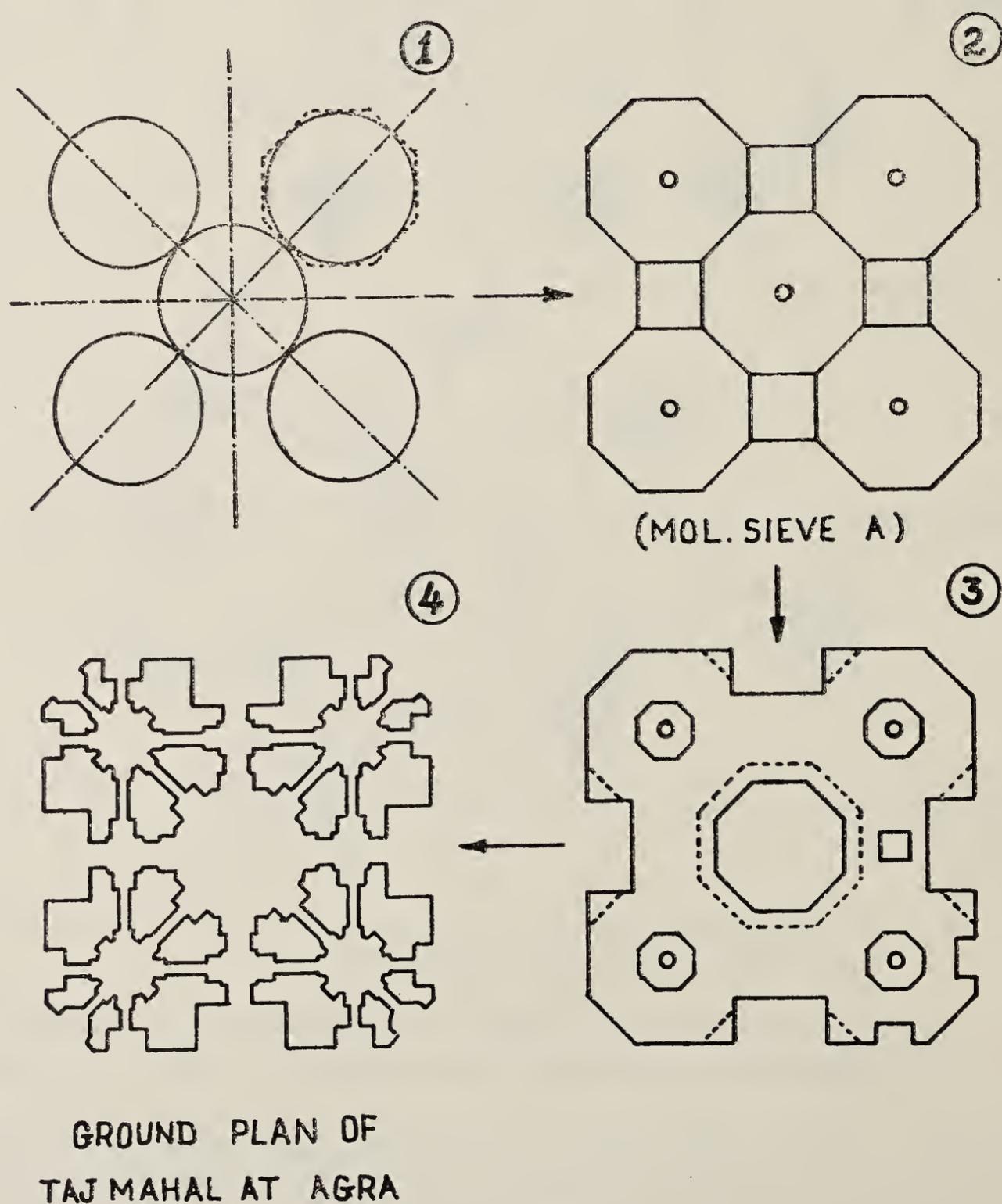


Figure 3. Octahedrons joined through squares, as in molecular sieve A, was used as the basic floor plan of Taj Mahal at Agra.

their difference is in their $\text{SiO}_2 : \text{Al}_2\text{O}_3$ ratio (figure 4). In these zeolites, the sodalite β -cages have the same pore structure as in A zeolites, but the super-cages are larger and have a diameter of 13 \AA and apertures of 8 \AA . A cross-sectional view of the 3 types of cages or hollow cavities in zeolite X and Y are shown in figure 5.

Silicon has a valency 4 and hence can take 4 oxygen atoms around it in a tetrahedral combination, maintaining electrical neutrality. When a trivalent aluminium atom is similarly bound to 4 oxygen atoms, the resultant aluminium tetrahedron will have a net negative valency. A positive charge or a cation is then required to neutralize this negative charge and provide overall electrical neutrality. Thus, stoichiometrically as many cationic charges will be needed as there are Al atoms in the zeolite. Synthetic zeolites are usually prepared and crystallized out as sodium zeolites. Hence initially the number of Na and Al atoms will be the same in any zeolite. The chemical formula and some characteristics of A, X and Y zeolites are

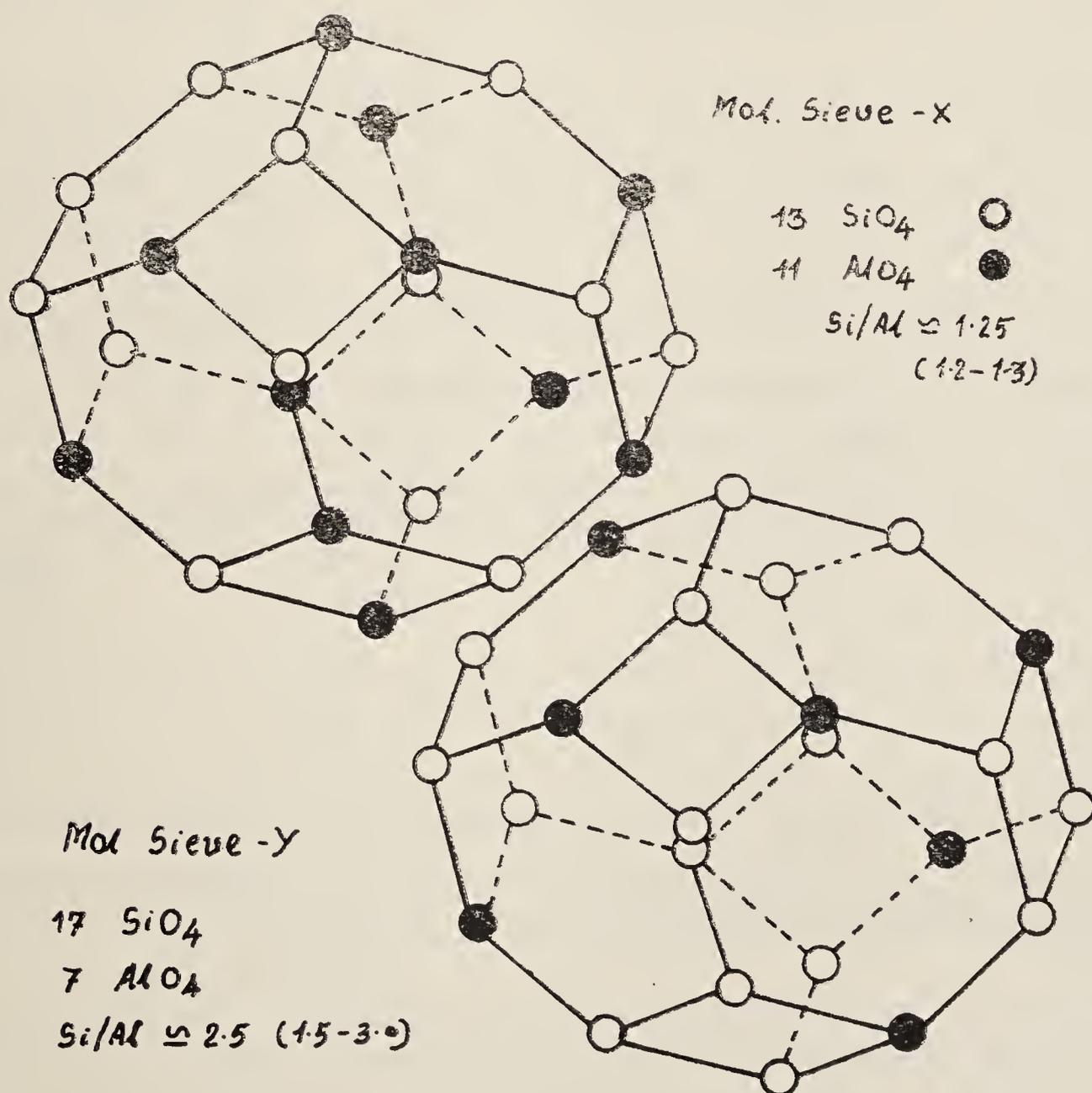


Figure 4. Molecular sieves X and Y are identical in structure. They differ only in their Si/Al ratio.

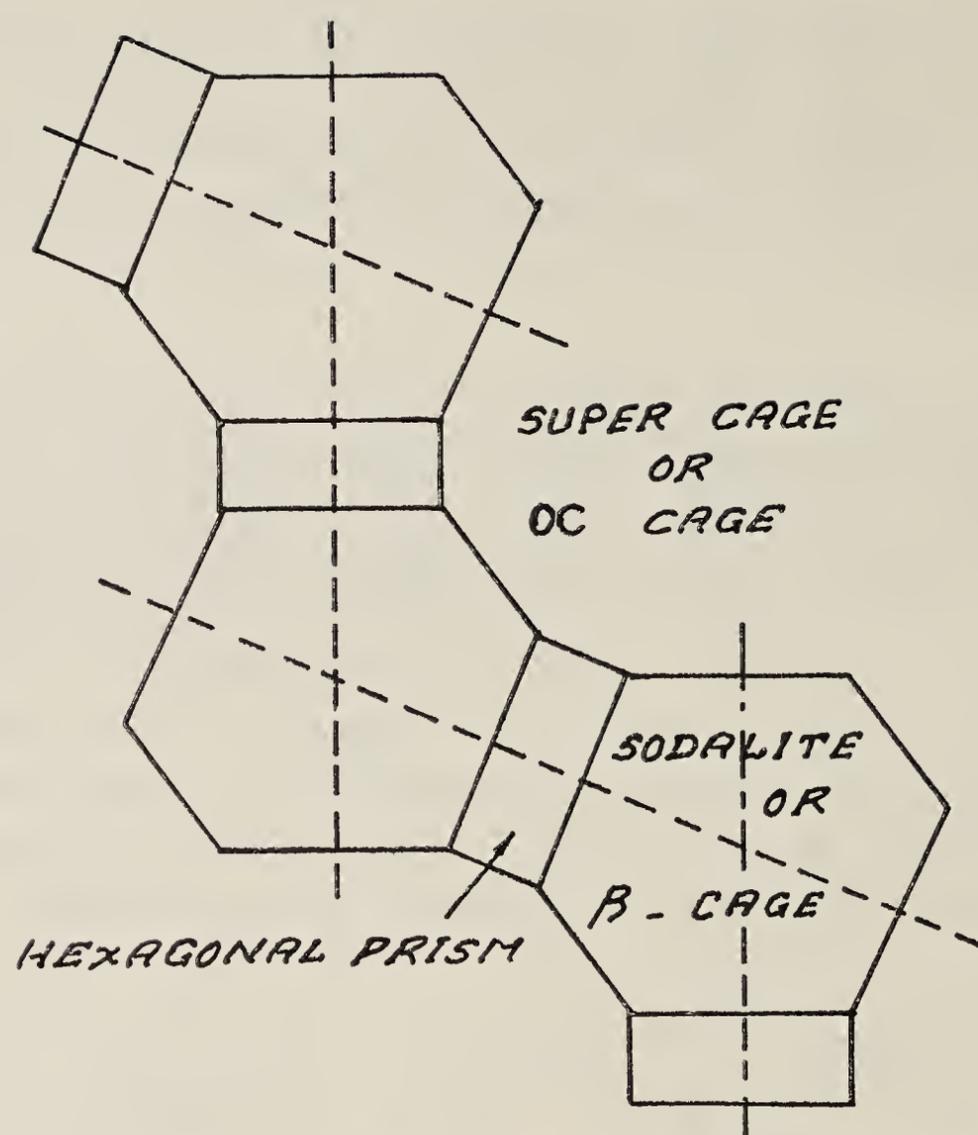


Figure 5. Cross-sectional view of the types of cages in molecular sieves X or Y.

Table 1. Properties of molecular sieves A, X and Y

Sieve A	$\text{Na}_{12} (\text{AlO}_2)_{12} (\text{SiO}_2)_{12} \cdot 27 \text{H}_2\text{O}$		
Sieve X	$\text{Na}_{86} (\text{AlO}_2)_{86} (\text{SiO}_2)_{106} \cdot 264 \text{H}_2\text{O}$		
Sieve Y	$\text{Na}_{56} (\text{AlO}_2)_{56} (\text{SiO}_2)_{136} \cdot 264 \text{H}_2\text{O}$		
Sieve	Mol. ratio $\text{SiO}_2/\text{Al}_2\text{O}_3$	α -Cage diameter	α -Cage aperture
A	2:1	11 Å	4 Å
X	2.5:1	13 Å	8 Å
Y	5:1	13 Å	8 Å

given in table 1. The large quantity of water in zeolites can be removed by heat treatment without destroying the crystal structure.

Zeolites have remarkable ability for ion-exchange. The sodium ions can be exchanged by monovalent, bivalent or trivalent ions (e.g. Ag^+ , Ca^{++} or La^{+++}). The direct replacement of the sodium ions by acids is, however, not possible because at pH below 4 aluminium ions are removed from the lattice and the structural framework will collapse. The safer way is to exchange the

Na⁺ ions with ammonium ions; on heating ammonia will be evolved leaving behind hydrogen ions in the zeolite. Exchange with other ions is usually carried out by treating with the corresponding salt solutions. Exchanging sodium by rare earth (RE) ions (mixture of La³⁺, Ca³⁺ etc.) is particularly important in the preparation of molecular sieve cracking catalysts. The RE-exchanged sieves have higher temperature stability than the other forms of sieves: the collapse temperature of RE-Y sieve is about 1000°C, compared to 700-800°C for Na-Y sieve.

2.2 MOLECULAR SIEVES AS CATALYSIS

The properties of molecular sieves which make them particularly suitable for use as catalysts are: their large surface area, well defined and uniform pore structure, well defined crystal structure, temperature stability, different forms prepared by easy ion exchange, and reproducibility of the various forms. The primary requirements of an industrial catalyst are its activity, selectivity and stability, all the three of which are met remarkably by zeolites in cases like catalytic cracking.

The major break-through in the use of molecular sieves as catalysts was their introduction as cracking catalysts by Mobil in 1962. For this purpose the sieves could not be used as such due to their very small size (a few microns) and their extreme activity. Both these difficulties were overcome by "diluting" the sieve by incorporating it (5-20% by wt.) into a conventional amorphous silica-alumina cracking catalyst as a carrier or matrix, which increased the particle size to 20-80 μ , usually required for the fluidized-bed operation in conventional catalytic crackers. Other criteria for catalyst preparation are: exchange pH 4-8.5, final sodium content <0.25 wt%, rare earth ion content 40-85% of exchangeable equivalents, and NH₄⁺ ion content for the remaining equivalents⁶.

Since catalytic cracking involves typical carbonium-ion reactions, the number of acid sites on the catalyst should be a guide to the activity of the catalyst. Moscou and Lakeman have found that the number of acid sites in a used ('equilibrium') amorphous cracking catalyst is about 10¹⁹ sites/g, while the corresponding figure for RE-Y sieve is 10²¹ sites/g. This explains at least qualitatively the fact that cracking activity of the RE-Y sieve is about 100 times that of the amorphous catalyst.

2.3 SUPERIORITY OF MOLECULAR SIEVE CRACKING CATALYSTS

The distinctive advantages of molecular-sieve catalysts over amorphous silica-alumina catalysts are: a) higher activity, b) higher stability to heat

and steam and to organic nitrogen bases and c) higher selectivity, i.e., producing more gasoline, less C_1-C_4 gases and less coke. In the United States, increase in gasoline production by only 1% means a gain of \$20 million and a saving of 27 million barrels of crude oil per year⁸. The actual increase in gasoline production by switching over to molecular-sieve catalysts is *at least* 7%! Such saving of crude oil is most welcome particularly in these days of hiked crude oil prices.

The cracking capacity of a catalytic cracker is limited by the capacity of the regenerator to burn off the coke formed in the cracking process, thereby restoring the activity of the catalyst as also producing the necessary heat for the endothermic reactions in the cracker. Since molecular-sieve catalysts produce less coke, there is less burn-off to be done in the regenerator. Even this burn off proceeds at a faster rate because the rare-earth ions in the molecular sieve serve as a catalyst for the combustion of coke⁹. The net result is a higher coke-burning capacity and consequently a higher through-put in an *existing* catcracker simply by switching over from amorphous to molecular sieve cracking catalysts. No wonder the American refiners hail the introduction of MS cracking catalysts as the greatest invention which gives maximum profits in minimum time with practically no new capital investment.

Table 2 gives typical performance of a catcracker which switched over from an amorphous cracking catalyst to a molecular sieve catalyst for naphtha.

Table 2. Performance of a catcracker on switching over from amorphous to MS cracking catalyst.

Feed density ($^{\circ}$ API) 27.6 Reactor temperature 500°C
Feed Kuwait naphtha.

		Amorphous catalyst	MS Catalyst
Throughput	tons/day	2,600	3,200
Catalyst-to-oil ratio		7	10
Catalyst addition rate	lbs/bbl	0.05	0.09
Conversion	Vol %	53.7	59.0
Conversion	wt %	52.0	57.3
C ₂ -lighter	„	3.5	2.1
C ₃ -total	„	4.3	3.8
C ₄ -total	„	6.4	7.6
Gasoline C ₅ to 205°C	„	33.3	40.7
Light cycle oil		21.6	19.5
Heavy cycle oil		13.9	10.0
Clarified oil		10.9	11.2
Coke		4.5	4.1

from Kuwait crude oil. The higher activity was used to increase the throughput by 23%. The increase in conversion contributed entirely to the gasoline yield.

The much higher activity of molecular sieves as compared to conventional amorphous cracking catalysts has led to radical changes in modern catcracker design specifically to take full advantage of this higher activity. In older catcrackers the catalyst-oil contact time (process time) in the fluidized bed used to be 3-10 minutes. Due to coke lay-down the cracking activity declines with the process time, hence the catalyst is considerably deactivated for the major part of the time it is in contact with the oil. With the more active MS catalyst, catalyst-oil contact times are kept as low as 10-60 seconds, when the catalyst is much more active due to the much less coke on it. Special 'Riser Crackers' have been designed by Universal Oil Products Co. (UOP) to get such short contact times and thus reap the full benefits of the MS catalyst. Most of the recently built catcrackers are of the Riser type.

In the United States, where the greatest demand is for gasoline, over 90% of the cracking capacity to-day is based on MS catalysts. In Europe, the MS catalysts now account for about 35% of the total cracking capacity; the change-over is slower since the demand for gasoline is relatively not so high, as in the United States, while light cycle oil and middle distillates have higher demands. In Europe many catcrackers follow a yearly cycle: they run on amorphous catalysts in winter when gasoline demands are low, but switch over to MS catalysts in summer when gasoline demands shoot up disproportionately.

2.4 CHARACTERISTICS COMMON TO AMORPHOUS AND CRYSTALLINE CRACKING CATALYSTS

With respect to catalytic properties as well as chemical properties, both amorphous and molecular sieve cracking catalysts have much in common¹⁰:

- a) Chemically, both are alumino-silicates.
- b) The character and direction of catalytic processes on both are comparable and equivalent. The activation energy values for cracking on both catalysts are comparable.
- c) For both, the alkali form is least active, the alkaline-earth form more active, and hydrogen form most active in hydrocarbon reactions.
- d) For both, the cracking activity is dependent to some extent on SiO₂/Al₂O₃ ratio.
- e) Cracking activity increases for both catalysts on addition of a little steam.

- f) The spectra of adsorbed pyridine on both catalysts are comparable. These and other facts, taken in total, suggest that the catalytically active sites of amorphous and crystalline aluminosilicates are apparently of the same nature and are due to the presence of acidic centres on the surface.

2.5 NATURE OF ACID SITES

In zeolites such acid centres can be formed in at least three different ways :

- Exchange of sodium with H^+ ions leading to the formation of acidic hydroxyl groups.
- Exchange of sodium with NH_4^+ ions followed by elimination of ammonia on heating when an H^+ ion will be left behind.
- Rare-earth ions introduced into the lattice by ion exchange can hydrolyse their water of hydration :



A two-dimensional representation of this acid formation in zeolite structure is shown in figure 6.

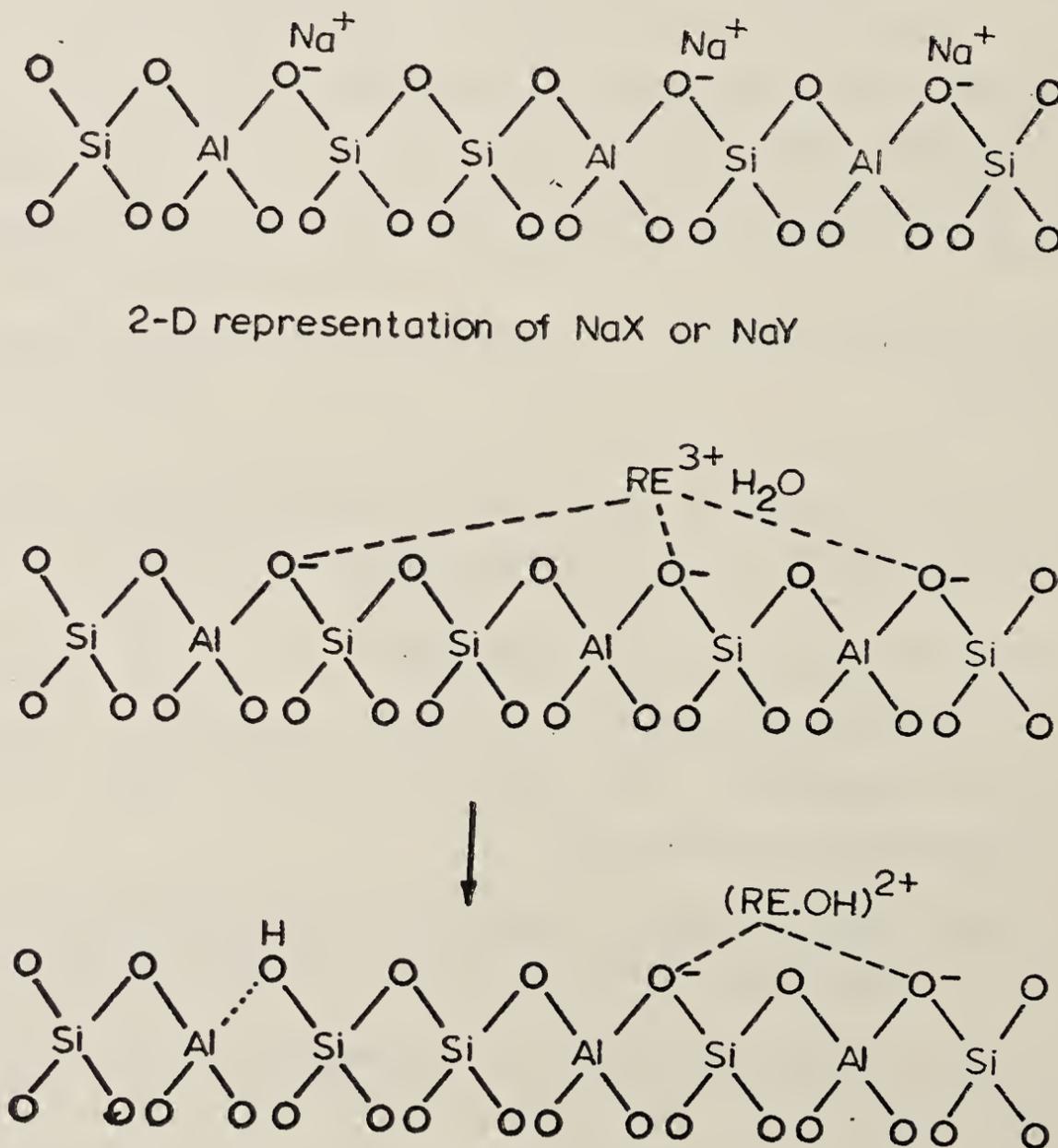


Figure 6. Formation of Bronsted acid sites in rare-earth exchanged X or Y sieves.

The acidity in zeolite can be of the Bronsted type or of the Lewis type and often a combination of both, depending on the degree of dehydration of the sample (figure 7).

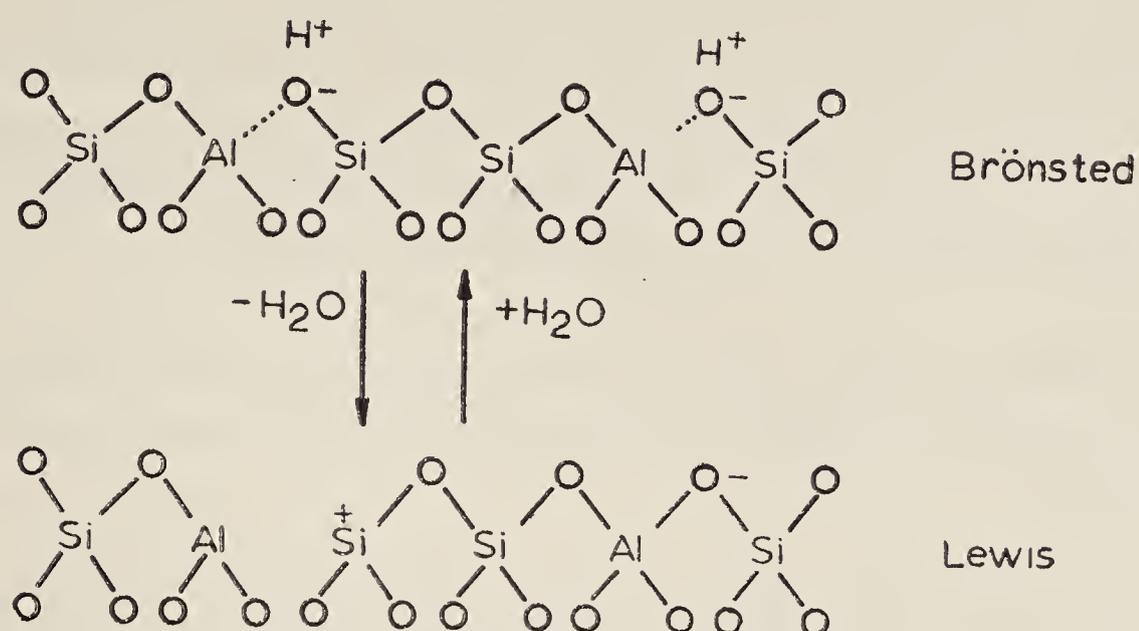


Figure 7. Inter-conversion of Bronsted and Lewis acid sites in molecular sieves under reaction conditions as in catalytic cracking (500°C , traces of steam present).

Recently Moscou and Lakeman⁷ have developed quantitative estimation methods for Bronsted acid sites on zeolite surfaces. These methods can distinguish not only between hydroxyl groups, non-acidic hydroxyl groups and water molecules but also between groups present in α cages and those in β cages of the zeolite structure. Two general reviews have also appeared recently on the determination of acidity and acid strength distribution in solid catalysts^{11,12}.

It has to be emphasized here that, just as in heterogeneous catalysis in general, in zeolites also only less than 5% of all the decationized (Na^+ removed) sites are involved in chemisorption and catalysis. The remaining 95% or more of the cation sites are relatively or totally inactive. Evidences for site heterogeneity have been obtained from a) X-ray crystallography¹³ b) activity per site in cumene cracking¹⁴ c) infrared spectra of adsorbed species^{15,16} d) Differential thermal analysis¹⁷ e) sorption of CO ¹⁸ and of ethylene¹⁹ and f) analytical differentiation of acid groups^{7,20}.

2.6 MOLECULAR SIEVES SHAPE—SELECTIVE CATALYSTS

The main factors determining the catalytic activity of zeolites are : a) the nature and degree of exchange of the cation, b) the structure of the zeolite lattice, c) the silica-alumina ratio, and d) the size of reactant and product molecules relative to the orifices or windows in the zeolite. Other conditions being the same, the last factor becomes particularly important²¹.

The use of molecular sieves as shape-selective catalysts implies that molecules of the proper dimension are continuously passing through the sieves. But, longer or branched or bulky molecules are held behind and prevented from reacting. Some typical examples for such shape selectivity are given in table 3. The super-cages or α -cages in zeolite are generally large enough to accommodate most hydrocarbon molecules. The molecular-sieve action is provided by the critical dimensions of the channels interconnecting the super-cages. Molecular shape selectivity can be achieved in two ways²¹ :

- a) *Reactant selectivity* : This occurs when the charge consists of two classes of molecules, one of which is too large to pass through the channels.
- b) *Product selectivity* : When various products are formed in the cages, only those with the proper size and shape can get out through the channels as products. If a molecular species of too large size is trapped in this way in the super-cage, thermodynamic equilibrium prevents its further accumulation there.

Table 3. Some typical examples for shape selectivity on MS catalysts. Branched or bulky molecules do not get access to catalytic sites and hence do not react at all :

A. Hydrogenation	on	Pt-carrier	Pt-Ca
n-Olefin	→	n-Paraffin	n-paraffin
Branched olefin	→	br. Paraffin	No reaction
B. Dehydration on Ca-A mol. sieve			
1-Butanol	→	Butene	
iso-butanol	→	No reaction	
Sec-butanol	→	No reaction	
C. Cat. cracking on Ca-A at 500°C			
n-Hexane	→	Products	
3-Methyl pentane	→	No reaction	

Sorption and diffusion rates also play a great role in catalysis over molecular sieves. Marked differences can be seen in the behaviour of even *cis* and *trans* isomers. The periodic behaviour of the diffusivities with the length of the molecule has also to be taken into account here, e.g., the diffusivity of C₂ to C₁₄ n-paraffins in erionite molecular sieve show two maxima, one at C₄ and the other at C₁₂, and a minimum at C₈; the values for C₄ and C₁₂ being almost two orders of magnitude higher than that for C₈. The n-paraffins emanating from cracking of n-C₂₂H₄₆ on erionite catalyst indeed show a similar picture, the yields are maximum for C₄ and C₁₁ and minimum for C₈ n-paraffins, thus

clearly suggesting the influence of product diffusivities on the yield and selectivity pattern⁴.

2.7 NATURE OF ACTIVE SITES IN MS CATALYSTS

The precise nature of the active sites responsible for the activity and selectivity of silica-alumina cracking catalysts is still a controversial subject. There are advocates for the Bronsted acid, the Lewis acid and a combination of these two as the species responsible for cracking activity. This field has been reviewed by Lombardo *et al*²², who give the major references to the wealth of conflicting evidences on this aspect of cracking catalysts. One view, however, deserves special mention.

For the last 20 years Trambouze and his colleagues in the Institut de Recherches sur la Catalyse, Lyon, France, have carried out extensive investigations on commercial samples of amorphous silica-alumina cracking catalysts. They have examined the effect of the nature and strength of the catalyst acidities on their cracking activity and selectivity. The ratio and strength of the acid regions were changed by thermal or chemical treatment (e.g. extraction of alumina with HCl). These studies led to the conclusion (for a review see Barthomeuf *et al*²³) that catalytic cracking activity is primarily due, not to the nature (Bronsted Lewis) of surface acidity, but to its strength. The selectivities of these catalysts are also due to the varying strengths of the acid centres. Recently Otoûma *et al*²⁴ have also reported similar results in the case of catalytic cracking by molecular sieves as well. Molecular sieve catalysts, however, have other specific features which deserve a more detailed examination.

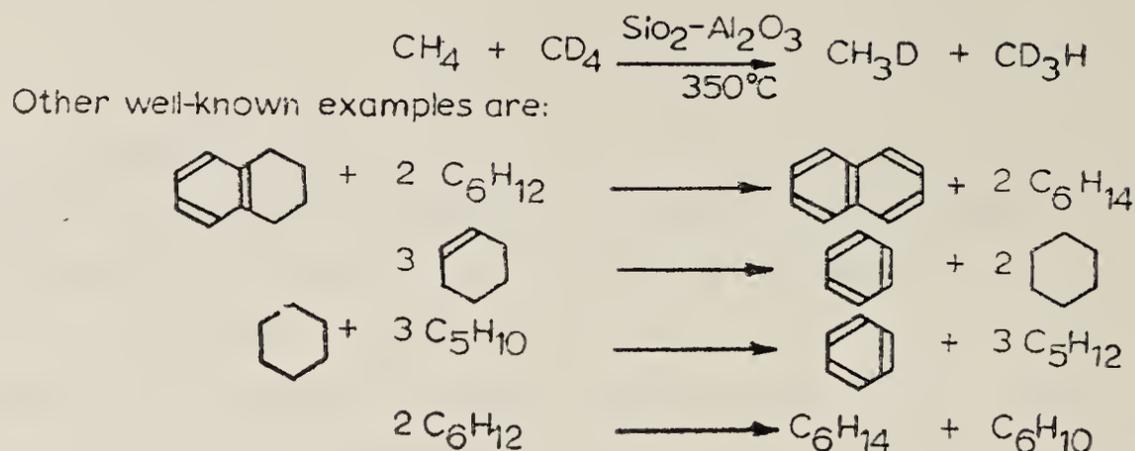
2.8 FACTORS GOVERNING SELECTIVITY OF MOLECULAR SIEVE CRACKING CATALYSTS

Three distinctive features of zeolitic molecular-sieve catalysts over amorphous alumino-silicates are their 1) molecular shape selectivity, 2) high hydrogen transfer efficiency and 3) extreme activity in readily converting olefins to hydrogen-rich and hydrogen-poor products, e.g., to paraffins and aromatics. The greater selectivity of molecular-sieve catalysts in catalytic cracking (more gasoline, less gas and less coke) can at least qualitatively be explained as a consequence of the above three unique features of zeolites.

2.9 HYDROGEN TRANSFER

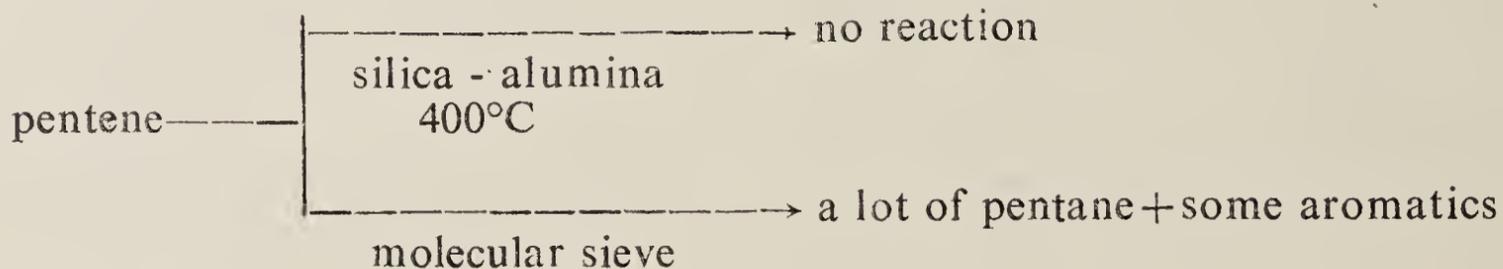
The term hydrogen transfer denotes the catalytic transfer of hydrogen directly from one type of hydrocarbon into another. The simplest example

for this is the exchange of H and D between CH_4 and CD_4 even at 350°C over amorphous silica alumina²⁵.



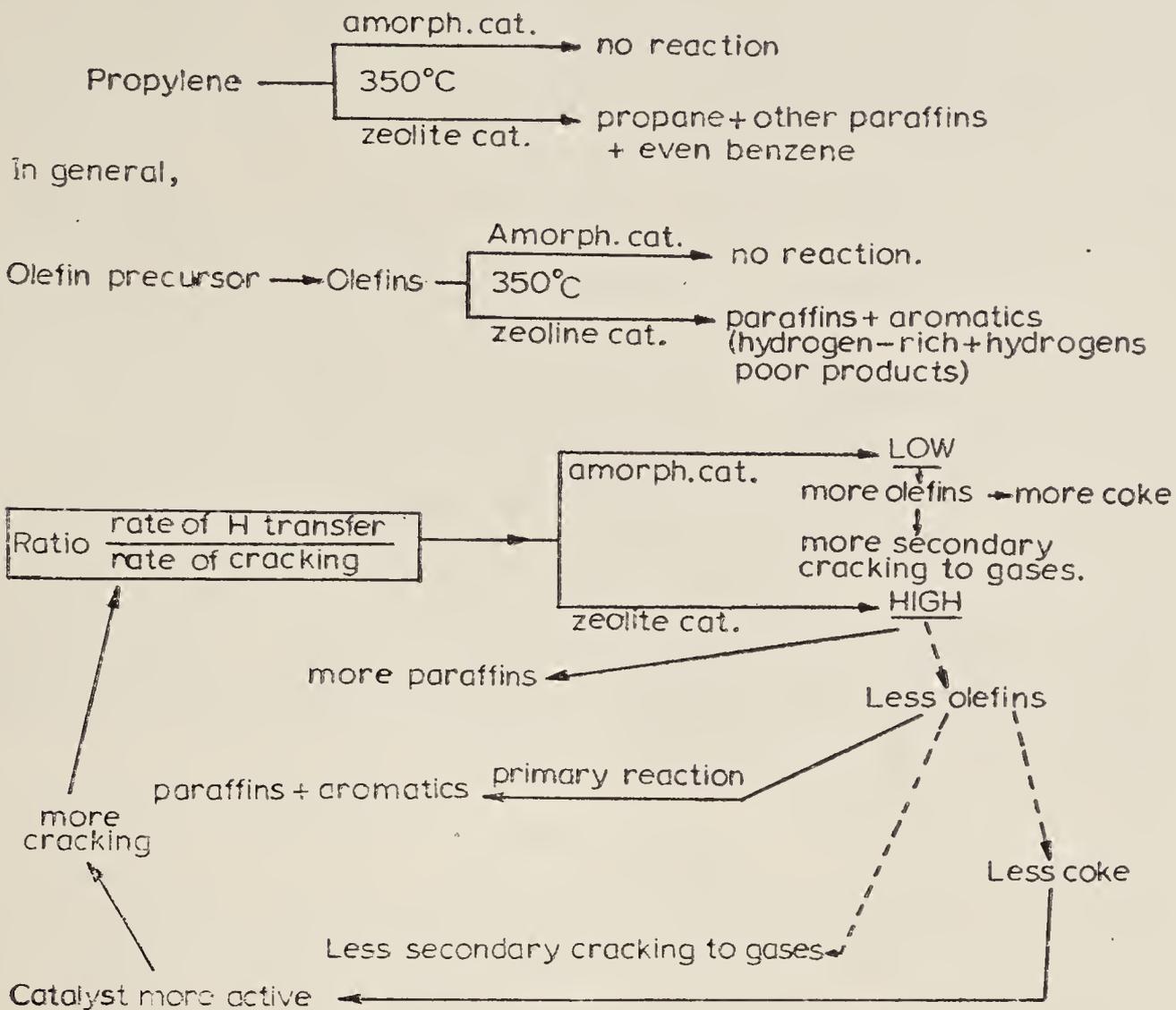
This type of hydrogen transfer is quite different from catalytic hydrogenation in which hydrogen from gaseous molecular hydrogen reacts catalytically with an olefin. *Hydrogen transfer decides whether an olefin is going to be desorbed as a paraffin or dehydrogenated to form polyolefins and ultimately coke.*

One basic difference between amorphous and crystalline (molecular sieve) alumino-silicates in hydrocarbon reactions is in the mechanism of hydrogen transfer on the latter, e.g.



This ready conversion of olefins to paraffins and aromatics is the most significant feature molecular sieve catalysts, contrasted to the dominating β -splitting carbonium-ion mechanism and the progressive conversion of olefins to polyolefins, coke-precursors and finally coke on amorphous silica-alumina. On zeolites the high rate of hydrogen transfer enables the quick saturation of the olefin and its being pushed off as a paraffin from the catalyst surface. Thus zeolite cracking catalysts give more paraffins compared to amorphous cracking catalysts. Gas ($\text{C}_1\text{-C}_3$) formation on zeolites is less because the C_5+ olefins are quickly converted to paraffins and desorbed before they can undergo further cracking to gases. Coke formation is also less because on zeolites the strongly coke-making olefins are too reactive and they also have a basically different reaction path, compared to olefins on amorphous catalysts. With less coke on the catalyst, the catalyst remains more active which, in turn, leads to more conversion. These major

differences between amorphous and zeolitic catalysts are summarized in the following reaction schemes.



2.9 MOLECULAR SIEVE CATALYST FOR AUTOTHERMAL CATALYTIC CRACKING

In a catcracker there is a delicate heat balance between the endothermic reactions in the cracker unit and the exothermic coke burn-off in the regenerator (figure 8). The capacity of the catcracker is limited by the coke-burning

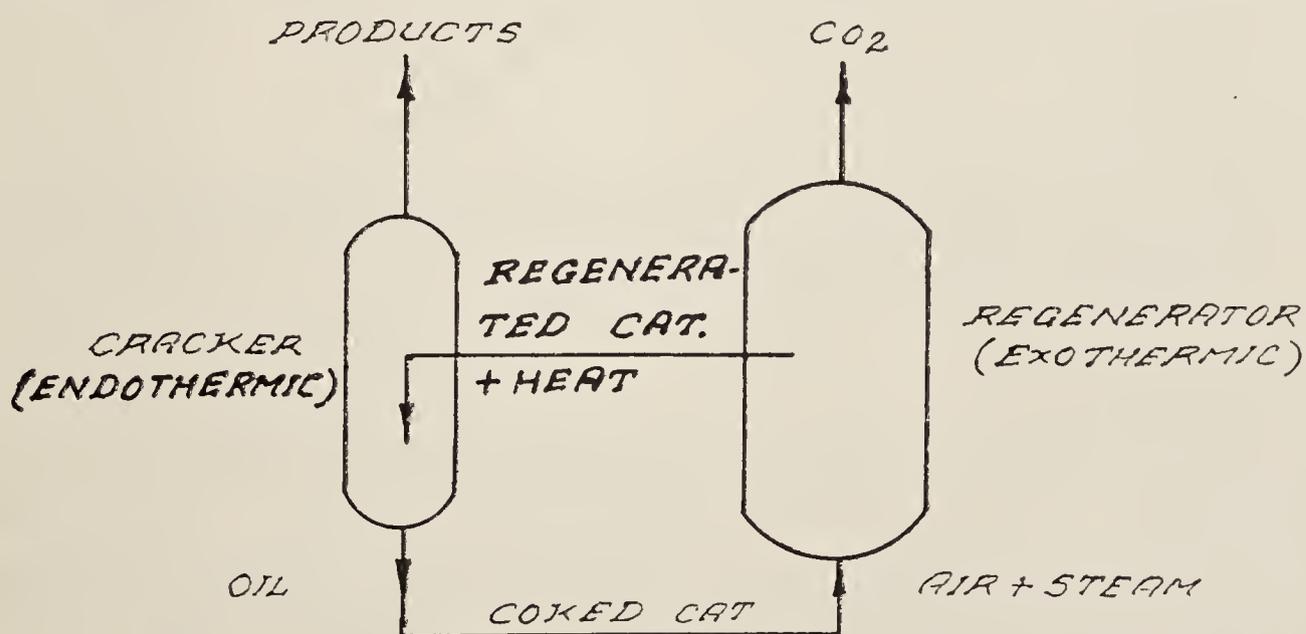


Figure 8. Thermal balance between endothermic cracking and exothermic coke burn-off or regeneration in a catalytic cracker.

of the regenerator. Mobil Oil²⁶ has tried to make the catcracker autothermal and thermally independent of the regenerator so that the greater activity of molecular sieve catalysts can be fully utilized instead of being limited as hitherto by the capacity of the regenerator. This is an outstanding example of molecular engineering of shape-selective catalysts, initiated by Weisz²¹.

In this catalyst the endothermic cracking of gas oil in the larger zeolite cavities is accompanied by a simultaneous exothermic combustion of only C_1 to C_3 gases selectively over platinum in smaller zeolitic cavities inaccessible to the larger molecules in the gasoline fraction. This compound catalyst is schematically shown in figure 9. By adjusting the oxygen supply, the combustion can be controlled so that the catalyst temperature can be maintained constant at any degree of conversion in cracking. In practical operation, the catalyst is first heated up by combustion of methane and oxygen to the cracking temperature and then gas oil is slowly introduced. As C_1 - C_3 gases formed from cracking undergo combustion, the methane supply is slowly reduced and finally stopped when heat balance is established between cracking and combustion and the reactor becomes "critical".

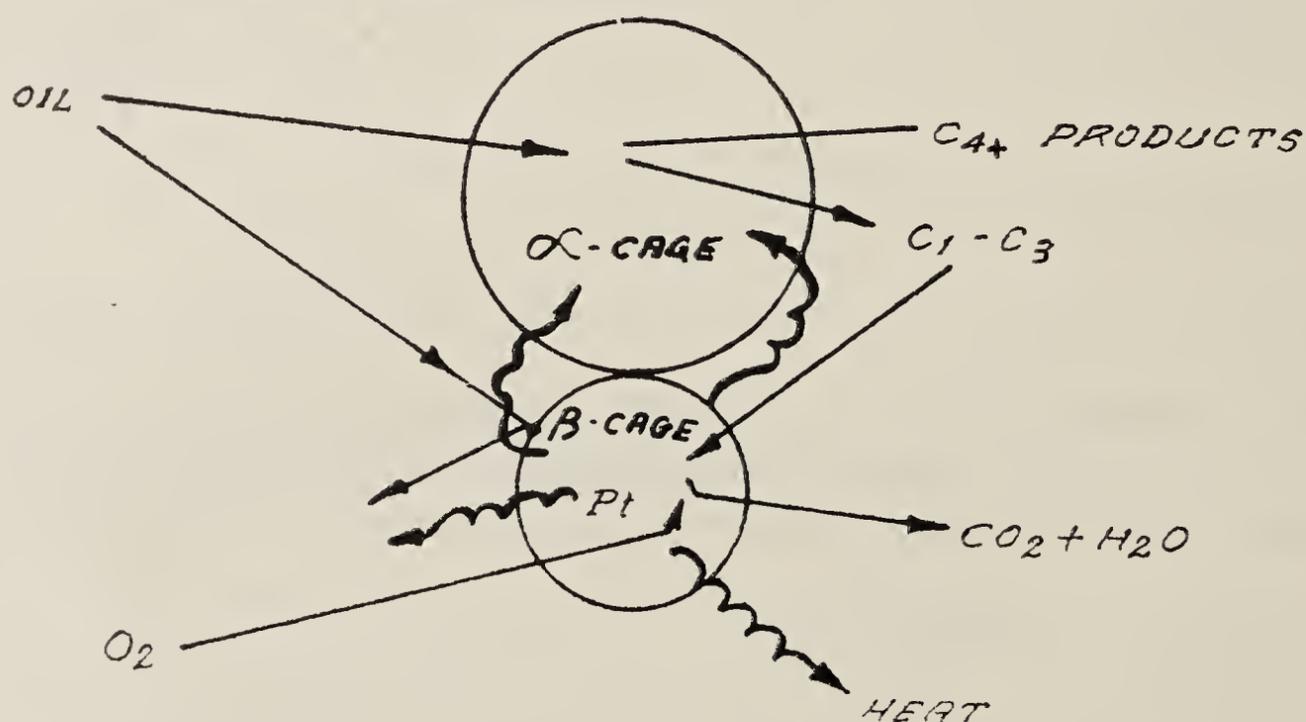


Figure 9. Principle of autothermal catalytic cracking by producing heat from the shape-selective combustion of C_1 - C_3 gases only in the smaller cavities of molecular sieve.

2.10 SELECTOFORMING

Conventional catalytic reforming is carried out on bifunctional catalysts like Pt-alumina. The main desired reactions here are the formation of aromatics from dehydrogenation of cyclohexanes, dehydroisomerization of cyclopentanes and dehydrocyclization of paraffins, and the skeletal isomerization of n-paraffins to iso-paraffins. The complete aromatization of virtually all initially available cycloparaffins leads to ultimate aromatic contents of 30-70% depending on the composition of the naphtha, i.e., its

initial naphthenes + aromatics contents. When still higher aromatics concentration (octane-number quality) is desired, reforming catalysts have been operated under more severe conditions, when a large part of the low-octane paraffins are eliminated by hydrocracking reactions to form gases. This reduction of paraffin concentration by hydrocracking, however, proceeds indiscriminately; both the unwanted n-paraffin and the highly desired high-octane iso-paraffin undergo cracking. Worse still, the iso-paraffin is preferentially cracked, since the cracking reactivity of primary : secondary : tertiary carbonium ions are in the ratio 1 : 2 : 20.

Introduction of a shape-selective catalyst in the final stage of reforming has led to a preferential cracking of only the n-paraffins, preserving the high-octane iso-paraffin content in the liquid reformat. This selectoforming process, developed by Mobil, gives a higher liquid reformat yield with a higher octane number. Selectoforming process is in use in four Mobil refineries in Europe. This improvement achieved in selectoforming is shown in figure 10. Further details on selectoforming have been given by Chen *et al.*²⁷.

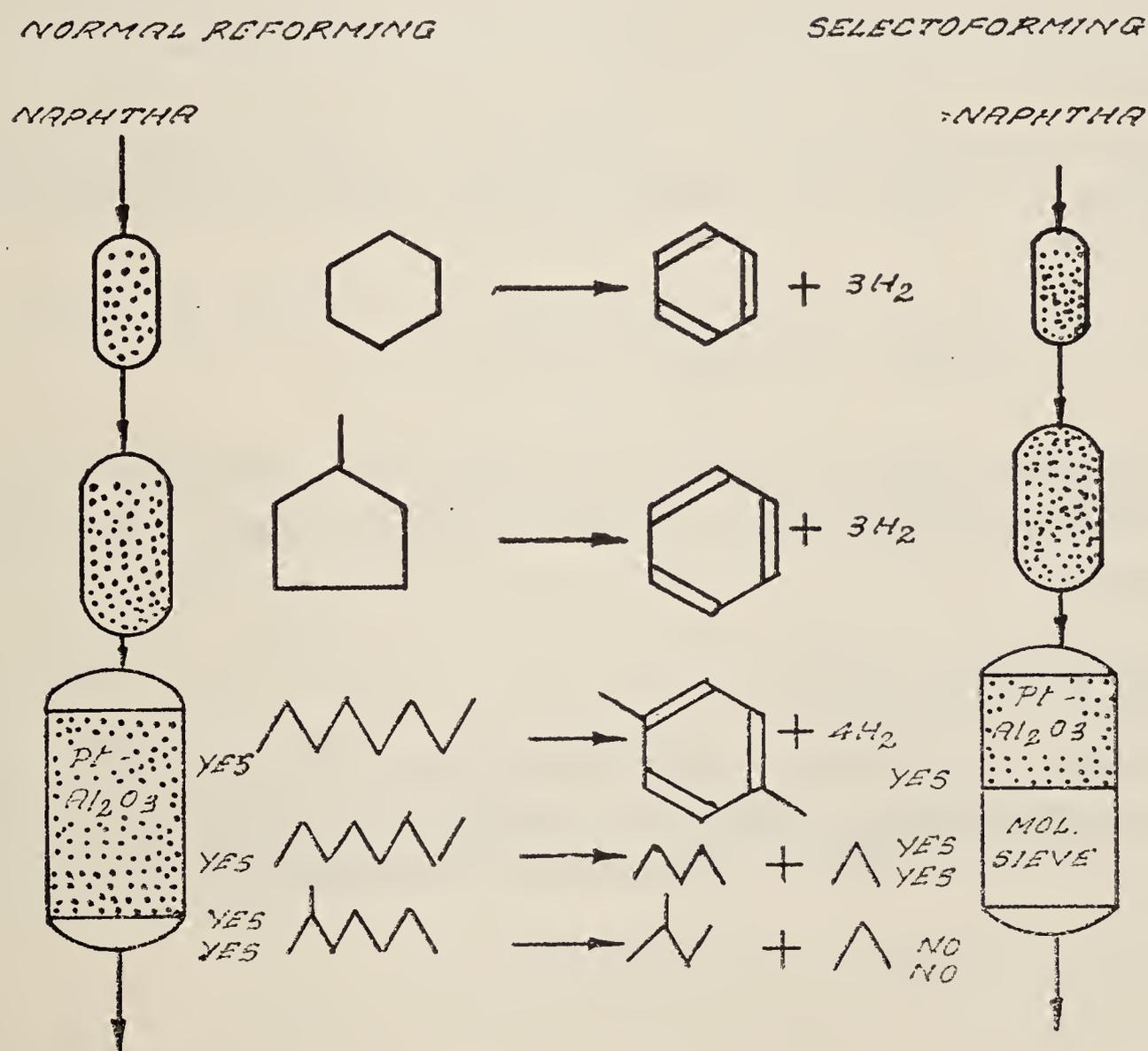


Figure 10. In selectoforming process the straight-chain paraffins are preferentially cracked off on a molecular sieve catalyst. Shape selectivity prevents the cracking of the branched paraffins which have high octane number and hence are highly desired in the liquid reformat product.

2.11 CATALYTIC REFORMING ON MOLECULAR SIEVES

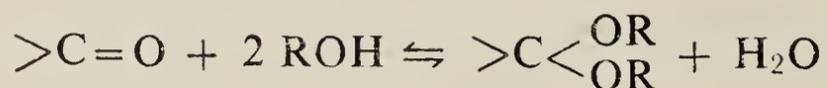
A more spectacular application of molecular sieves in catalytic reforming is reported in a Mobil patent²⁸. Reforming is carried out here *in the absence of platinum and in the absence of hydrogen pressure*. The advantages claimed are :

- 1) High iso- to normal-paraffin ratio, exceeding thermodynamic equilibrium. Consequently high octane number, reducing the need to produce high aromatic content.
- 2) Conversion of naphthenes to aromatics by hydrogen transfer rather than dehydrogenation produces a reformat of reduced aromatic content. For the same high octane number, a high isoparaffin content is preferred over a high aromatic content. (High ratio of aromatics to isoparaffins causes deterioration of gasoline quality as measured by various performance indices as the Rumble Rating).
- 3) Avoidance of the highly endothermic dehydrogenation reaction eliminates the necessity for additional heating devices in the reactor system.
- 4) Reforming reaction can be carried out in the absence of additional hydrogen. Hence no hydrogen recirculation and expensive high-pressure equipment.
- 5) No pretreatment of the feedstock is necessary, reforming may be carried out in the presence of nitrogen and sulphur.

This atmospheric-pressure reforming on molecular-sieve catalyst, if proved successful on a commercial scale, will be a major breakthrough in petroleum processing.

2.12 SYNTHESIS OF ACETALS

Roelofsen *et al*²⁹ have found that acetals can be prepared in high yield from the corresponding aldehydes or ketones and alcohols using molecular sieves to shift the equilibria by adsorption of the water formed.



Examples include acetals of acetone, cyclohexanone, isobutyraldehyde and benzaldehyde, using proton-exchanged Zeolon-500 or mordenite as catalyst. Here actually the outer surface of the sieve catalyzes the acetal formation while the inner surface selectively adsorbs and traps the water formed.

2.13 ZEOLITES AS OXIDATION CATALYSTS

While the sodium ions in zeolites have to be substituted by other metallic or hydrogen ions to get catalysts for carbonium-ion reactions of hydrocarbons, for oxidation of hydrocarbons the sodium zeolites are found to be the most active. For instance, the sequence of activity for X-type molecular sieve for oxidation of hexane isomers was found³⁰ to be $\text{NaX} > \text{MnX} > \text{CaX}$. Kinetics of oxygen uptake were different for NaX and CaX. No reaction occurred in the absence of oxygen, but with oxygen present, carbon deposition occurred as an important side reaction. Cracking varied in the order $\text{CaX} > \text{MnX} > \text{NaX}$. It was proposed that non-heterogeneous processes were initiated at the zeolite surface. The superior oxidation activity of NaX was attributed to its greater ability to initiate reactions by H-atom abstraction and to sustain free-radical reactions, whereas CaX and MnX favour carbonium-ion reactions.

The above work emphasizes the need to pay more attention to NaX and similar alkali-metal zeolites in catalytic reactions where radical mechanisms can be expected. The X-sieve should be superior to the Y-sieve in this respect, since the latter is a more vigorous carbonium-ion catalyst. The ability of zeolites to physically adsorb hydrocarbons at high temperatures also makes them potential agents for heterogeneous initiation of homogeneous reactions.

3. CONCLUDING REMARKS

Zeolitic molecular sieve catalysts have added new dimensions to heterogeneous catalysis, particularly with regard to super-activity, shape selectivity and higher hydrogen-transfer efficiency. In these respects they are the first inorganic catalysts to approach even remotely the performance of biochemical or enzyme catalysts. Hence they serve to some extent to bridge the vast gap between chemical and biochemical catalysts. In the meantime, industrial applications of molecular sieves as adsorbents, desiccants, molecular separators and heterogeneous catalysts are opening up entirely new vistas in chemical, petroleum and petrochemical industries.

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Homogeneous catalysis by metal complexes in solution

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Homogeneous catalysis has gained importance in recent years due to the advancements in the theories of the co-ordination bond and the development of techniques for the synthesis of new and novel coordination and organometallic compounds. These compounds have been used quite successfully as catalysts in a variety of reactions¹, such as hydrogenation and oxidation of alkenes and alkynes, carbonylation and carbon monoxide insertion reactions of a variety of organic compounds, polymerization of alkenes and recently the metathesis or disproportionation of alkenes.

Homogeneous catalysis has certain advantages and disadvantages over the related field of heterogeneous catalysis which has got a greater, wider and a well established industrial base. One of the greatest advantages of homogeneous catalysis is the amenability of the reactive species to experimental studies. The structure and thermodynamic parameters of the species in solution can be studied by a variety of methods like equilibrium data, u.v., i. r., n.m.r., e.p.r. and Mossbauer spectroscopy and recently the application of X-rays² in structural investigation of the species in solution. The kinetic data can be correlated to the nature of the species in solution and a reasonable mechanism suggested for the reaction. A good catalyst should be thermodynamically stable to be formed in solution and kinetically labile to participate in a reaction. The structural parameters and the correlation of the energy levels to the reactivity pattern of a catalyst have just started to be understood by chemists. The ultimate goal would be to seek the best theoretical compromise between the thermodynamic stability and the lability of the catalytic species in solution and to arrive at the optimum conditions for the best tailor-made catalyst. With the recent developments in the theory and experimentation of coordination compounds this goal is not too far away.

A major disadvantage of homogeneous catalysis is the problem of the recovery of the catalyst from the product of reaction. This severely hampers

the use of a homogeneous catalyst for industrial purposes because recyclization is either difficult or would be achieved with the loss of catalytic efficiency. This difficulty has been overcome in certain cases by the use of polymer attachment to the active catalyst which can be removed from the reaction products by gel filtration, membrane filtration or reverse osmosis. These processes have yet to be developed and it will take some time before they could be industrially exploited.

Some of the reactions in which homogeneous catalysis has either made a mark in industry or offer a good potential for industrial uses are as follows :

1. Nitrogen fixation
2. Oxidation and hydration of the olefins
3. The oxo reaction
4. Polymerization of olefins by soluble Zeigler catalysts and coordination compounds
5. Olefin metathesis
6. Polymer based homogeneous catalysis.

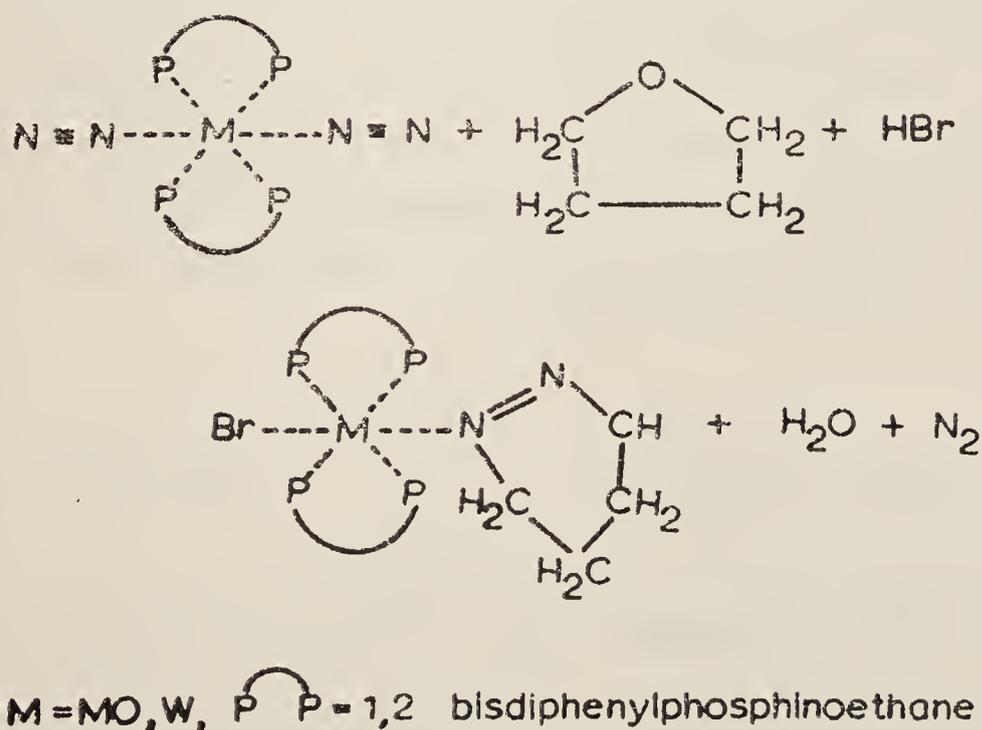
1. NITROGEN FIXATION

Molecular nitrogen is an extraordinary stable¹ molecule with the bond dissociation energy of 225 K cal/mole. Fixation of nitrogen involves the production of nitrogen compounds from molecular nitrogen. The extraordinary stability of N-N bond is reflected in the methods of its fixation which involves an input of energy for the activation and the subsequent dissociation of the N-N bond. One of the well known methods for the fixation of nitrogen is the Haber's process which requires the stringent conditions of 300-350° and 320-400° atmospheres in the presence of a heterogeneous iron catalyst. In contrast, the biological fixation of molecular nitrogen by enzymic activation in the soil and root nodules for plants proceed by a homogeneous pathway and take place at ambient temperature and pressure. The natural fixation of nitrogen is important in maintaining the fertility of the soil and a balance of free and fixed nitrogen in the geo- and biospheres.

During the past fifteen years there have been several attempts in various laboratories to understand and copy the bacterial process of fixation of nitrogen in homogeneous solutions at ambient conditions. Such a process is expected to surpass Haber's process in energy expenditure and efficiency and would be a breakthrough in the green revolution of the world. The first streak of hope for this breakthrough came with the discovery of the first transition metal dinitrogen complex (coordinated molecular nitrogen is referred to as dinitrogen) by Allen and Senoff³. During the past eleven years a wide variety of molecular nitrogen complexes of transition metals have been

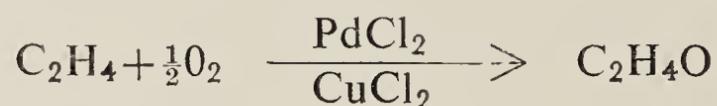
prepared by several investigators¹ and to date the list includes about hundred and twenty compounds, a dozen of which have been fully characterised by X-ray crystallography. Molecular nitrogen in these complexes is coordinated to the metal ion in a linear manner as is the case in the isoelectronic molecule CO. The metal-dinitrogen bond is however much weaker than a metal-carbonyl bond and there is not much reduction in the $N \equiv N$ bond order in these compounds, as compared to a $C \equiv O$ bond in metal carbonyls. It is because of this reason the coordinated nitrogen does not undergo a facile reduction with mild reducing agents and requires powerful reducing agents like $LiAlH_4$, $NaBH_4$ or metallic lithium or aluminium for reduction. Though stoichiometric reduction of coordinated nitrogen to hydrazine or ammonia has been achieved by a variety of costly reducing agents like sodium naphthylide⁴⁻⁶ or metallic aluminium⁷ and AlX_3 in nonaqueous solutions, nobody has achieved to date a true catalytic reduction of dinitrogen to ammonia under ambient conditions. Fixation of nitrogen in aqueous solution has met various degrees of success by Haight and Scott⁸, Yotsimirskii and Pavlova⁹ and recently by Taqui Khan and Martell¹⁰, none of these processes are catalytic and the yield of ammonia varied from 0.05-0.4 mole per mole of the catalyst. At present, the difficulty in a true catalytic process seems to be the recycling of the reducing agent to give a steady supply of reducing electrons to coordinated dinitrogen at the proper potential. Attempts to attack coordinated dinitrogen by oxygen through a mixed ligand dioxygen-dinitrogen complex¹¹ are also being continued and are expected to yield some significant results.

Besides fixation of nitrogen, activation of molecular nitrogen in homogeneous phase can lead to insertion of nitrogen in an organic compound to yield heterocyclic nitrogen compounds. Metal ion catalysed insertion of dinitrogen in tetrahydrofuran¹² to give coordinated tetrahydropyridazido group ($C_4H_7N_2$) is a very significant step towards this end and offers a potential field of research in homogeneous catalysis.

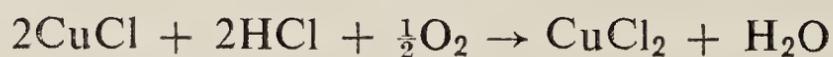
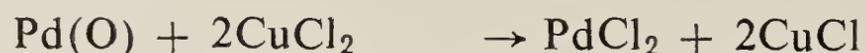


2. OXIDATION AND HYDRATION OF THE OLEFINS.

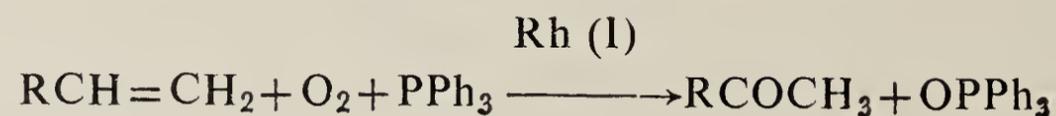
In the case of oxidation and hydration of olefins, the metal ion catalyst participates in a two electron oxidation-reduction cycle¹³⁻¹⁵ in the presence of a suitable oxidizing agent. The most important and versatile metal ion used for the oxidation of alkenes has been Pd (II) because of its noted property of formation of strong π -complexes with the alkenes. In Walker's process Pd (II) catalyzes the oxidation of ethylene to acetaldehyde and substituted alkenes to aldehydes or ketones. The presence of Cu (II) as catalyst in the system oxidizes palladium (O) back to palladium (II) and keeps the process continuous. In the presence of Cu(II), the oxidation of ethylene may be represented by the net reaction.



Propylene and higher alpha olefins give a mixture of aldehydes and ketones. Internal olefins usually give ketones as the main product. The reaction takes place smoothly at room temperature. The rate of oxidation of a particular olefin depends on the nature of the olefin, the nature of the palladium (II) compound employed, the acidity of the medium and the nature and the concentration of anions in solution¹³. The oxidation of ethylene with the Pd (II)-Cu (II) catalytic system may be represented by the sequence of reactions :

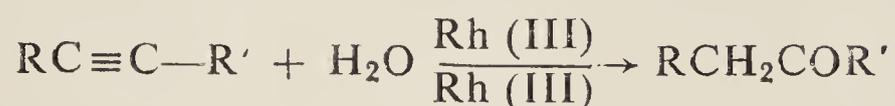


Direct epoxidation of the olefins by molecular oxygen have been achieved with those metal ion catalysts that form a dioxygen complex with molecular oxygen. Oxygen atom transfer takes place within the coordination sphere of the metal ion resulting in a high yield of the epoxide or a ketone. Rhodium (I)¹⁶ and ruthenium (II)¹⁷ complexes are good catalysts in this reaction. The reaction results in the oxidation of one or two moles of the olefin by the metal catalyst and molecular oxygen.



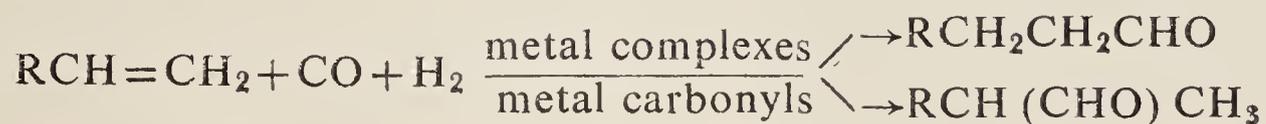
In case where one molecule of the olefin is oxidized, a molecule of the coordinated ligand like triphenylphosphine also gets oxidized to the phosphine oxide.

Hydration of alkynes is catalyzed by Ru(III) or Rh(III) chloride in aqueous-ethanolic medium^{18, 19}. Hydration of alkynes involves no change in the oxidation state of the metal ion catalyst. The net reaction involves the addition of hydrogen and hydroxyl ions across the triple bond, followed by rearrangement of the bonds to form an aldehyde or ketone. The yields of the products are above 80% in most of the cases.

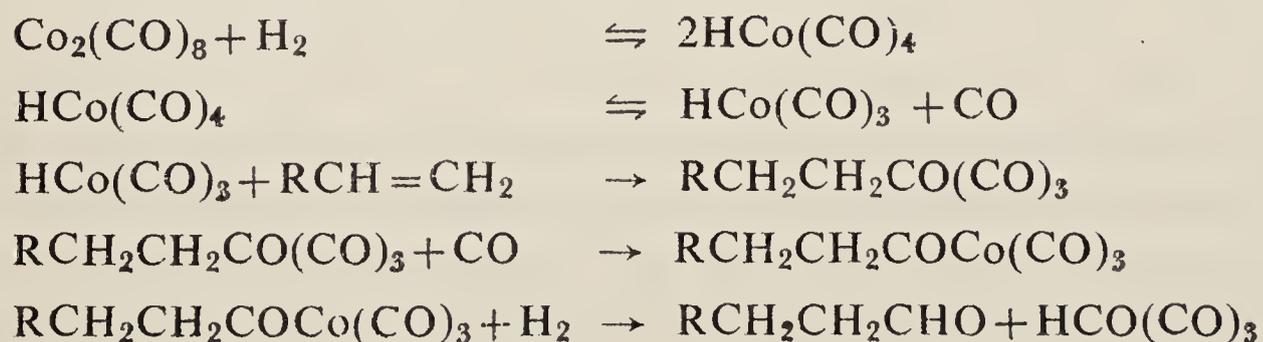


3. THE OXO REACTION

The oxo reaction was developed by Otto Roelen^{20,21} through a modification of the Fischer-Tropsch synthesis in order to produce aldehydes or ketones, rather than hydrocarbons as a main product. The reaction is also referred to as hydroformylation and involves the addition of hydrogen and formyl groups across the double bond of an alkene. The general oxo reaction may be represented as follows :



The oxo reaction is catalyzed by a variety of metal carbonyls and metal complexes like cobalt carbonyls²², rhodium carbonyls²³, iron carbonyls²⁴, $\text{RhCl}(\text{PPh}_3)_3$ ²⁵, and $\text{RuH}(\text{CO})\text{Cl}(\text{PPh}_3)_3$ ²⁶. The primary products of the oxo reaction are aldehydes or ketones which can be readily converted to secondary products such as alcohols by subsequent hydrogenation. The oxo reaction proceeds over the temperature and pressure ranges of 50-200° and 100-400 atmospheres. A 1 : 1 molar ratio of CO and H₂ is usually employed. Both straight and branched chain aldehydes are obtained, the ratio depending on the temperature and the nature of the catalyst. Breslow and Heck²⁷ and Heck²⁸ have postulated the following mechanism for the oxo reaction :



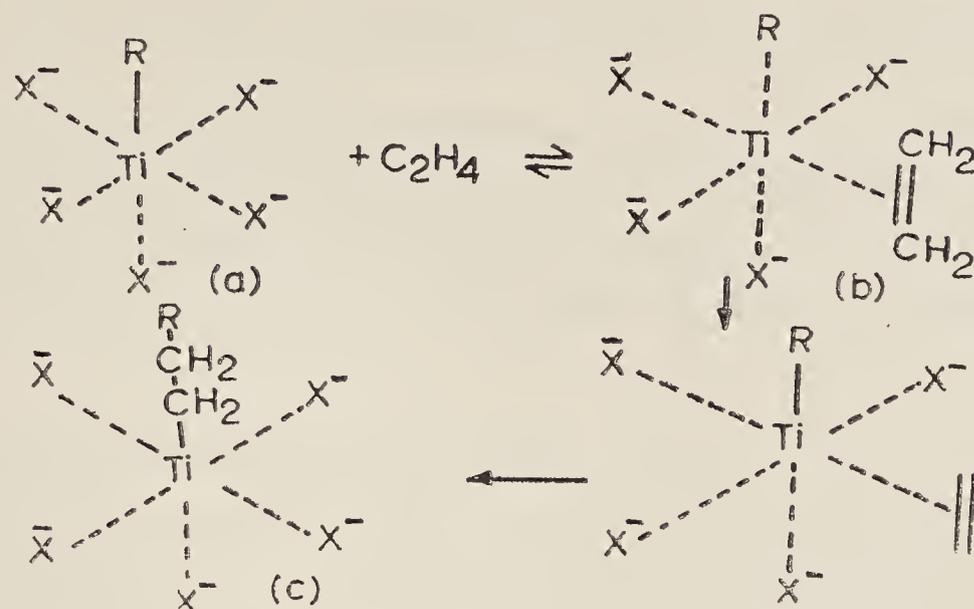
The actual catalytic species in the reaction is the coordinately unsaturated hydridocobalttricarbonyl $\text{HCo}(\text{CO})_3$ formed by the dissociation of $\text{HCo}(\text{CO})_4$. In the olefin insertion step $\text{HCo}(\text{CO})_3$ is added across the double bond of $\text{RCH}=\text{CH}_2$ to form the σ -bonded alkyl species $\text{RCH}_2\text{CH}_2\text{Co}(\text{CO})_3$. In the next step, insertion of CO takes place in the metal-C bond to form the acyl compound $\text{RCH}_2\text{CH}_2\text{COCO}(\text{CO})_3$ which on hydrogenolysis gives the aldehyde and the catalyst. The use of rhodium carbonyls^{23,25,26} permit the reaction to be conducted at 50 atmospheres and room temperature. Evans *et al*²⁶ have used $\text{HCORuCl}(\text{PPh}_3)_3$ for the hydroformylation of alkenes at ambient temperature and pressure and obtained good yield of the straight chain aldehydes. Iron carbonyls require a higher temperature (180°) for the reaction and are accordingly used for the hydrogenation of aldehydes to alcohols under oxo conditions. From a small beginning of five million pounds in 1948, the commercial potential of the oxo process is more than about 1000 million pounds to date. About 70% of the oxo production capacity is utilized for the synthesis of C_8 , C_{10} and C_{13} alcohols that are generally used as plasticizers, and for the production of detergents and lubricants, the remainder is involved in C_4 aldehyde production. The metathesis of hydrocarbons and the Zeigler catalysts provide inexpensive raw material olefins for the one step oxo synthesis of alcohols.

4. THE POLYMERIZATION OF ALKENES AND ALKYNES

The polymerization of alkenes and alkynes to long polymer chains of high molecular weight have been achieved by Zeigler catalysts^{29,30} which consists of an organometallic compound of titanium or vanadium of the composition $\text{R}_n\text{MCl}_{6-n}$ and an aluminum alkyl $\text{AlR}_n\text{Cl}_{3-n}$. Depending on the catalyst, stereospecific or non-stereospecific polymerization of the olefin takes place at the ambient conditions. Tris-2-4-pentanediono complexes of V(III) and Cr(III) in the presence of $\text{AlR}_n\text{Cl}_{3-n}$ have also been used^{31,32} as soluble Zeigler catalysts. Besides, Zeigler catalysts, Ru(III) chloride, Rh(III) chloride, Bis(allyl)- NiX_2 and ReCl_5 have also been used¹ for the polymerization of unsaturated hydrocarbons.

Polymerization of alkenes and alkynes proceed through a sequence of steps³³ in which a series of the hydrocarbon molecules are inserted in a metal-ligand bond leading to polymers of different molecular weights. In stereospecific polymerization the coordination requirements of the metal ion imposes stereospecificity on the growing polymer chain. Polymerization actually takes place on the transition metal centre, the aluminium cocatalyst usually alkylates the transition metal ion and prevents its

further reduction to the metallic state. The mechanism of polymerization may be represented by the following :



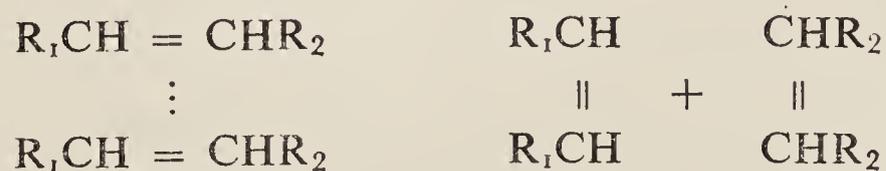
The insertion of an olefin in a metal-alkyl bond in (a) takes place through an intermediate formation of a complex (b) which rearranges to form a new metal-alkyl complex (c). The process of chain growth continues till it is terminated either by a chain transfer to the monomer unit or to the solvent.



The stereospecificity of the polymer is controlled by the coordination requirements of the metal ion which allows the olefin to coordinate in a specific stereo configuration.

5. METATHESIS OF THE OLEFINS

One of the most important reactions from the view point of petroleum industry is the metathesis of olefins³⁴⁻³⁷: an essentially trans alkydenation reaction.



The reaction is catalysed by heterogeneous catalysts which consist of transition metal oxides or carbonyls deposited on high surface area supports or homogeneous catalysts that are mostly molybdenum, tungsten or rhenium compounds low in valence electrons (d^2 - d^4 configuration) and capable of seven

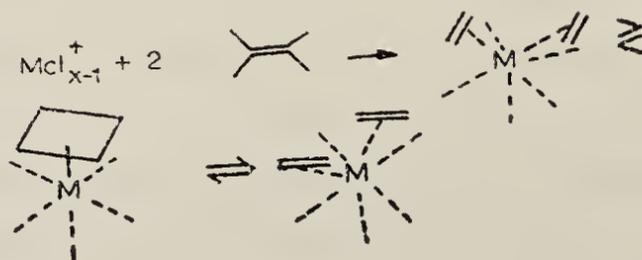
coordination. The presence of an aluminum lewis acid is essential for this reaction which proceeds initially by an ion pair formation of the type :



The rate of metathesis of olefins increases with the conductivity of the reaction mixture thus lending support to the ion-pair formation. The double bond scrambling proceeds smoothly at room temperature with the energy of activation³⁸ as low as 6-8 K cal/mole.

Photolysis of $W(CO)_6$ in CCl_4 by radiation of 335 nm ($1T_{2g} \rightarrow 1A_{1g}$) afforded³⁹ $W(CO)_5Cl$ which caused a 50% metathesis of trans-2-pentene to 2-butene and 3-hexene mainly in the trans form. The metathesis was independent of temperature and ceased when CCl_4 was replaced by hexene in the initial irradiation. In the latter case it was believed that the active tungsten species allowed coordination of the olefins in the transposition so that metathesis reaction cannot take place. The species $W(CO)_5Cl$ however, eliminated cis CO groups due to an increased trans effect of CO over Cl and caused olefin coordination in cis positions resulting in 50% metathesis. Photolysis of $W(CO)_6$ or $Mo(CO)_6$ thus seems to be a very convenient method for the metathesis of olefins and in this case the presence of aluminium lewis acid is not necessary.

Though several mechanisms^{40,41} that differ in details of the energetics and the symmetry requirements of the transition state have been proposed, a feature that is common to all these mechanisms is the requirement of a seven coordinate geometry of the metal ion and the availability of cis positions³⁹ to be coordinated by the olefin residues. As in Zeigler system, the active catalytic centre is the cationic molybdenum or tungsten species that coordinate with the molecules of the olefin in the cis positions to form a cyclic quasicyclobutane or pseudocyclobutane transition state in which all the four carbon atoms are equally related to the transition metal ion. The reaction thus proceeds in a concerted manner.



Since the bond energies of the various hydrocarbon species are almost the same, the distribution of the various species at equilibrium is governed by entropy consideration. The rate of metathesis reaction decreases³⁷ in the order:



The reaction has got a wide application in petroleum industry where a surplus of any one of the hydrocarbons can be converted to other products. The process for the conversion of surplus propylene to ethylene and butene has been industrialized. Metathesis of alkenes is also used in the preparation of macrocycles and catenanes.

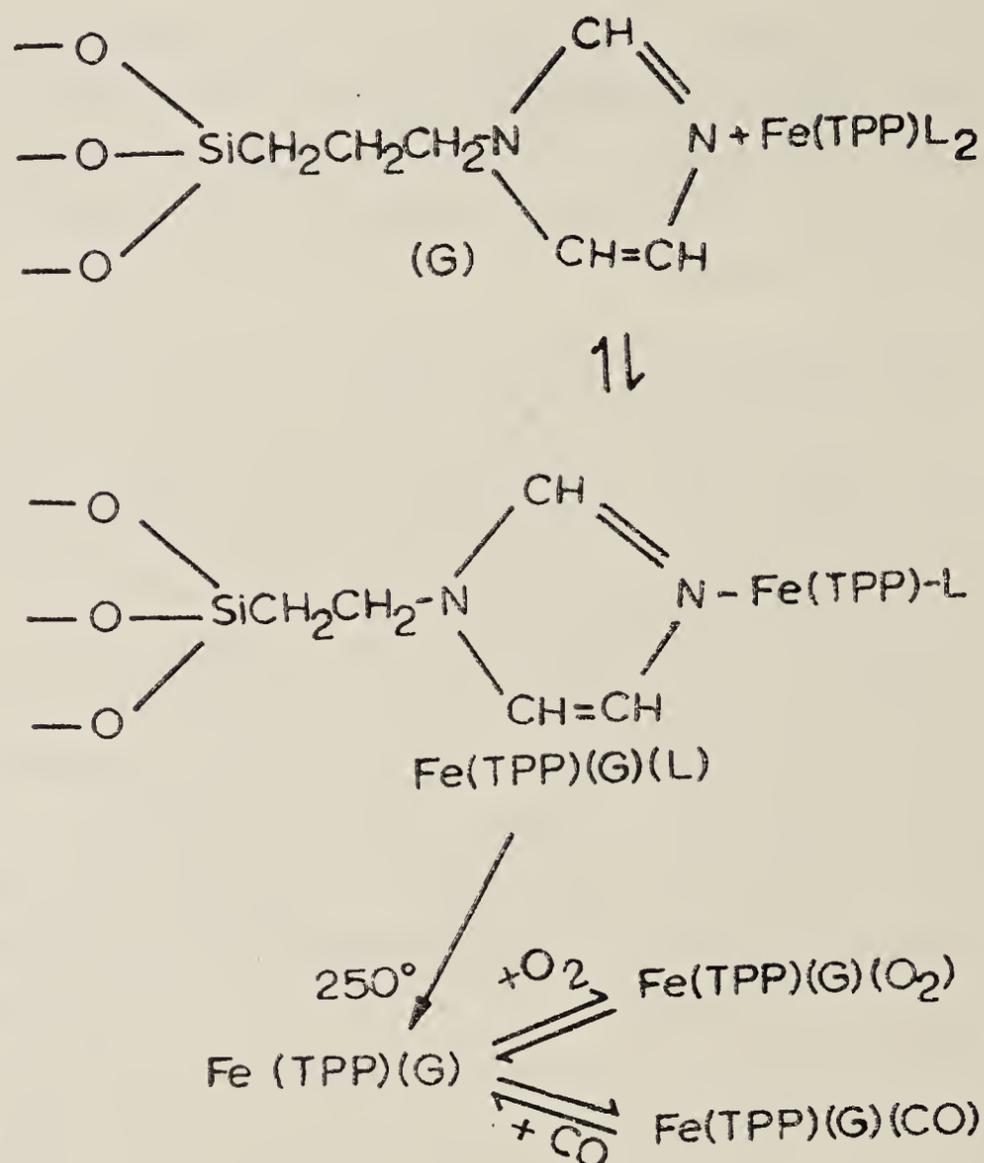
6. POLYMER BASED HOMOGENEOUS CATALYSIS

Homogeneous catalysis has gained lot of importance in recent years by the development of polymer attached catalysis. The active catalytic centre in these reactions is a simple coordination compound bound either to an inorganic carrier material or an organic crossed linked polymer. The polymer bound catalytic site has various degrees of accessibility to the substrate and the catalytic process becomes similar to a metalloenzyme where the polypeptide bound prosthetic group of the enzyme is the active site and the polypeptide chain improves the catalytic activity by providing polarizing groups or a hydrophobic surrounding. The major disadvantage of catalyst separation in homogeneous catalysis is overcome here by the facile removal of the catalyst from the reaction medium by filtration, gel filtration or membrane filtration and the catalysts can be recycled without a significant loss in catalytic activity.

The inorganic polymer supports used to date are mainly porous glass support⁴² or bound silica gel^{43,44}. The glass support polymers are prepared⁴² by treating a suitable glass surface with trichloro- or triethoxy silanes to form glass-Y₃-Si-R-Y surface groups. By reaction with metal halides or suitable metal complexes, stable polymer bound complexes of the type [glass-O-Si-R-Y]_m-MCl_n-l_n (M=Ti, Mo, V, L=cyclopentadienyl group) are obtained. These polymers are stable at higher temperatures and do not swell in organic solvents.

Silica gel modified imidazole^{43,44} attached iron (II) tetraphenylporphyrin Fe (TPP) (G) has been found⁴⁵ to absorb O₂ and CO reversibly to form Fe (TPP)(G)(O₂) or Fe(TPP)(G)(CO), respectively. Polymer bonding has made it possible for the first time to prepare a 1:1 Fe (II)-O₂ complex without dimerization to an oxo bridged Fe(III)-O-Fe (III) species. In the silica gel attached Fe(II) porphyrin, the rigid support of the polymer prevents the dimerization of the complex to the oxo bridged species cited above and makes possible the formation of a simple Fe(II)-O₂ complex similar to oxyhaemo-

globin. Silica gel attached 3-imidazolyl propyl (G) groups are prepared^{43,44} and then reacted with dipyridinetetraphenylporphyriniron (II), complex $\text{Fe}(\text{TPP})\text{L}_2$ to form $\text{Fe}(\text{TPP})(\text{G})\text{L}$. By heating $\text{Fe}(\text{TPP})(\text{G})\text{L}$ to 250° the active complex $\text{Fe}(\text{TPP})(\text{G})$ is obtained by the loss of pyridine (L).



These resin bound porphyrin and related complexes provide a method to duplicate natural oxygen carriers in the laboratory and study their model reactions.

Lattice silicates and zeolites⁴⁶⁻⁴⁸ provide another important inorganic base for the study of a variety of polymer-based catalyzed homogeneous reactions. Since zeolites, lattice silicates and molecular sieves have openings of a particular order of magnitude, molecular sieve-based catalyst can provide specificity and selectivity in catalytic reactions of a mixture of isomers with subtle differences in their size and complexing properties. The field has opened up vast avenues of research for future.

A variety of organic polymer supports have recently been used for catalytic reactions. These include functionalized soluble polystyrene, polyethylene glycols, polyvinyl chloride, styrene-divinylbenzene polymer and nylon.

Polystyrene based tris(triphenylphosphine)-chloro-rhodium (I) and hydridocarbonyltriphenylphosphine-rhodium (I) can completely hydrogenate⁴⁹ alkenes at 22° and one atmosphere of H₂. Polystyrene based⁴⁹ RhH (CO) (PPh₃) can hydroformylate 1-pentene to C₆ aldehydes at 22° and one atmosphere pressure of CO + H₂ (1:1). The rate of hydrogenation of 1,5 cycloocta 1,5 diene over styrene-divinylbenzene anchored IrCl (CO) (PPh₃) was much faster⁵⁰ than the homogeneous hydrogenation with IrCl (CO) (PPh₃)₃ indicating that the polymer attachment has increased the accessibility of the substrate olefin to the catalyst. Nylon supported⁵¹ RhCl₃ · 3H₂O, H₃RhCl₆, Rh (CO)₂Cl₂ and H₂IrCl₆ were found to be quite effective in the hydrogenation of benzene to cyclohexene (10%). The optimum temperature for the reaction is 140° with the order of catalytic activity Ir ≫ Rh. Styrene-divinylbenzene bound polymer system of the type [polymer]_n-(C₅H₅) MCl_n (M = Ti (IV), Zr(IV), Mo(V), Nb (V) and W(VI)) have been prepared⁵² by first binding cyclopentadiene to a styrene-divinylbenzene (20%) copolymer and then converting it to the cyclopentadienyl anion. The resin bound cyclopentadienyl anion is then treated with the corresponding metal chlorides to get the rigid polymer bound complex. The catalysts may be reduced by one or two electrons without undergoing dimerization and are about six times as reactive as the corresponding reduced metal halides in the hydrogenation of olefins.

The examples of the polymer based homogeneous catalysts cited here are by no means exhaustive and complete and are meant to indicate the vast scope of such reactions. The field has lot of promise in industrial catalysis and model enzymic catalysis in future and can duplicate many enzymic systems especially in the synthesis of useful products like carbohydrates and proteins.

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