

ABSTRACTS OF PAPERS PRESENTED AT THE 49TH ANNUAL MEETING OF THE INDIAN ACADEMY OF SCIENCES AT PUNE, 7-9 NOVEMBER 1983.

The 49th Annual Meeting of the Indian Academy of Sciences, founded in 1934 by the late Prof. C. V. Raman, was held at the National Chemical Laboratory, Pune from 7th to 9th November 1983.

The objective of the Academy continues to be to promote the progress and uphold the cause of science, both pure and applied, by *inter alia* publishing the results of important scientific researches through its journals, proceedings and transactions; publishing books, memoirs and reviews on scientific subjects; undertaking and directing scientific enterprises of all-India significance; organising scientific discussions through congresses, conferences, symposia and seminars; and securing and administering funds, grants and endowments for the furtherance of scientific research.

Publication occupies the pride of place amongst all the activities of the Academy and is of a pioneering nature. At present it brings out the following eleven journals: 1. *Proceedings* — Chemical Sciences, 2. *Proceedings* — Earth and Planetary Sciences, 3. *Proceedings* — Mathematical Sciences, 4. *Proceedings* — Plant Sciences, 5. *Proceedings* — Engineering Sciences, 6. *Proceedings* — Animal Sciences, 7. *Pramana* - a Journal of Physics — in collaboration with Indian National Science Academy and Indian Physics Association, 8. *Bulletin of Materials Science* — in collaboration with the Indian National Science Academy, 9. *Journal of Biosciences* 10. *Journal of Astrophysics and Astronomy* and 11. *Current Science* (a Fortnightly Journal of Research.)

The annual meetings of the Academy serve to bring together the Fellows spread all over the country and enable them to make personal contact with one another and exchange ideas and information. To commemorate the work and memory of Prof. C. V. Raman, the Academy instituted the Raman Chair in 1972; This is a Visiting Professorship supported by the interest from a Rs. 6 lakhs endowment by the Department of Science and Technology, Govt. of India. The Visiting Professorship is for a period of 3-6 months. Eminent scientists from any country and working in any scientific discipline can be invited to occupy this chair.

Back in 1943 the Academy established the prestigious Raman Research Institute at Bangalore. Today this Institute is an independent autonomous research institute of repute and functions under a public charitable trust created in 1971.

Recently the Academy has undertaken an important task of encouraging young scientists by instituting Associateship available to scientists below

the age of 35 years, for a period of 5 years. They are called Young Associates of the Academy. This is an important step forward in encouraging the talented young scientists. Plans are afoot to celebrate the Golden Jubilee of the Academy next year in a befitting manner and to consolidate and expand its activities even more, to further the cause of science in India.

At its 49th Annual Meeting, held at NCL, Pune, Dr. L. K. Doraiswamy, Director of National Chemical Laboratory, Pune, welcomed the gathering and appealed to the Academy to actively participate in the identification of areas of thrust in Science and Technology and to produce guidelines for Science Policy in the Country.

In his Presidential address, Prof. Ramaseshan discussed many aspects of materials science. He said that materials science had assumed greater importance in the modern age in view of the specific materials needed for the atomic weapons, space exploration and electronics. Prof. Ramaseshan explained that materials science was not just physics, chemistry or metallurgy; but it was an interaction and application of different disciplines to the problems of materials.

The application of solid-state physics and chemistry could improve the dry torch-light batteries. The knowledge of plasma physics can influence organic synthesis and polymer chemistry. It would perhaps be possible in the next five years to manufacture newsprint by using inorganic-polymer composites which could be recycled. The most exciting thing that has happened in the field of simple materials, he said, was the discovery that the cement was weak because of the air holes entrapped in it. But this has now been eliminated by a suitable surfactant. As a result, the strength of the cement is 10 times greater than the one without the surfactant.

The following programmes of scientific lectures and discussions took place on 7th forenoon: "Energy conservation — An organic chemist's attempts" by N. S. Narasimhan, University of Pune, "Unification of the forces of nature" by G. Rajasekaran, University of Madras, "Development and differentiation in plants" by M. M. Johri, Tata Institute of Fundamental Research, Bombay.

A Symposium on "Frontiers in organic synthesis" was held in the afternoon in which the following topics were discussed: "Some aspects of steroid synthesis" by G. S. R. Subba Rao, Indian Institute of Science, Bangalore, "Synthetic design of platonic hydrocarbons" by G. Mehta, University of Hyderabad, Hyderabad, "Car-3-ene, a versatile resource for important indus-

trial fine chemicals" by Sukh Dev, Multi-Chem Research, Institute, Baroda; "New stereocontrolled synthetic routes to bridged ring and alicyclic compounds" by U. R. Ghatak, Indian Association for the Cultivation of Science, Calcutta, "Synthesis of biologically active compounds with a difference" by A. V. Rama Rao, National Chemical Laboratory, Pune.

The following are the scientific talks and discussions held during the forenoon on the 8th: "Some oceanographic features of the Antarctic Ocean" by S. Z. Qasim, Department of Ocean Development, New Delhi; "Geological set-up around Dakshin Gangothri Antarctica and snow ice studies" by V. K. Raina, Geological Survey of India, Lucknow; "Chemical and environmental studies on ice and waters" by S. G. Prabhu Matondkar, National Institute of Oceanography, Dona Paula; Meteorological aspects of Antarctica" by C. R. Sreedharan; "Biological investigations in Antarctic ecosystems" by A. H. Parulekar, National Institute of Oceanography, Dona Paula. During the afternoon session the lectures consisted of "Glass transition: a veritable clue to the nature of glassy state" by K. J. Rao, Indian Institute of Science, Bangalore; "Non-covalent interactions and molecular association involving biomolecules" by M. Vijayan; "Intracranial vascular malformations" by P. N. Tandon.

On the last day, viz 9th, a Symposium on "Tissue Culture" was held during the forenoon. The following papers were presented: "Photosynthesis in cultured cells" by A. Gnanam, Madurai-Kamaraj University, Madurai; "Induction of haploids: achievements, problems and possibilities" by Sudhir Sopory, Jawaharlal Nehru University, New Delhi; "Isolation and fusion of protoplasts and somatic fertilization" by S. S. Bhojwani, University of Delhi, Delhi; and "Tissue culture and micropropagation" by A. F. Mascarenhas, National Chemical Laboratory, Pune. In the afternoon session the following lectures were presented "Neutrinos in the universe" by R. Cowsik, Tata Institute of Fundamental Research, Bombay; "Modelling and simulation in polymer engineering: A scientific approach to technology absorption" by R. A. Mashelkar, National Chemical Laboratory, Pune; "Diamagnetism — a surprise in theoretical physics" by N. Kumar, Indian Institute of Science, Bangalore; "Correlation of the tectonic belts in Kumaun Lesser Himalaya: A geochronological approach" by K. Gopalan, Physical Research Laboratory, Ahmedabad.

Two evening lectures were held on the 7th and 8th. Dr. M. G. Deo, Cancer Research Institute, Bombay, spoke on "Antileprosy vaccine — the global scene"; and Dr. B. Nag, Jadavpur University, Calcutta, on "Microprocessors".

Neutrinos in the Universe

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THE study of neutrinos in a big-bang universe yields very restrictive limits on the mass, decay properties and the number of neutrino-types. If they should have a rest mass of ~ 10 eV then they would be the most important constituents of the universe, gravitationally and would trigger the formation of large scale condensations like galactic clusters. The early results are briefly reviewed and extended to cover recent theoretical speculations on the properties of neutrinos and also the paradoxical observation of dark matter on a variety of scales from clusters, through binary galaxies, down to dwarf spheroidals.

The 'hot big-bang' theory¹ envisages the present universe as evolving from an initial singularity, which occurred about $\tau_u \approx 1.2 \cdot 10^{10}$ years ago, into its present state containing $\leq 10^{-32} \text{ g cm}^{-3}$ of baryonic matter and radiation at $\sim 2.7^\circ \text{ K}$ filling it homogeneously and isotropically, on the average. Also, the present universe is expanding at the rate of $H_0 \approx 55 \text{ km sec}^{-1} \text{ Mpc}^{-1} \approx 2 \cdot 10^{-18} \text{ sec}^{-1}$ which implies a critical density for closure of $\rho_c = H_0^2 / 8\pi G \approx 2 \cdot 10^{-30} \text{ g cm}^{-3}$. Extrapolating from the present state into the past, qualitatively one expects the temperature and densities to increase, ad infinitum. Of particular interest here is that the expected temperature is $\sim m_\pi c^2$ and $\rho_{\text{tot}} \sim 1 \text{ g cm}^{-3}$ when the universe was just one milli second old and this time marks roughly the epoch after which all the elementary particle interactions proceed essentially as we expect them from laboratory studies. Before then, with higher temperatures and densities, one takes recourse to yet speculative Grand Unified Theories for the description of the interactions and the dynamics. It is within the frame work of such a cosmology that many of the following results are derived.

Limit on neutrino masses: The temperature and density increase sufficiently rapidly as we approach the big-bang singularity that even neutrinos, with only the weak interactions, attain thermodynamic equilibrium. If their masses are sufficiently small, less than 1 MeV, then their evolution subsequent to decoupling conserves particle number, and indeed the density in phase space, because the annihilation cross-sections are exceedingly small. Their present temperature and number density is related to the observed temperature of the relict radiation as:

$$T_\nu(0) = (4/11)^{1/3} T_\gamma(0) \approx 1.9^\circ \text{ K} \quad (1)$$

$$N_\nu = N_{\bar{\nu}} = 16\pi g_\nu \zeta(3) [KT_\nu(0)/h]^3 \approx 200 \quad (2)$$

Even though $g_\nu = 2$ for massive neutrinos the right-handed component which decouples earlier because of smaller cross-sections may have a slightly lower population so that $1 < g_\nu \lesssim 2$. Each neutrino species contributes $2N_\nu m_\nu$ to the present density of the universe and their total contribution cannot exceed the total density in all forms implied by H_0 and τ_ν ; thus²⁻⁵

$$\sum_i m_\nu \leq \rho_{\text{tot}}(H_0, \tau_\nu) \{32\pi\zeta(3)(KT_\nu/h)^3\}^{-1} \quad (4a)$$

$$\leq 50 \text{ eV}$$

The sum extends over all the neutrino types like ν_e, ν_μ etc. with masses less than 1 MeV.

More massive neutrinos, if they are stable, will suffer annihilation with their anti-particles at a rate comparable to the expansion time after decoupling and their expected number density now decreases rapidly with increasing mass. Yet their contribution to the ρ_{tot} would be too high unless their mass⁶ is more than 2 GeV.

$$m_\nu > 2 \text{ GeV} \quad (4b)$$

Can neutrinos of mass in the range $100 \text{ eV} \leq m_\nu < 1 \text{ MeV}$ exist but not contribute to the mass density of the universe because they decay away into other forms? First, accelerator and astrophysical studies place rather stringent limits on the decay rate of $\nu \rightarrow x + \gamma$ and one has to consider decays of the type $\nu_h \rightarrow \nu_L \nu_L \nu_L$ where ν_h and ν_L represent heavy and light neutrinos. If such decays are there then each heavy neutrino would contribute at least three lighter neutrinos. Of for example $m_{\nu_h} \gg m_{\nu_\mu} \gg m_{\nu_e}$ then the expected number density of ν_e in the universe would increase with respect to that given by (2) at least seven fold for purely direct decays and thirteen-fold for purely cascading decays. With such an enhancement the limit on the mass of the lightest neutrino

$$m_{\nu_L} < 50/7 \text{ eV or } m_{\nu_L} < 7 \text{ eV} \quad (4c)$$

Number of neutrino types: In the hot big-bang about 25% of the baryons are synthesised into Helium nuclei, the exact fraction is controlled by the weak-interaction rate, the neutron life-time and the expansion rate of the universe the last of which is proportional to the square root of the energy density in all forms. In the relativistic radiation dominated epoch at $\tau_0 \approx 300 \text{ sec}$ each light neutrino type contributes $\sim 2\sigma T^4$ to the energy density, σ being the Stefan constant. With more species of neutrinos the density will become correspondingly higher and the universe would expand rapidly so that not many neutrons have a chance to decay within the time the universe takes to cool to a temperature of $\sim 10^9 \text{ }^\circ\text{K}$ below the photodissociation temperature of deuterium which forms the intermediate step in the Helium synthesis. In order that too much He may not be synthesised⁷ it is claimed⁸

that the number of neutrino types should be less than three.

Radiative Stability of Neutrinos: In the decay process $\nu \rightarrow x + \gamma$ the photon carries away a fraction $\eta = 1/2 (1 - m_x^2/m_\nu^2)$ of the total energy of the neutrino and the rate of the reaction can be determined by observing this photon. Such studies at reactors and particle accelerators indicate that neutrinos are sufficiently stable so that they escape, without decay, the astrophysical objects like the sun, the central stars of planetary nebulae and the supernovae which emit neutrinos copiously. The absence of any electromagnetic signal that can be attributed to the decay of the neutrinos⁹⁻¹⁵ from these objects and from the cosmological background yield the results:

$$\begin{aligned} \tau_0/m_\nu &> 10^{17} \text{ sec/eV for all } \nu\text{-flavors} \\ \tau_0 &> 10^{25} \text{ sec if } m_\nu \sim 10 \text{ eV} \\ \Gamma(\nu_e \rightarrow x + \gamma)/\Gamma(\text{total}) &< 10^{-15} \\ \Gamma(\nu_\mu \rightarrow x + \gamma)/\Gamma(\text{total}) &< 3 \cdot 10^{-6} \end{aligned} \quad (5)$$

Neutrino dominance in the Universe and Galactic clusters: Consider now the fact that the density of all visible matter in the Universe, ρ_v , is only a small fraction $\rho_c/100$ of the closure density and therefore the visible matter would have negligible effect in the early formation of condensates compared with neutrinos should they have a finite rest mass of even a few electron volts¹⁶. If indeed the neutrinos have triggered the initial condensations then evidence for this could be obtained by studying the present day dynamics of clusters. Astronomical observations of the velocity dispersion of galaxies in most clusters do indicate the presence of large amount of unseen mass in them. In modeling¹⁶ the distribution of this unseen mass as a self-gravitating Fermi-gas it has been pointed out that in a potential well of depth U the maximum number density is not given by the well-known Thomas-Fermi formula $N_\nu = 4/3\pi^2(2m_\nu U/h^2)^{3/2}$ but one half of this value¹⁰ if these neutrinos have evolved conserving their phase-space density from a thermal distribution, in equilibrium with radiation very early in the universe. It is easy to show that for such a self gravitating sphere

$$m_\nu^8 \approx 3h^6/G^3 R^3 M \quad (6)$$

For $m_\nu \sim 5-10 \text{ eV}$ the unseen mass M in the clusters and their distribution with a typical scale size R is reproduced well.

Missing mass on smaller length-scales: Further careful study has shown that unseen mass which provides additional gravitational binding exists also on the scale of the binary galaxies¹⁷, the Galaxy and even on the scale of dwarf-spheroidal galaxies¹⁸⁻²⁰. A substit-

tion of these masses and length scales in (6) yield unacceptably high neutrino masses, m_ν , as much as ~ 400 eV in the worst cases. The resolution of this paradox has been sought in terms of other forms of unseen matter like back-holes. However, it is interesting and gratifying to note that all these missing mass on different scales can be accommodated within the frame-work of neutrinos of a unique mass dominating the clustering in the universe. The essential elements of this explanation are, a) neutrinos condense only on the scale of the clusters b) the galaxies and other systems merely perturb the phase-space distribution of the neutrinos c) the perturbed phase-space distribution, derived self-consistently, exhibit many fascinating features like non-dispersive propagation of density perturbations and generate the requisite gravitational binding even on small scales. The mathematical theory based on collisionless Boltzmann equation appropriate to neutrinos with only weak interactions will be presented elsewhere

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Aspects of Steroid Synthesis

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ONE of the main strategies in the synthesis of natural products is the construction, functionalisation and fragmentation of ring systems to obtain the desired target molecule. Diels-Alder reaction has been widely employed for the construction of ring systems and this reaction involves the addition of a conjugated diene to a dienophile. There are certain limitations to this reaction since a β , β -di substituted dienophile does not participate in additions even with a reactive diene. Further preparation of conjugated dienes present difficulties in view of their ready polymerisation. Cyclohexadienes both conjugated and unconjugated, in particular, the l-methoxy derivatives are readily available by the metal-ammonia reduction of aromatic ethers and these have been successfully used in cyclo-addition reactions to obtain adducts which are transformed into the desired products. We have extensively used them for a synthesis of a variety of natural products and I would like to describe some of these syntheses in this lecture.

l-Methoxycyclohexa-1,4-diene, obtained by the metal-ammonia, alcohol reduction of anisole contains a reactive vinyl ether double bond, and an isolated double bond in its structure and one or other or both these features explain its reactivity towards electrophilic reagents and dienophiles. Selective oxidation of the enol-ether bond affords a substrate having a double bond with Z-configuration. This method has been used for stereoscopic generation of Z-olefins. An extension of this process is the oxidative cleavage of l-methoxy-5-methylcyclohexadiene to give a synthon which was coupled with β -ionone resulting in the synthesis of retinolic acid derivatives.

l-Methoxycyclohexa-1,3-diene, which is readily obtained by the base catalysed conjugation of the corresponding 1,4-diene is very reactive as a diene in Diels-Alder and Alder-Rickert reactions. The adducts formed are usually regiospecific and undergo cleavage with the fission of one or two carbon-carbon bonds depending on the nature. Later work showed that the unconjugated dienes can be directly employed for the Diels-Alder reaction indicating that the conjugation proceeds through a charge transfer phenomenon. The use of catalysts like dichloromaleic anhydride, tris-

triphenylphosphine rhodium chloride accelerates the conjugation process and hence the cycloaddition. Thus addition of methyl vinyl ketone to 1-methoxycyclohexa-1,3-diene results in an adduct which on acid hydrolysis affords a ketoenone. This undergoes a base catalysed intramolecular Michael reaction yielding a *cis*-dione. Reaction of dimethyl acetylene dicarboxylate with 1-methoxycyclohexa-1,3-, or 1,4-diene yields an adduct which spontaneously undergoes rapid aromatisation giving 3-methoxyphthalic acid. Addition of alkyl or arylacetylenic esters with these dienes have been investigated to produce 6-alkyl or 6-aryl salicylic esters. This reaction has been exploited for the synthesis of a number of naturally occurring orsellinic acid derivatives which are derivatives of lichens. Based on this principle, the synthesis of macrolides, lasiodiplodin, Curvularin and Zearalenone has been achieved.

The mesomeric anions, obtained from the methoxycyclohexadienes with bases, can be regiospecifically alkylated or arylated. The resulting compounds, on acid hydrolysis, afford 2-substituted cyclohex-2-en-1-ones which are otherwise difficult to obtain. This principle has been exploited in the total synthesis of a sex pheromone, *Z*-heneicos-11-en-6-one. The intermediate alkylated diene undergoes cycloaddition with alkylacetylenic esters resulting in an alkylated salicylic ester. Thus alkylation of 1,5-dimethoxycyclohexa-1,4-diene with *n*-butyl bromide followed by cycloaddition with methyl 1-octynoate afforded an adduct which readily aromatised to give methyl 3-butyl-2,4-dimethoxy-6-pentyl benzoate. This compound on dimethylation followed by decarboxylation afforded a stenphol, a natural polyketide of fungal origin. This method allows us to prepare a number of compounds which are intermediates in polyketide biosynthesis.

Reaction of 1-methoxy-4-methyl cyclohexa-1,4-diene with acrolein afforded an adduct which was reacted with 3-methoxyphenylpropyl magnesium bromide to yield an alcohol. This was oxidised to the ketone which on acid hydrolysis afforded an unsaturated ketone. This, on treatment with base afforded *cis*-diketone which was cyclised to the tetracyclic ketone having *C/D-cis* geometry. The tetracyclic ketone has been successfully converted into equilenin and oestrone thus completing our own total synthesis of aromatic steroids. This process will be extremely useful in that several unnatural D-homo steroids can be readily made with a *cis*-C/D ring junction and find their way as drugs.

Finally the adducts obtained from 5-methoxy-1-methyl-4,7-dihydrohydrindane and chloroacrylonitrile afforded a tricyclic ketone on hydrolysis. This has been converted into a spirocompound by the oxidative cleavage of the double bond, which has been trans-

formed into acorone, thus leading to a stereospecific total synthesis of spiro-[4,5]-decanes. Acid catalysed rearrangement of the alcohol obtained from the above ketone leads to the formation of two compounds (i) an unsaturated ketone and (ii) a saturated ketone. These compounds are precursors for the total synthesis of Zizaene and Cedrene—two different types of complex sesquiterpenes biogenetically derived from the same precursor.

In this lecture, I have tried to summarise the strategies of syntheses of natural products using dihydrobenzenes. Most of the work presented here is unpublished. I am only a spokesman of the work carried out by my able and illustrious colleagues during the last five years at the Indian Institute of Science and I thank all of them for their performance.

Energy Conservation—An Organic Chemist's Attempts

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SYNTHESIS of drugs is one of the important industrial activities of a country. Development of new and more efficient synthetic methods for drugs are therefore most desirable.

Several drugs incorporate benzene-fused heterocyclic ring systems. The usual methods of synthesis of such ring systems are acid catalysed. The reactions involved proceed, in the aromatic ring, at positions which have more electrophilic reactivity. If one wants the reactions to occur at the less reactive positions, the more reactive positions have to be blocked and after the reaction at the desired position is effected, the block has to be removed. Due to such restrictions several benzene-fused heterocyclic compounds either cannot be synthesised or can be synthesised only through lengthy routes by these acid catalysed methods.

In contrast to the above, heteroatom directed aromatic lithiation reactions, where a lithium atom is introduced in an aromatic ring, in the presence of a substituent carrying an atom with unshared electron pair, due to the unique mechanism operative, proceed at positions not favoured in acid catalysed reactions. Through the organolithium compounds, which have greater reactivity towards electrophiles at positions carrying the lithium atom, reaction can be promoted at positions which are otherwise less reactive.

Using aromatic lithiation reactions new synthesis of several heterocyclic ring systems have been developed by the author. These include synthesis of phthalides, naphthofurans, furocoumarins, benzocoumarins, iso-

coumarins, isochroman-3-ones, oxaphenaline, isoquinolines, phenanthridines, dibenzo-(oxa, thia and di)-azepines, furoquinolines, pyranoquinolines, etc. The superiority of the new methods is that they can be used to synthesise several compounds, including natural products, not readily accessible by the usual methods, in simple steps and by considerably shorter routes and these involve considerable energy conservation. The compounds synthesised by the newer methods include meconine, mellein, kigelin, methoxycoumarins, several furoquinoline alkaloids and berbine alkaloids of the type tetrahydropalmatine.

Diamagnetism: A Surprise in Theoretical Physics.

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AFTER a brief discussion of the Bohr-van Leeuwen Theorem on the absence of classical diamagnetism, the subtle role of confinement, implicit in the theorem, is elucidated for the classical as well as for the quantum case. Possibility of non-zero diamagnetism in a system of charged particles (electrons and holes) undergoing rapid recombination-regeneration process is pointed out. Finally the non-dissipative nature of the diamagnetic currents in the context of the Quantized Hall Conductance is commented upon.

Paramagnetism resides in the unpaired spins of electrons with the concomitant *permanent* magnetic moments, (the Bohr magneton $\mu_B = -e\hbar/2mc$). These elementary magnetic dipoles (μ) tend to align gyroscopically parallel to the applied field and hence the positive susceptibility (χ), e.g. χ_{Para} (Langevin) $= n\mu^2/3k_B T$ of paramagnetic atoms and ions of number density n , or χ_{Para} (Pauli) $= 2\mu_B^2 g_F$ of degenerate electron (metals) having a density of states g_F at the Fermi-level. Diamagnetism is, however, different. It is an *induced* effect arising from the modification of the *orbital* motion of the charged particles in response to the applied field. Like all reactionary effects these induced currents are so directed as to generate a field opposing the applied field (Lenz' Law) and hence the diamagnetic susceptibility $\chi_{\text{dia}} < 0$, e.g. χ_{dia} (Langevin) $= -ne^2/6mc^2 \sum \langle r_i^2 \rangle$ of atoms and ions and χ_{dia} (Landau) $= -e^2 k_F/12\pi^2 m^* c^2$ of metals. Diamagnetism is universal, but is generally 10^{-1} – 10^{-3} times the paramagnetic effect and is, therefore, masked by the later. There are, however, notable exceptions, eg the semimetallic B_i because of the smallness of effective mass m^* . Typically $\chi_{\text{dia}} \sim 10^{-6}$. Given the spin of electrons, paramagnetism is essentially a classical effect. Diamagnetism is on the other hand *classically inad-*

missible. Thus paramagnetism is strong but simple. Diamagnetism is weak but subtle. As rightly emphasized by Peierls¹, diamagnetism is a surprise in theoretical physics. I will now try to amplify this claim.

First we must come to terms with the Bohr-van Leeuwen theorem on the absence of diamagnetism in a classical system of charged particles in equilibrium with fixed boundary. Proof is simple. We note that a pre-existing static magnetic field does not alter the energy of charged particle. For every possible motion in the absence of field, there exists a unique motion having the same energy in the presence of the field. Thus the energy as well as the entropy and, therefore the free energy is independent of the magnetic field, and hence no diamagnetism. Formally this can be seen from the relation between the magnetization M and the free energy F in the presence of a field $\mathbf{B} = (B, 0, 0)$, or the Vector potential $\mathbf{A}(0, Bx, 0)$:

$$M = -\frac{\partial F}{\partial B}, \quad F = -k_B T \ln Z, \quad (1)$$

$$Z = \int_{-\infty}^{\infty} e^{-\beta E} g_c(E) dE,$$

$$g_c = \frac{1}{N!} \frac{1}{(2\pi\hbar)^{3N}}$$

$$\int \dots \int \delta[E - H(p, q)] dp dq,$$

$$\text{with } H = \sum_i \frac{1}{2m} \left(\mathbf{p}_i - \frac{e}{c} \mathbf{A}(q_i) \right)^2 + V(q).$$

Clearly, for a finite system, A is bounded. A straightforward change of variable $\mathbf{p}_i \rightarrow \mathbf{p}_i - \frac{e}{c} \mathbf{A}(q_i)$ makes the vector potential disappear from the reckoning of the phase-space density-of-states $g_c(E)$, and hence $M=0$. (The proof holds generally except when there are constraints involving momenta). Now this contradicts the intuitively immediate real space-time picture. Under the Lorentz force $(-|e|c)\mathbf{v} \times \mathbf{B}$, the projected particulate motion normal to B is a circular orbit and contributes a non-zero moment giving

$$\chi = -\frac{1}{4\pi} \frac{(1/2 m v_{\perp}^2) n}{(B^2/8\pi)} \quad (2)$$

This, however, diverges as $B \rightarrow 0$. Besides, it is independent of charge, and must be wrong. (We recall that the static magnetic field does no work on the particle). But what has gone wrong and where? As was pointed out long ago by Lorentz and Bohr, the error lies in the improper treatment of the incomplete orbits lying within a Larmor radius of the boundary. The point is that the currents due to the closed orbits well within the sample, all add up to zero (like the Maxwell cyclical currents) giving only a 'skimming' current at the

surface. The particle near the surface, however, performs a 'skipping' orbit with the guiding centre performing a retrograde motion in a sense opposite to that of the skimming current. It is now plausible and indeed demonstrable that the two cancel exactly not only globally but in detail. The surprise lies in the fact that this space-time picture involves the boundary in an essential way while the statistical mechanical proof makes hardly any such reference to the boundary. Besides, the naive athermal picture in terms of the circular orbits is hardly tenable as the true motion of the particle in interaction with the 'bath' must be chaotic. Thus this scenario has 'time' in it but no temperature, while, the statistical mechanical picture has 'temperature' but is timeless. What is required is a stochastic treatment that interpolates between the two. This is provided by the time-dependent statistical mechanics *a la* Langevin equation of motion of the charged particle in the magnetic field²:

$$\frac{d^2 z}{dt^2} = -\Gamma \frac{dz}{dt} + F(t),$$

where $z = x + iy$, $\Gamma = \frac{r}{\hbar} + i\omega_c$ and

$$F(t) = \frac{1}{m}(f_x(t) + if_y(t)), \text{ with } \omega_c = \frac{|e|\hbar B}{mc}$$

$$\text{and } \langle f_\alpha(t) f_\beta(t') \rangle = \delta_{\alpha\beta} 2 k_B T \gamma \delta(t-t')$$

it can readily be shown that the magnetization

$$\lim_{t \rightarrow \infty} \langle m(t) \rangle \equiv \lim_{t \rightarrow \infty} \frac{-|e|\hbar}{2c} \text{Im} \langle Z^* Z \rangle = \frac{-|e|\hbar}{mc} \frac{k_B T \omega_c}{(\gamma/m)^2 + \omega_c^2}.$$

Now, one finds that the mean-squared displacement $\langle |Z(t)|^2 \rangle \rightarrow \infty$ as $t \rightarrow \infty$ and hence the particle must interrogate the confining boundary, however remote. We can impose confinement by introducing a harmonic potential well $1/2 \omega_c^2 m |Z|^2$. Then we find indeed that $\langle m(t) \rangle \rightarrow 0$ as $t \rightarrow \infty$. The harmonic potential well does simulate a 'soft' boundary, but, unfortunately, it represents a small system rather than an extensive one. This is readily remedied by considering a 'homonium' model for the real coulombic system where all coulombic interactions — both the electron-electron as well as the electron-ion, are replaced by the harmonic interaction. The stochastic problem remains solvable exactly, in terms of the Jacobi coordinates, and confirms the above result.

The foregoing treatment of diamagnetism or rather its absence depends explicitly on the existence of a Hamiltonian. Now there is no classical Hamiltonian describing a system of non-permanent charged parti-

cles undergoing a birth-death process, *e.g.* electrons and holes undergoing rapid pair-production and recombination. This is like chemical equilibria where one knows that the maxwellian distribution does not hold if the reactions are fast enough. It is, however, possible to treat such a system stochastically. Assuming the velocity distribution to be isotropic at birth, and a poissonian birth-death process we find a non-zero diamagnetism that turns out to be large under certain conditions². This however needs further attention.

Quantum mechanically non-zero diamagnetism is expected. The argument following Eq(1) now breaks down because of non-commutation. The integral in $g_c(E)$ gets replaced by a discrete sum over eigenvalues and this discreteness makes the cancellation incomplete. However, the surprise continues in that, the well-known Landau treatment gives the correct result even though he ignored the boundary in finding the energy eigenvalues. On the other hand each Landau level (occupied) ought to, at least naively, contribute a diamagnetic moment of the order of one Bphr magneton, the summary effect of which is far in excess of the Landau value. Again the boundary causes the cancellation — partial this time. This is resolved by noting that the Landau levels in the bulk are degenerate with respect to the position of the centre of the orbit. But as the orbit approaches the boundary, the eigenvalue begins to climb and, of course, it is emptied when it crosses the Fermi level (or the chemical potential). One can use the Feynman Hellmann stress theorem to evaluate the change in the eigenvalue as one moves from the bulk interior towards the surface. This turns out to be proportional to just the magnetic moment due to the surface currents! This formal identity is independent of the shape of the boundary. One could also analyse the fine structure of the surface currents using the linear response theory³.

And now some comments on the non-dissipative nature of the diamagnetic amperian currents *vis-a-vis* the dissipative ohmic currents. The fact that the static magnetic field does no work on the charged particle implies that these currents must be non-dissipative. (In this connection it is amusing to note that the usual argument that a *perfect* diamagnet should be a superconductor really does not hold). In point of fact magnetic field destroys the time-reversal invariance, and hence the eigenstates as well as the equilibrium state may be current carrying. (One should note here that the eigenstates in the presence of an electric field alone are not current carrying). The ohmic current due to an electric field involves an irreversible process in which an electron from below the Fermi level makes a transition to an empty state above it — the initial and the final states must bracket the Fermi level, and thus only

electrons near the Fermi level participate (i.e. the exclusion principle). For the diamagnetic current no such transitions are required and all the electrons participate (e.g. the $-e^2/mc \nabla^2 \Psi$ term in the current). These amperian currents are, however, "molecular" and can not be tapped except in the presence of a crossed electric field. Thus a two-dimensional electron system in the x,y plane with a magnetic field along the z-axis and an electric field along the y-axis will have an "amperian" current flowing along the x-axis that should be non-dissipative. This is guaranteed from the fact that the system is now invariant under combined time-reversal and reflection in the y-z plane. This situation can be realized in the inversion or the accumulation layer of a MOSFET or a heterostructure and has indeed been a subject of intense discussion recently in the context of Quantized Hall Conductance. One would expect the dissipation to set in when the electric field times the Landau Orbit radius exceeds the Landau level spacing. (Of course, in the presence of an electric field the discrete Landau levels disappear into a continuum but the relevant matrix elements develop an equivalent structure). On an atomic level, a hydrogen atom in the ground state in the presence of magnetic field is an ideal example of non-dissipative amperian currents and one can even define an Azimuthal Hall Conductance with respect to the radial coulomb field.

Finally, we return once more to the diamagnetism of non-permanent charged particles. One could well ask for the diamagnetism of the virtually or thermally created electron-hole (positron) pairs in an extra strong magnetic field $\sim 10^{12}$ – 10^{13} gauss, or indeed the diamagnetism of the Dirac vacuum regarded as an insulator with filled valence band. There may be more surprises to come.

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Intracranial Vascular Malformations

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"What the mind does not know the eyes do not see"

THERE are two main types of intracranial vascular malformations, (i) saccular or berry aneurysms and (ii) arteriovenous malformations (AVM). The former a

result of a defect in the wall of an artery, consists of a localised bulge of the intimal layer forming a tiny balloon. In an arteriovenous malformation there is a developmental defect resulting in a direct communication between the arterial and the venous system, which thus forms a turgid, tortuous, worm-like collection of blood vessels. One of the major consequences of such malformations is their propensity to bleed resulting in haemorrhage either in the subarachnoid space (SAH), the brain parenchyma or both.

Till a decade ago it was generally believed that intracranial vascular malformations, aneurysms and arterio-venous malformations, specially the former are uncommon amongst Indians. Careful planned investigations during the past few years have already demonstrated that this may not be the case. It is of interest to note that while the existence of such lesions has been known for more than a century, the methods for definite clinical diagnosis and successful attempts at surgery have only been possible after 1930's. With rapid advances in anaesthetic management and surgical techniques, including use of operating microscope, excellent results can now be achieved through surgical treatment of these lesions.

Our studies on the subject are briefly summarised below. It may be stated that several colleagues in the Department of Neurosurgery and Pathology have contributed to these studies.

1) *Pathological-Anatomical Studies*: Anatomical variations in the Circle of Willis obtained at routine autopsies were studied. The incidence of such anomalies was identical to the reports from countries like USA, Japan etc where aneurysms are found to be more frequent. Similarly microscopic studies of the junctional areas revealed comparable incidence of medial defects in the wall of the arteries at the base of the brain. Both these factors have been implicated in the pathogenesis of intracranial saccular aneurysms.

2) *Etiology of Subarachnoid Haemorrhage (SAH) in India*: Hospital based incidence studies carried out at six centres in the country, revealed that contrary to existing belief saccular aneurysms constitute the commonest cause of SAH (40–50%), followed by A.V. malformations (7–8%).

3) *Clinical Studies*: (a) Intracranial Saccular Aneurysms: During the last 10 years 121 cases of aneurysms, 89 of which were submitted to surgery have been investigated. Six cases had more than one aneurysm and six had giant aneurysms (more than 2.5 cm in diameter). All these patients had at least one episode of intracranial bleed. 13 surgically treated patients died, a mortality still undesirably high but far better than any conservative treatment. It is now generally agreed that surgical treatment is the safest method of

preventing recurrent haemorrhage, which remains a life long threat in conservatively treated patients. (b) Arteriovenous malformations (AVM)—68 cases of AVM, studied by us had resulted in SAH in 37 cases, and intracerebral haemorrhage in 12. Epileptic seizures, progressive neurological deficit and raised intracranial pressure are some of the other presenting features. It was only in 1936 that Olivecrona from Stockholm reported the first successful excision of such a malformation. There is still some debate about the best method of treatment. However, surgical excision provides the best over all results. Amongst the 52 patients who were submitted to surgery, we lost only 2, inspite of the fact that several of these were very large. Use of operating microscope has helped reduce the mortality.

New Stereo Controlled Synthetic Routes to Bridged-Ring and Alicyclic Compounds

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THE bridged-ring systems, incorporated in carbocyclic skeleta, are widely distributed in large groups of natural products having interesting physiological properties. Apart from this important aspect, bridged-ring systems are valuable models for conformational and spectroscopic studies. In addition, a great deal of promising synthetic methodologies for the stereocontrolled development of other ring systems have been realised through these intermediates. Despite extensive studies in this area, the problem of devising simple and general synthetic methods for functionalized bridged-ring and polycyclic systems remains a major challenge in organic chemistry. The work done in our laboratories during the recent years on the intramolecular alkylation of unsaturated diazomethyl ketones¹ and particularly those on the alkylation-rearrangement reactions has turned out to be potentially important for generation of the basic structures of the plant hormones-gibberellins², polycyclopentanoids³ and a number of other diterpenoids⁴ and diterpene alkaloids⁵. In continuation of our studies on carbenoid reactions we have recently developed for the first time, bis [2,4-pentanedionatonickel (II)] as highly efficient homogeneous catalyst in intramolecular α -ketocarbenoid addition reactions⁶ of a number of cyclic γ , δ -unsaturated diazomethyl ketones leading to the key intermediates for tetracyclic gibbane and the related bridged-bicyclo[3.2.1]octanones. This homogeneous catalyst has been found to be equally effective for the regioselective intramolecular carbon

hydrogen insertion reactions⁷ resulting in the established⁸ synthetic intermediates for certain diterpenoid alkaloids and C₂₀-gibberellins. In conjunction with the diazo-ketone alkylation reactions we have further developed a highly efficient and simple method for the synthesis of functionalized bridged-ring systems by chemoselective degradation of an aromatic ring to carboxylic acid by ruthenium-catalyzed periodate-oxidation⁹. As a prelude to a biomimetic synthesis of the complex carbocyclic bridged-ring systems present in the highly bio-active diterpenoids aphidicolin and stemodin, and the biogenetically related diterpene, stemarin, several approaches to the construction of bicyclo (2.2.2) octanone systems incorporated in tetracyclic systems were undertaken by using intramolecular cationic cyclizations. The works along this lines, in fact, showed great promise with realization of a new simple general stereocontrolled synthetic route to such bridged-ring systems¹⁰ and finally to the carbocyclic skeleta¹¹ of stemodin and stemarin. This strategy appears equally feasible for the synthesis of aphidicolin skeleton. An unexpected turn taken in the course of these studies led to the discovery of a fascinating intramolecular carbon-carbon bond formation reaction from $\gamma\delta$ and $\beta\gamma$ -unsaturated methyl ketones with methyl- or ethylorthoformate in the presence of perchloric acid, leading to a general method of bridged bicyclo [3.3.1] nonanone and cyclopentenone annulations¹². The synthesis of bridged-bicyclo [3.3.1] nonane system has also practical importance as we have already reported¹³ that a bridged-ring hydrocarbon, (\pm)-des-*N*-morphinan is a powerful attractant for some insects. This is the first synthetic bridged-ring hydrocarbon found to be an insect attractant.

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Induction of Haploids: Achievements, Problems and Possibilities

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THE realization of the importance of haploid plants in agriculture and fundamental biology led to the development of different techniques for inducing them. However, it was only after the development of anther culture technique¹ that hopes were raised to obtain haploids in many plants including even in agriculturally important plants. So far anthers of over 170 species have been cultured with success in inducing the pollen grains to form calli, embryoids and plants².

Of the different parameters tested to optimize success in anther culture, the most important are the genetic background of the plant, the physical factors and the nutritional conditions^{2,3}. It is still not clear if the recalcitrant nature of certain genetic clones is due to the lack of 'genes' for callusing and embryoid production or due to the lack of our understanding of the factors which would 'switch on' those genes. Different physical factors which influence the process of androgenesis have been reported. Many species require darkness but others need to be transferred to light or maintained in it throughout. The action spectrum studies are still lacking in order to find out which pigment systems are involved in light requiring species. A pre-treatment of buds to cold temperature has been

found to induce the response in some non-responding species and increase the response in otherwise responding plants. The optimal culture temperature is, however, in the range of 25–30°C in varied species. Besides these two important physical factors, the effect of centrifugal force, size of the culture flask, the number of anthers per culture vessel and the way the anthers are positioned on the medium also influence the response. Of the chemical factors, role of iron, sucrose and hormones has been worked out in some details. Iron has been found to be essential and works better if supplied with a chelating agent EDTA or EDDHA. Without sucrose it is difficult to get the response. The effect of sucrose can also not be mimicked with mannitol or even with glucose. In many reports, hormones have not been included in the medium but our own studies and those of others have shown that hormones, especially cytokinins and auxins, either alone or in combination are required for pollen grains to divide and develop into haploid plantlets. Lately, activated charcoal and polyvinyl polypyrrolidone have been included in the medium to increase the response. The extract of potatoes has also been used alongwith some hormones to induce androgenesis in some crop species⁴.

Besides achieving success in a number of species by manipulating different parameters, haploid plants have also been used in breeding and the haploid cells have been employed for induction of desired mutations⁵. It has been possible to get high yielding varieties in rice, wheat, and *Nicotiana*, virus and nematode resistant clones to potato, and variants with less alkaloid content in seeds of *Brassica napus*. Also drug, toxin, and temperature resistant mutants have been selected using haploid cells. Recently, haploid cells of *N. sylvestris* were used to select for virus resistance. Haploid cells have been especially of great use in obtaining auxotroph lines in different plants, which had been very difficult to get earlier.

In spite of recording achievements, the utilization of haploids has not been what was expected. There are still many problems which have to be overcome. It is not possible to get the pollen divide and form haploids in many genetic clones and therefore one has to standardize the conditions for each individual clone.

In many species anthers respond by producing callus and not direct embryoids. It is difficult to differentiate callus and moreover the callus stage leads to instability of chromosomes resulting in the production of more diploid, aneuploids and higher ploidy plants, than the required haploids. In such cases one is not sure of the homozygous nature of the plants. Even in cases where direct embryoids are produced, not all of them grow into plantlets. One problem, especially for

cereals, has been the production of large number of albino haploid plants. In many species, unreduced microspores are produced which can lead to the production of heterozygous plants. Since there are so many variable factors, it is even difficult to reproduce the results exactly and even today in many plants the yield of plantlets per planted anther is rather low and varies. With the advancement of techniques it is the hope of workers in this area that many of the problems would be solved and it should be possible to use haploids more efficiently. Already, genetic markers have been used to establish the homozygous nature of the plants. Possibilities have been raised to use microspores for inducing mutations directly. It is hoped that screening 'Gametoclonal' variations would be useful. For example, early maturing variants in rice have been raised by this technique⁶. Above all the possibility of using genetic transformation experiments with haploids are being done. Infact recently, it was pointed out that in order to get stable transformants, only haploids be used⁷.

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Tissue Culture and Micropropagation

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Two major discoveries have mainly been responsible for the emergence of micropropagation, as a method for clonal multiplication of plants. The first, arising as a result of an observation made by Skoog and Miller¹ in 1957 showing definite evidence that shoot and root differentiation in callus and organized cultures is under the regulation of two main groups of chemicals, the cytokinins and auxins. The second advance being from the research of Morel² in 1960 where shoot tip cultures of an orchid were found to be a potential source for the production of large numbers of identical orchid plants.

Plantlet formation by micropropagation can follow two separate pathways. In the first, plantlets can be produced from the primary explants either directly³ or via an intermediate callus stage⁴. The second pathway before plantlet formation involves an intermediate stage of somatic embryogenesis⁵, where the embryos resemble zygotic embryos in many respects.

Conditions for use of Tissue Culture for Micropropagation

The use of tissue culture for micropropagation should be used under very specific conditions (i) for the mature elites, particularly where seed raised plant-trees show wide variation as in teak⁶ and *eucalyptus*⁷ (ii) for hybrids mutant/varient plants, haploids⁸ etc produced by tissue culture or other methods (iii) for newly developed plants normally propagated vegetatively, where by conventional methods low multiplication rates are obtained as in turmeric⁹ where multiplication from rhizome is as low process.

Criterion for success

The main criterion for success in the development of a process of micropropagation lies in the correct formulation of the different ingredients in the medium for stimulation of the explant either to develop shoots, roots or plantlets or of callus and embryoids.

Generally apical or axillary vegetative buds are preferred for micropropagation because of a high level of organogenesis obtained combined with a high genetic stability of the regenerated plantlets. This method has also proved amenable for some of the older trees besides being of value for both the isolation and later multiplication of disease free plants as in sugarcane^{10,11}.

Stages for plantlet formation

There are mainly 3 stages for successful plantlet formation from the explants (i) the primary explant, either an apical or axillary bud is stimulated, under the influence of hormones mainly a cytokinin to develop a large number of shoots, (ii) each of these shoots can then be transferred to the second stage culture medium, generally containing an auxin for their rooting, (iii) the resulting plantlets formed can then be treated like seedlings and transferred to a normal unsterile environment. The high rates of multiplication are achieved by excising the individual shoots from stage (i) and transferring them to fresh media at regular intervals, whereby large numbers of shoot cultures can be maintained for conversion to whole plants when required. Based on the degree of shoot multiplication obtained at the first stage pheno-

menal increases in the overall production of plants can be achieved.

Commercial application

Currently micropropagation is being commercially exploited for the production of a large number of ornamental and herbaceous plants, fruit and also recently forest trees. An ideal small scale commercial laboratory for producing about a million plants per year would occupy about two thousand sq ft. utilizing the services of about 10 technicians working 200 days a year with an average production of about 70,000 plants a year per worker. However, in any commercial process a prerequisite and an important factor is the economics of the process, which also requires careful research and planning. For instance, starting from 1000 explants it has been calculated, that if there is a 2% contamination rate at each of the three stages, and a multiplication ratio of 1:40 at stage (ii), then one can expect a total loss of profit of Rs. 840, assuming the cost of each plant to be 0.35 paise¹². This is the result of a drop in the overall production of plants from 40,000 to 37,600. In a similar manner cost calculations, comparing several other parameters can also be made. Such studies would be invaluable for any commercial venture.

In conclusion, one can visualize the gradual development of micropropagation into a low cost technology for plant production, whereby direct embryogenesis from suspension cultures or explants would substitute for organogenesis. With such methods which are still very rare, the 3 labour intensive stages would be totally eliminated, paving the way for the conveyor belt technology and mechanization for large scale production and sowing of plants. A beginning has already been made in this direction.

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Synthesis of Biologically Active Compounds with a Difference

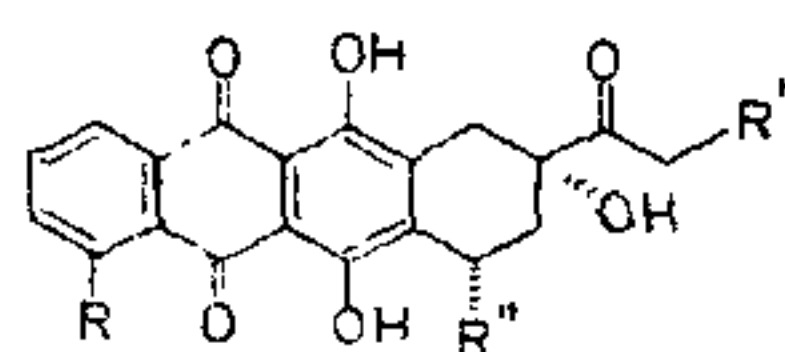
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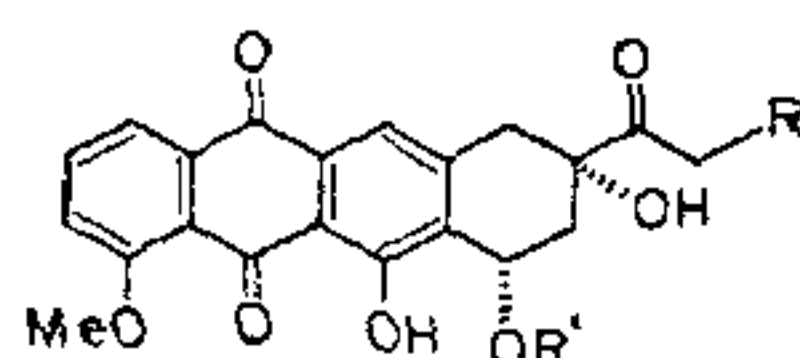
NATIONAL Chemical Laboratory (NCL) has formulated several programmes of research and three such projects are: (a) development of anticancer agents (b) organic synthesis, and (c) developing new plant growth regulators. The progress made in these areas dealt with in this article.

Anthracycline antibiotics

During the past one decade, the anthracycline antibiotics such as daunomycin (1) and adriamycin (2) have emerged as antineoplastic agents for the treatment of broad spectrum of human cancers¹. While they are presently produced by fermentation, the challenge of developing them through practical and total synthetic routes coupled with the quest for a superior analogue, has been continued. Recent discovery of the improved antineoplastic activity of synthetic anthracycline, 4-demethoxydaunomycin (3)² as compared with naturally occurring 1 and 2 have stimulated considerable synthetic effort towards corresponding aglycone, 4-demethoxydaunomycinone (4)³. In most of these studies 4-demethoxy-7-deoxydaunomycinone (5) was the target molecule. As the functionalization of A ring to give 4 has been well established and the resolution of the racemic 5 to give the desired optically active aglycone was achieved⁴ our efforts have been directed to allow the preparation of 5 in multigram quantities so that the clinical efficacy of the synthetic analogue 3 can be evolved⁵⁻¹⁰



- 1 : R = OMe; R' = H; R'' = O-daunosaminy
 2 : R = OMe; R' = OH; R'' = "
 3 : R = R' = H; R'' = O-daunosaminy
 4 : R = R' = H; R'' = OH
 5 : R = R' = R'' = H
 11 : R = OMe; R' = H; R'' = OH



- 8 : R = H; R' = daunosaminy
 9 : R = OH; R' = "
 10 : R = R' = H

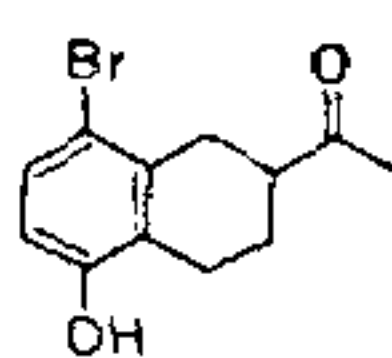
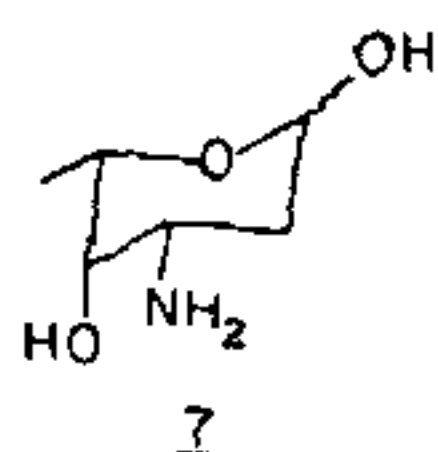
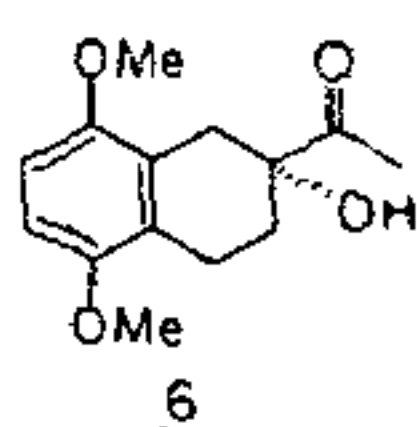
Our strategy was to build tetracyclic system by AB + CD coupling by making use of the key intermediate, 2-acetyl-5,8-dimethoxy-1,2,3,4-tetrahydro-2-naphthol (6). We have further demonstrated that 4-demethoxy-7-deoxydaunomycinone (5) can be made by fusion of 6 with an intimate mixture of phthalic anhydride $\text{AlCl}_3\text{-NaCl}$ (5:1) followed by usual work up⁵. 5 thus obtained was converted into 4-demethoxydaunomycinone (4). The coupling of 4 to *L*-daunosamine (7) is being looked into. The synthesis of 7 was achieved by two elegant approaches starting either from *D*-glucose or *D*-glucosamine^{11,12}.

Although daunomycin (4) and adriamycin (2) continue to enjoy the clinical effectiveness in the treatment of human cancers, their main disadvantage of having irreversible cardiomyopathy have prompted the search for new antibiotics that show decreased side effects and/or increased antitumor activity. This has resulted in the isolation of new anthracycline antibiotics lacking a hydroxyl group at 11-position (11-deoxydaunomycin (8) and 11-deoxyadriamycin (9))¹³. We have achieved a general regiospecific synthesis of 11-deoxydaunomycinone (10) involving the Diels-Alder reaction¹⁴. We have also demonstrated the synthesis of 10 starting *m*-cresol¹⁵. In addition, we have recently reported a regiospecific and common approach for the synthesis of daunomycinone (11) and 11-deoxydaunomycinone (10) starting from a common synthon (12) made from *m*-cresol¹⁶.

3-Methoxyphthalic anhydride, desired for the synthesis of 10 and 11 was made by a two step synthesis by a Diels-Alder reaction of 2-methoxyfuran and maleic anhydride¹⁷.

Capability building in organic synthesis: Total synthesis of macrolides

The synthesis of macrolides is currently under inten-

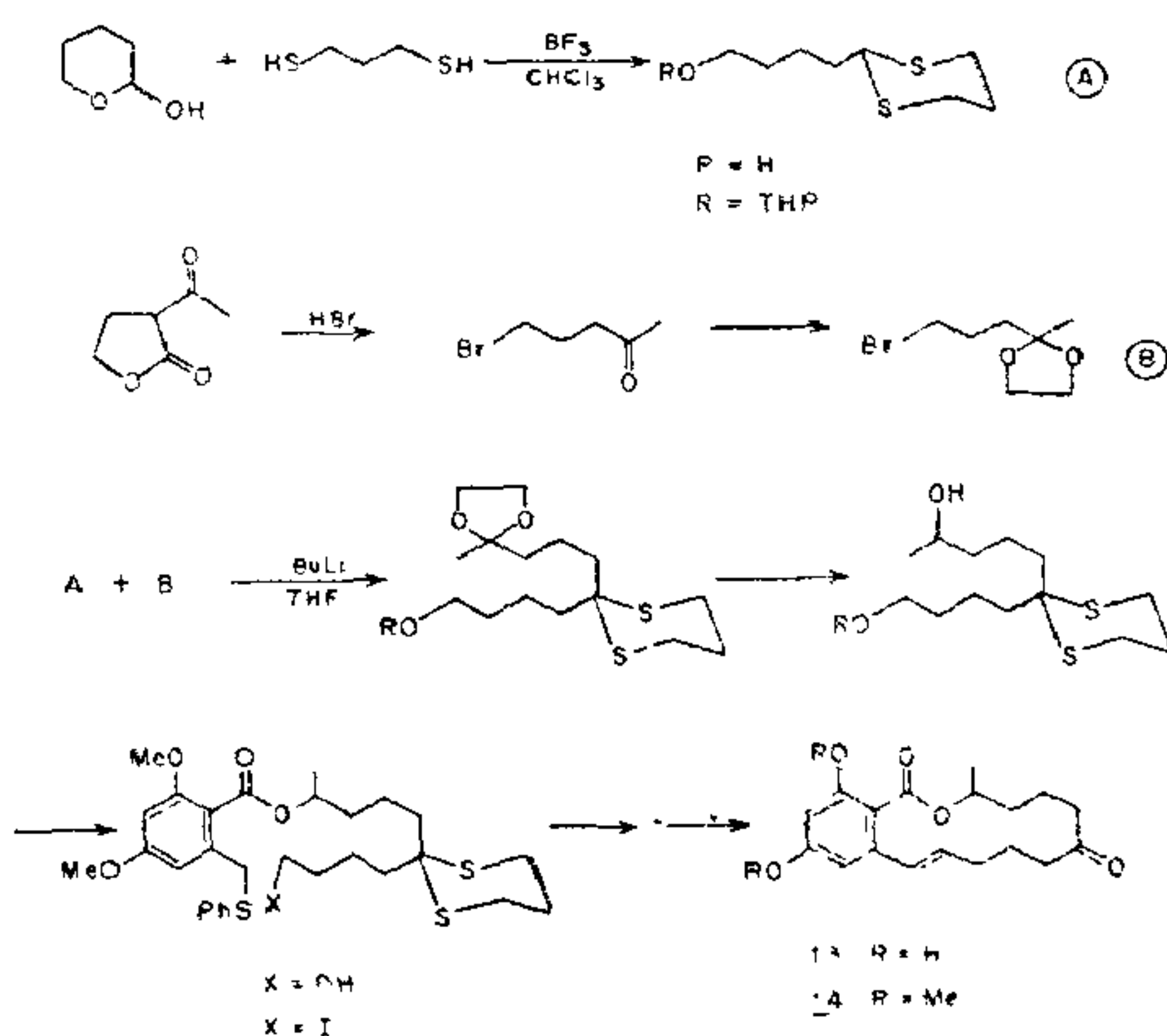


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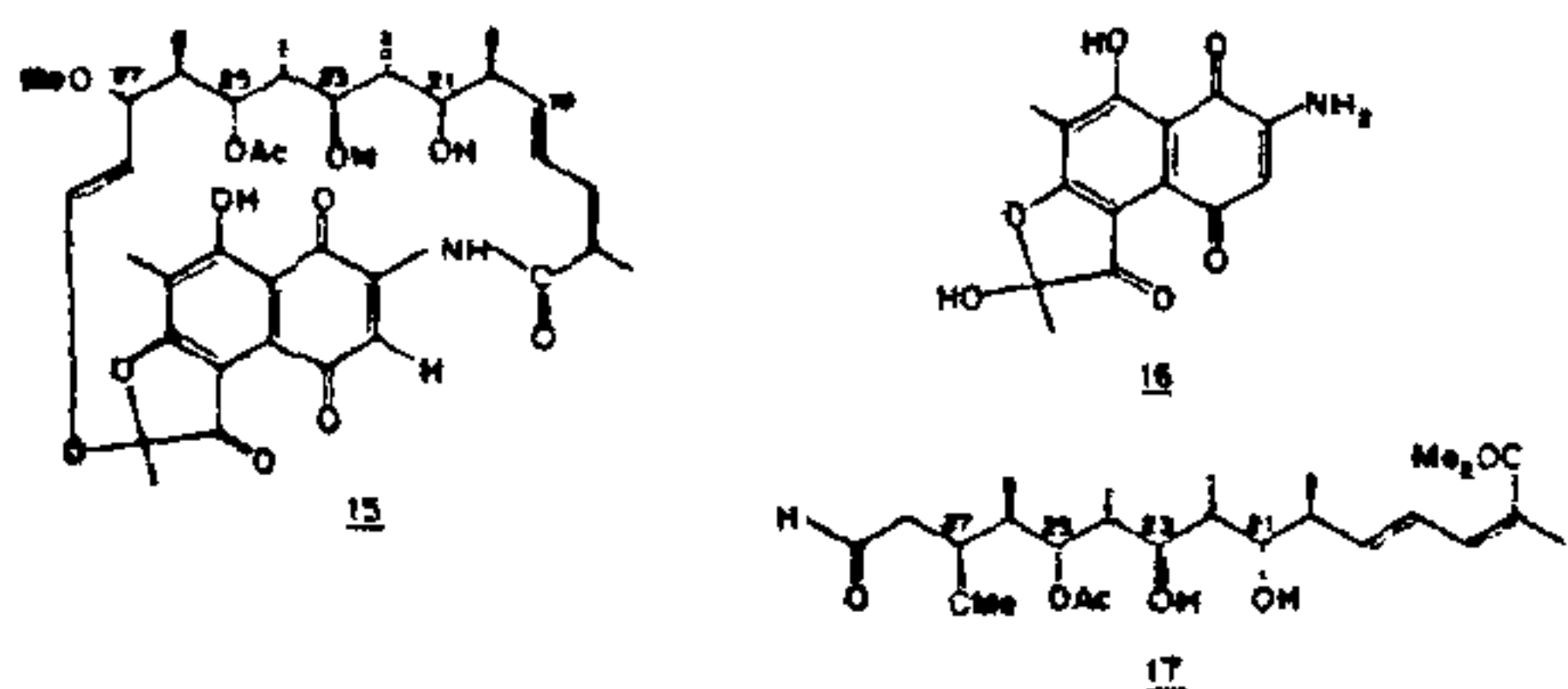
sive investigations because of their diverse biological activities. Recent developments in their synthetic strategies made the task easier for the synthesis of several naturally occurring macrolides. We have recently undertaken the total synthesis of a number of macrolides and two examples are cited below:

Zearalenone (13) an antibiotic with anabolic and uterotrophic activity, was the first macrolide to be synthesised by both Merck and Syntex groups. The aliphatic portion has been obtained utilising 2-hydroxypyran and 2-acetyl- γ -butyrolactone (Scheme 1). The final condensation of this segment with the aromatic part and subsequent transformation led to (\pm)zearalenone dimethyl ether (14)¹⁸.

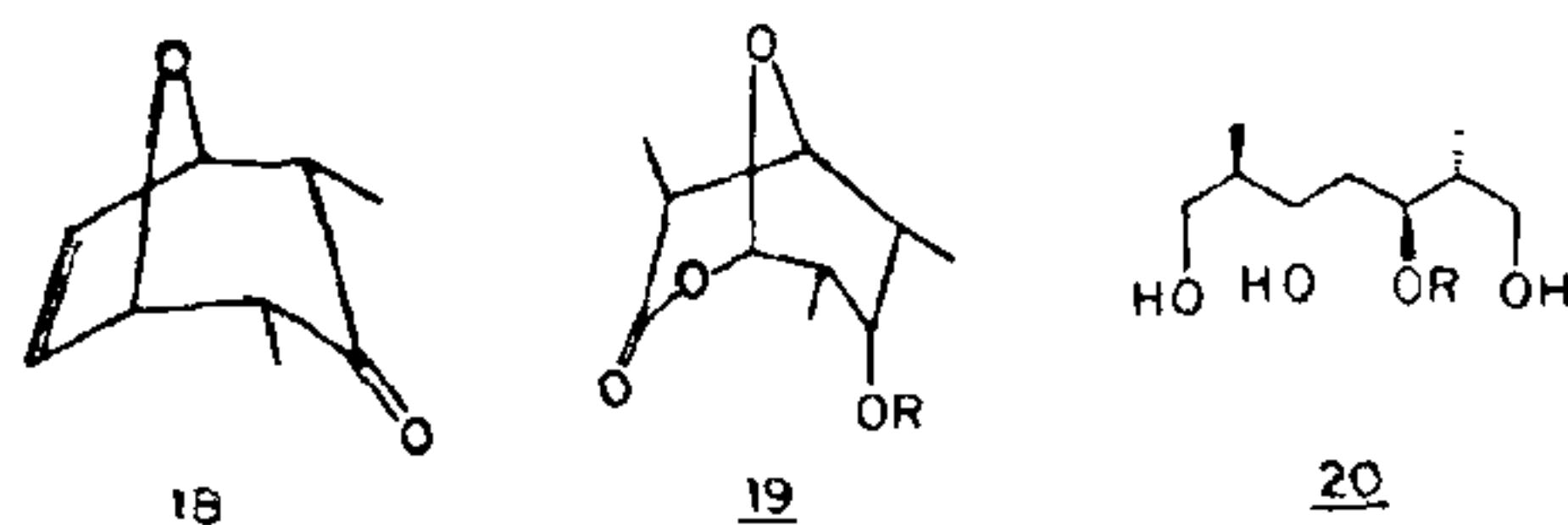
Total synthesis of Zearalenone



Refamycin S (15) belongs to a novel class of antibiotics, ansamycins, which constitutes an aliphatic bridge linking two non-adjacent positions of an aromatic nucleus. The synthesis of 15 is a challenge to any organic chemist and was reported recently by Kishi and his colleagues from Harvard University¹⁹. We have initiated the synthesis of 15 one year ago and made good progress both at the naphthaquinone unit (16) and the aliphatic part (17). Unlike the approach adopted by Kishi and others, wherein the stereocontrol of the aliphatic chain was achieved via acyclic intermediate, our approach is by involving cyclic intermediate (18) which was further elaborated to 19 which on LAH reduction gave the aliphatic segment (20) corresponding to carbon atoms C₂₁ to C₂₇ of the refamycin S¹. Further elaboration of 20 to 17 is in progress.



Scheme 1



Plant growth regulators

Global demand for higher crop production coupled with constant increase in the cost of nitrogen fertilizers had led in recent times to an increased interest in plant growth regulators. One such interesting finding was by Ries *et al.*²¹ who demonstrated that application of alfalfa (*Medicago sativa*) increased yields of tomatoes, cucumber, rice, corn and several other crops. The active principle responsible for the enhanced growth was identified as 1-triacontanol. With an intention to assess its importance in large scale trials at a reasonable cost. We have worked out its total synthesis by two different and practical approaches. In our first approach, 1-triacontanol was synthesised starting from stearic acid and by two successive addition of six carbon units through enamine intermediates²². Our second approach was based on the successive alkylation of tosylmethylisocyanide followed by acid hydrolysis and subsequent reduction of the keto-ester to give 1-triacontanol²³ (See scheme 2).

Having made 1-triacontanol in large quantities, we have found to our surprise that it had not responded to many of the systems we have tried. This fact was further strengthened from a number of recent controversies regarding 1-triacontanol as a plant growth regulator, which led us to believe that 1-triacontanol may

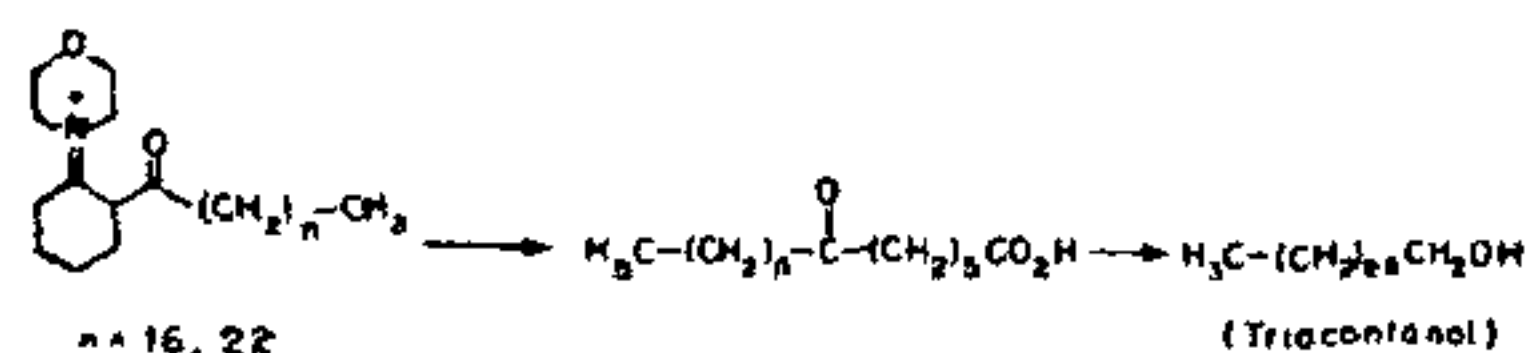
not be the only active principle in alfalfa responsible for the increase in growth. The above findings led us to probe into the known chemical constituents of alfalfa which may be responsible as plant growth regulator. One of the interesting observations was that Vitamin K₁ was first isolated from this source. Later findings have shown that Vitamin K₁ is distributed in all higher plants, although its role is not well established.

As menadione (2-methyl-1,4-naphthoquinone) is more active compared to Vitamin K₁ in its general biological properties and to make it water soluble, we made its bisulphite adduct and assessed for its growth regulatory activity in a number of plant tissues, where it was seen to bring about significant increase in growth. Further studies were carried out to investigate its role to auxin metabolism. Besides lowering the activities of the enzymes involved in IAA oxidation, it also brought about a 3-4 fold increase in the IAA levels of plants²⁴. Finally to assess the commercial utility of menadione bisulphite, field trials were carried out using tomato plants. Initial experiments showed large increase (> 50%) in the yield of tomatoes²⁵. Its effect on other crops is being looked into.

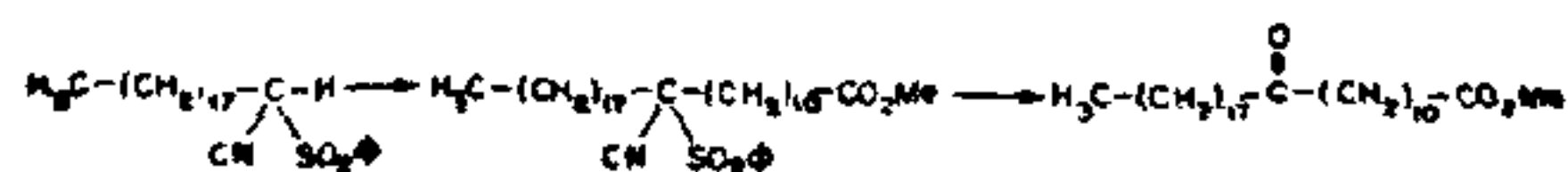
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Scheme 2

1st Method



2nd Method



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Somatic Hybridization and Cybridization

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SOMATIC cell fusion gained prominence as a novel approach to produce rare hybrids of sexually incompatible and sterile parents following the report by Cocking and his co-workers, in 1970, that plant protoplasts can be fused¹ by NaNO_3 . Since then research activities in the area of somatic hybridization have dominated the field of plant tissue culture. Somatic hybridization involves a series of steps outlined in figure 1.

Isolation of large quantities of viable protoplasts has been reported for most crop plants². This has been

facilitated by the commercial availability of many powerful hydrolytic enzymes of fungal origin. Most recent introduction to the list of these enzymes is Pectolyase Y-23 with which mesophyll protoplasts of tobacco could be liberated within 30 min³. There is no serious technological problem associated with this step 1 of somatic hybridization.

Cell fusion in animals has been carried out since 1962 using Sendai virus⁴, which is ineffective for fusing plant protoplasts. Of the various other methods tried to fuse plant protoplasts, high pH (10)/high Ca^{+2} (50 mM CaCl_2) and polyethylene glycol (PEG) have yielded promising results.

A prerequisite for protoplast fusion is the establishment of intimate contact between plasma membranes of two cells over a large surface area (adhesion). However, because of the common negative surface charge normally protoplast membranes cannot be maintained in close proximity ($< 10 \text{ \AA}$). The electric potential of the membranes, depending on the species, varies from -10 mV to -35 mV. This does not change with the age of the plant and the time of the year⁵, although it may show slight variation with the cell cycle phase⁶. Repulsion between two protoplasts is removed and their fusion favoured by cations such as Ca^{+2} . 100 mM CaCl_2 completely neutralises the surface charge and favours good adhesion followed by fusion of plant protoplasts⁵.

As a fusogen, PEG has become more popular than high pH/high Ca^{+2} method. Application of PEG induces instantaneous adhesion of plant protoplasts; fusion of the membranes occurs during the removal of PEG. PEG also brings about good fusion of animal cells and animal cell with plant protoplast. It has been suggested that PEG treatment results in the appearance of smooth areas free of intramembranous particles at the site of fusion and some sort of perturbation by possible displacement of membrane⁷ bound Ca^{+2} . Protoplast fusion by PEG is now a class room exercise, and many somatic hybrids and cybrids have been produced by this method.

During the past 5 years Zimmermann and his co-workers⁸⁻¹⁰, in West Germany, have done considerable work to develop an electrically stimulated protoplast fusion method. These studies have recently culminated in the production of an automatic

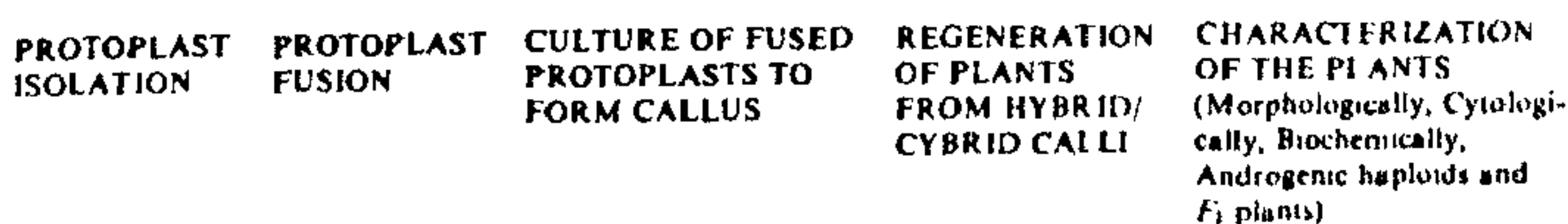


Figure 1.

machine, called "Zimmermann Electrofusion System", which is claimed to be 10,000 times more effective than other biological or chemical fusion methods. As assessed by their cytoplasmic energy status (ATP/ADP) electrically fused protoplasts maintain a high viability. Electrically fused protoplasts of *Petunia inflata* also formed wall and divided.

Incompatibility recognition system, as found in flowers, does not occur at the level of the plasma membrane allowing fusion of protoplasts from highly unrelated plants. However, the most crucial and, currently, a bottleneck in somatic hybridization is the culture response of the heterokaryons following protoplast fusion. Of key importance in somatic hybridization is to be able to sort out and follow the development of the small number of heterokaryons (<10%) from a much larger population of unfused protoplasts and homokaryons. For this several approaches have been used which include special media, complementing albino parents, culture of mechanically isolated hybrid cells, and labelling the parent protoplasts with different fluorescent probes.

By culturing fused protoplasts several hybrid cell lines and somatic hybrids have been raised between sexually compatible and incompatible parents². *Datura stramonii*, a somatic hybrid between *D. innoxia* and *D. stramonium* (sexually incompatible) produces more dry mass per acre and has scopolamine yield less dependent on climate and soil conditions than either of the parents¹¹. This is only a rare example of useful gene transfer. Generally, somatic hybrids with complete genomes of unrelated plants are neither stable nor desirable. Emphasis is, therefore, shifting to controlled transfer of gene blocks or individual genes by introducing isolated chromosomes or purified DNA into plant protoplasts. However, the technology of genetic transformation in higher plants is only at the developmental stage and, unlike protoplast fusion, is not yet routine. Partial genome transfer through protoplast fusion may be possible by using donor's protoplasts in which DNA has been fragmented by irradiation and the normal protoplasts of the recipient¹².

Protoplast fusion followed by plant regeneration is proving a powerful tool to study organell genetics and produce alleloplasmic lines or cybrids¹³. In somatic hybrids segregation of cytoplasmic organelles is very common. Being random, it results in the formation of novel cytoplasm-nucleus combinations. Segregation of chloroplasts occurs independent of mitochondria. Cybrids show recombination of mitochondrial DNA. Many agronomically important traits, such as male sterility, and resistance to phytotoxin, herbicides and pesticides are controlled by extra-nuclear genes. Controlled, unidirectional transfer of these characters

without the interference of the nucleus of one of the parent is now possible through protoplast fusion. Interspecific and intergeneric transfer of male sterility has been achieved via cybridization.

Genetic engineering techniques, including protoplast fusion, involve modifying genome of single cells. It must be possible to grow these modified cells into whole plants. Cellular totipotency seems to be a dominant feature. So far the choice of parents in protoplast fusion studies has been made on the basis of the ease with which they can be manipulated in cultures rather than the desirability of the hybrid. This is because of the difficulty is culturing protoplasts of cereals and regenerating plants from protoplasts of many important crop plants.

In conclusion, during the past decade considerable technological advancement has been made but much remains to be achieved before the technique of protoplast fusion can fulfil its promises. A most important outcome of the work done so far is the appreciation of the limitation of somatic hybridization as conceived originally and the recognition of realistic possibilities through isolated protoplasts.

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Chemical and Environmental Studies in Ice and Waters in and along Antarctica

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UTILIZING the unique opportunity provided by the First Indian Expedition to Antarctica during the austral summer of 1981-82 some chemical investigations were carried out on the ice and waters of the Antarctic continent and along a NE-SW transect in the southwestern Indian Ocean from 32° S latitude to the edge of the Antarctic continent.

These studies included estimations of several chemical components in ice and lake water on land and in waters of the Southern Ocean. The chemical components were: several essential nutrients, heavy metals and a few major elements. In addition, a study of aerosol were also conducted to examine the effect of aerial transport of particles.

(i) Ice shelf, freshwater lake and polynya

Chemical characteristics, such as, salinity, temperature, dissolved oxygen, phosphate-phosphorus, nitrate-nitrogen, nitrite-nitrogen, silicate-silicon, calcium, magnesium, potassium, fluoride, bromide, iodide, iron, zinc, copper manganese, nickel, cobalt, lead, and cadmium were measured upto 5 m depth in the ice shelf, in the surface waters of a freshwater lake and at several water depths in the polynya.

An increasing downward gradient was found in the ice in respect of most of the constituents. This was caused by the downward diffusion of salt in the ice. A reducing condition was observed at depths in the ice, caused by the gradual escape of gases upwards. Surface concentration of some of the elements appeared to be influenced by atmospheric transport and deposition. No significant difference was observed in the

constituents at some distance away from the edge of the ice shelf.

Concentrations of the heavy metals in the ice and in the lake and in a Himalayan glacier at varying heights were observed to be of a similar order (table 1). These features indicate that both Antarctica and the high altitudes of the Himalayas have clean and unpolluted environment.

Using these two as the southernmost and the northernmost datum points, a watch can be maintained on the health of the Indian Ocean.

Hydrochemical studies of the polynya in Antarctica indicate that the surface layers are influenced by melting of ice but an oceanic layer exists below 150 m depth. Chemical characteristics of the lower layers have many similarities with those of the deeper waters of the Southern Ocean. The height of the ice shelf was about 20 m above the sea surface and extended to about 150 m depth into the sea. Particulate matter resulting from the melting of ice probably adds to the reserves of nutrients and heavy metals of the sea water.

(ii) Aerosol

Measurement of aerosol particles along a transect in the Western Indian Ocean, between 09° 45'N and 69° 59'S latitudes, showed a gradual decrease of particles towards Antarctica.

The concentration varied from $65.3 \pm 1.9 \mu\text{g}/\text{m}^3$ air at 8° 37'N latitude to 0 in Antarctica. Peaks were recorded at places where the atmosphere was influenced by continental air. A peak was observed at the zone of Antarctic Convergence which may be the effect of the flow of surface air from Antarctica towards the equator. Katabatic wind is probably the cause of another peak observed near the Antarctic continent. An inverse relation has been observed between the wind speed and the aerosol content which is presumably due to scrubbing effect and dispersion.

(iii) Southwestern Indian Ocean

Chemical parameters analysed in the water samples,

TABLE I

Concentrations of a few heavy metals (in $\mu\text{g}/\text{l}$) in ice and water, Princess Astrid Coast, Antarctica and in a glacier in the Kashmir Himalayas.

Station	Fe	Zn	Cu	Mn	Ni	Co	Pb	Cd
Antarctic Ice	1.4	14.4	3.0	6.8	2.0	0	0	0
Antarctic Lake	50.5	13.6	3.4	5.6	3.2	0	0	0
Polynya - 10 m	2.0	46.8	3.1	0	3.7	1.4	2.6	1.5
Himalayan Glacier (mean)	82.5	4.5	4.1	17.0	1.8	1.1	3.1	0

collected upto a maximum depth of 4000 m at 17 oceanographic stations, were used to identify the regions of polar divergence, antarctic convergence and sub-tropical convergence. Within the limits of the available data, the polar divergence could be identified between 60° and 63° S. Antarctic convergence was observed slightly south of 52° S. Similarly, the boundary of the sub-tropical convergence was located at around 41° S. Downward gliding of the nutrient-rich water along the continental slope of Antarctica was also recorded.

The change in the oxidative ratios ΔAOU : ΔSi : ΔN : ΔP were 285:58:19:1 by atoms. A comparison of these with similar ratios from the Northern Indian Ocean indicated excesses of 19% nitrate and 45% silicate in the Southern Indian Ocean, while the phosphate concentrations in both these regions were found to be quite similar. Reserved fractions of phosphate and nitrate decreased significantly from the antarctic waters, south of the antarctic convergence, to the sub-antarctic waters found north of this region. This indicates that there is an increase in the intensity of oxidation from south to north. Similar values of reserved silicate in both the regions indicate their origin from oxidation and probably from other processes. Applying an empirical relationship between silicate and AOU, it was observed that the lower ranges of silicate concentrations were due to its consumption by siliceous algae, north of the polar divergence. Negative differences between the observed and calculated values of AOU suggest a transfer of oxygen from deeper to intermediate layers. Oxidative ratios do not indicate any difference in the rate of oxidation of organic matter in the Indian Ocean, north and south of the equator upto the zone of antarctic convergence.

Biological Investigations in Antarctica Ecosystems

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FIRST (1981–82) and the second (1982–83) Indian Scientific Expedition to Antarctica, gave an unique opportunity for biotic characterization of a multitude of ecosystems (Oasis; freshwater lakes; fast ice (icy sea shelf and 'Polynya') pack ice; open ocean and a number of subantarctic islands from a scientifically little known area, between 37° to 72° south latitude and 5° to 45° east longitude.

Deploying the trophic ecology energy flow approach attempts were made to study the composition, distribution, abundance and productivity potentials of different ecosystems and their contribu-

tion to Antarctic food web. Accordingly, floral and faunal components, in relevance to environmental oscillations, were explored.

Biotic components included bacteria, mosses, lichens, phytoplankton, zooplankton, benthos, krill, seabirds and marine mammals and the associated life process characterization, such as, chlorophyll and pheophytin pigment content; estimation of particulate organic carbon (POC); measurement of live carbon or adenosine tri-phosphate (ATP) and the rate of biological production and utilization at different trophic levels in the terrestrial, limnetic, glacial and marine ecosystem.

Some of the important findings were as follows:

- i. Under the tremendous biennial fluctuations in light intensity, lack of organic soil, and wind chill stress, the microflora, dominated by petrophilous lichen (*Acrospora* sp.) and moss (*Bryum* sp.), flourishes in the crevices and pores of rocks and boulders, in the Oasis ecosystem of Dakshin Gangotri (Antarctica).
- ii. Ice free exorheic lakes, forming a self-contained ecosystem, supported a high bacterial population ($12 \times 10^4 - 5.9 \times 10^6$ /ml), characterized by *Bacillus*, *Micrococcus* and *Corynebacterium*. Chlorophyll *a* content varying from 0.11 to 0.38 $\mu\text{g/l}$ supported a high rate of photosynthesis, ranging from 0.14 to 0.68 $\text{mgC/m}^3/\text{hr}$.
- iii. Fast ice (icy shelf and Polynya) and pack ice, constituting an independent but complex ecosystem, is dominated by unicellular algal organisms, distinctly different from the planktonic flora. While the plant pigment concentration was rather poor (0.027–0.067 $\mu\text{g/l}$), the primary productivity (0.24–0.48 $\text{mgC/m}^3/\text{hr}$) was reasonably high. Sea ice was poor in POC and ATP content. Highest surface chlorophyll value of 1.5 mg/m^3 and a production rate of 2.65 $\text{mgC/m}^3/\text{day}$, characterized the pack ice ecosystem.
- iv. Phytoplankton of the open sea environment, was contributed by 27 species of diatoms and 1 species each of dino and silicoflagellate, respectively. Average primary production rate of 3.72 $\text{mgC/m}^3/\text{day}$ in coastal waters was several times higher than that (0.95 $\text{mgC/m}^3/\text{day}$) in the off-shore areas. Bacterial counts and ATP content, displayed a direct relationship, with a significant part of POC, represented as detritus.
- v. The 68 species under 6 taxa, constituted the zooplankton. Standing crop, varied from 14 to 624 $\text{ml}/1000 \text{ m}^2$. Secondary production, exhibiting a distinct zonality, fluctuated between 8.4

mgC/m³/day (coastal waters) and 249.8 mgC/m²/day (open sea). Light induced diurnal periodicity of vertical migration of zooplankton, was less pronounced, in the 'Polynya'.

- vi. Bottom fauna or benthos in the depth range of 200 to 3600m, was characterized by 12 meiofaunal and 3 macrofaunal taxa, with population density ranging from 118 to 29774, with a high mean count of 6760/m². Biomass production varying from 1.18 to 566.40 g/m². was exceptionally high at shallow depths and exponentially decreased with increasing depth and also away from the continent.
- vii. High content of carbohydrates and organic carbon in the seafloor sediments, indicate the selective preservation of metabolites, during oxidation, under extreme low temperature regime.
- viii. Observations on 13 species of seabirds and 5 species of marine mammals, reveal that the distribution and abundance is limited by such environmental parameters, as: sites for breeding and nesting, low temperatures, high winds (especially, for seabirds) and availability of food.
- ix. Trophic relationship and energy flow in any Antarctic ecosystem, whether, oasis, limnetic, glacial or marine, is centered around the significant role of the key organism-Antarctic Krill-having uneven distribution with congregation of high standing crop, in the open sea and relative impoverishment in the coastal region.

Glass Transition: A Veritable Clue to the Nature of Glassy State

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SUPERCOOLED liquids congeal into an amorphous solid known as glass at the glass transition temperature, T_g . Glass transition is generally reversible. The transition of the glass to the supercooled liquid is characterized by rapid step-like rise of heat capacities and thermal expansivities in a manner reminiscent of a second order phase transformation in crystalline solids. A number of efforts¹ have been made to understand the nature of this transition but with no completely satisfactory explanation of the phenomenon. All such approaches to glass transition have assumed homogeneous disordered structure of glasses.

Glasses are x-ray amorphous and x-ray scattering techniques yield radial structure functions. The only

definitive information obtained from scattering techniques is local coordination numbers around the heavily scattering atoms in the glass. Use of other techniques like EXAFS has extended the structural information available to partial local structures around different types of atoms in a glass. Intelligent modelling has often provided more useful structural information. In recent times computer simulation has indicated interesting structural features such as presence of nonspace filling groups known as amorphous in glass forming systems².

Glasses are indeed microscopically homogeneous and isotropic. But structures in the submicroscopic range of the order of a few angstroms (20 Å–100 Å) do not seem to be homogeneous. Several experimental evidences, notably high resolution electron microscopy of ionic and metallic glasses, suggest strongly the presence of ordered regions of the dimensions of 20–100 Å. Glasses are therefore heterogeneous over that scale of distances. Ordered regions in glasses appear to be held together by continuous matter whose structure is truly amorphous. It would therefore be more close to reality to consider glasses as microheterogeneous in which 'more ordered' regions which we call as 'clusters' are bonded by 'less ordered' regions which we call as 'connective tissue', ordered regions having typical dimensions of 20–100 Å^{1,2}.

Glass transition models should therefore recognize the presence of such microheterogeneity. A qualitative difference would arise in treatment of glass transition phenomenon itself. If such a model of glass transition³ can explain known experimental facts with regard to various physical quantities and their variation through glass transition, it would serve to establish firmly that glasses are in general microheterogeneous⁴. Glass transition study could therefore serve as a clue to the nature of glassy state.

In the cluster model of glass transition^{3,6} more ordered clusters are conceived as bonded by less ordered tissue. Further, tissue is less dense and the particles in the tissue vibrate in highly anharmonic potential wells. Glass transition occurs when these clusters melt away into the tissue where particle motions characteristic of a fluid originate. In order to render mathematical treatment tractable, several simplifying, but reasonable, assumptions are made. More specifically, the features of the model are as follows. (a) Clusters are spherical and of equal size. (b) Particles in the tissue vibrate in anharmonic wells. As a result of the anharmonicity several potential wells are connected (coalesce) at thermally accessible energies. (c) Vibrational motion of particles excited into higher energy states of multiply connected potential regions gradually evolve into translational motion. (d) Particles on the surface of clusters which share the anhar-

monic potentials of the tissue region gradually 'drop' into the tissue region decreasing the volume of the clusters. (e) When all clusters shrink by shedding their particles into the tissue, the glass transition to a (highly viscous) supercooled liquid is complete.

The relative size of a shrinking cluster, ξ as referred to its size at 0° K decreases rapidly from unity to zero over a short span of temperature which defines the transition region. Variation of ξ is quite similar to the variation of order parameters in cooperative transformations in crystalline solids. If one were to treat the diameter of a cluster as a measure of phonon coherence length, it could indeed be considered an order parameter and glass transition may be treated in the framework of Landau theory. The observed variation of heat capacity and thermal expansivity are indeed in the nature of such 'mean field' systems to which Landau theory is appropriate.

In this simple and quite general model of glass transition, T_g is related to vibrational excitation energies in the tissue region. Vibrational excitation energy of about 1 kcal (400 cm⁻¹) suggests a value of T_g 600 ± 50° K with reasonable values of other model parameters. Most lithium ion containing oxyanion glasses where Li⁺ ion exhibits a typical cage like vibration with a frequency of ~ 400 cm⁻¹, have T_g values in this range. Cluster model can also elegantly account for the presence of two activation energy regions of conductivity observed in many ionic glasses. It can account for the pressure effect on the conductivities of ionic glasses, for the occurrence of subglassy relaxations, loss of Mossbauer intensities, variation of T_g 's in alkali phosphate glasses, variations in band shapes in absorption spectra corresponding to external modes etc.

Formation of clusters upon cooling the melt may be caused by the familiar nucleation process; however rapid growth of nuclei is somehow inhibited. It could also result from freezing out of ordered fluctuations. Presence of such ordered clusters circumvents Kauzmann paradox and obviates the necessity for speculating an amorphous ground state for which neither experimental nor theoretical basis exists. It can also easily account for crystallisation of several glasses at temperature quite close to T_g itself upon heating.

Investigation of glass transition with the cluster model has thus resulted in providing strong indications regarding the microheterogeneous nature of glassy state.

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Geological and Glaciological Studies at and Around Dakshin Gangotri-Indian Research Station at Antarctica

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1 GEOLOGICAL STUDIES

THE Geological evolution of Antarctica has followed a course similar to that of other southern continents—being the part of once Gondwana Land. Lot of studies in recent years, have been devoted to Antarctic geology more so with the advent of the International Geophysical year (1957-58) and much knowledge has been gained since then, mainly as a result of extensive research during the expeditions of several countries. Indian contribution till now had been practically nil. Geological studies initiated in this remote continent during the 1st and 11nd Indian Expedition, however, have added to the existing status of our geological knowledge.

Geologically, Antarctica has been divided into two main structural provinces, a stable mass comprising

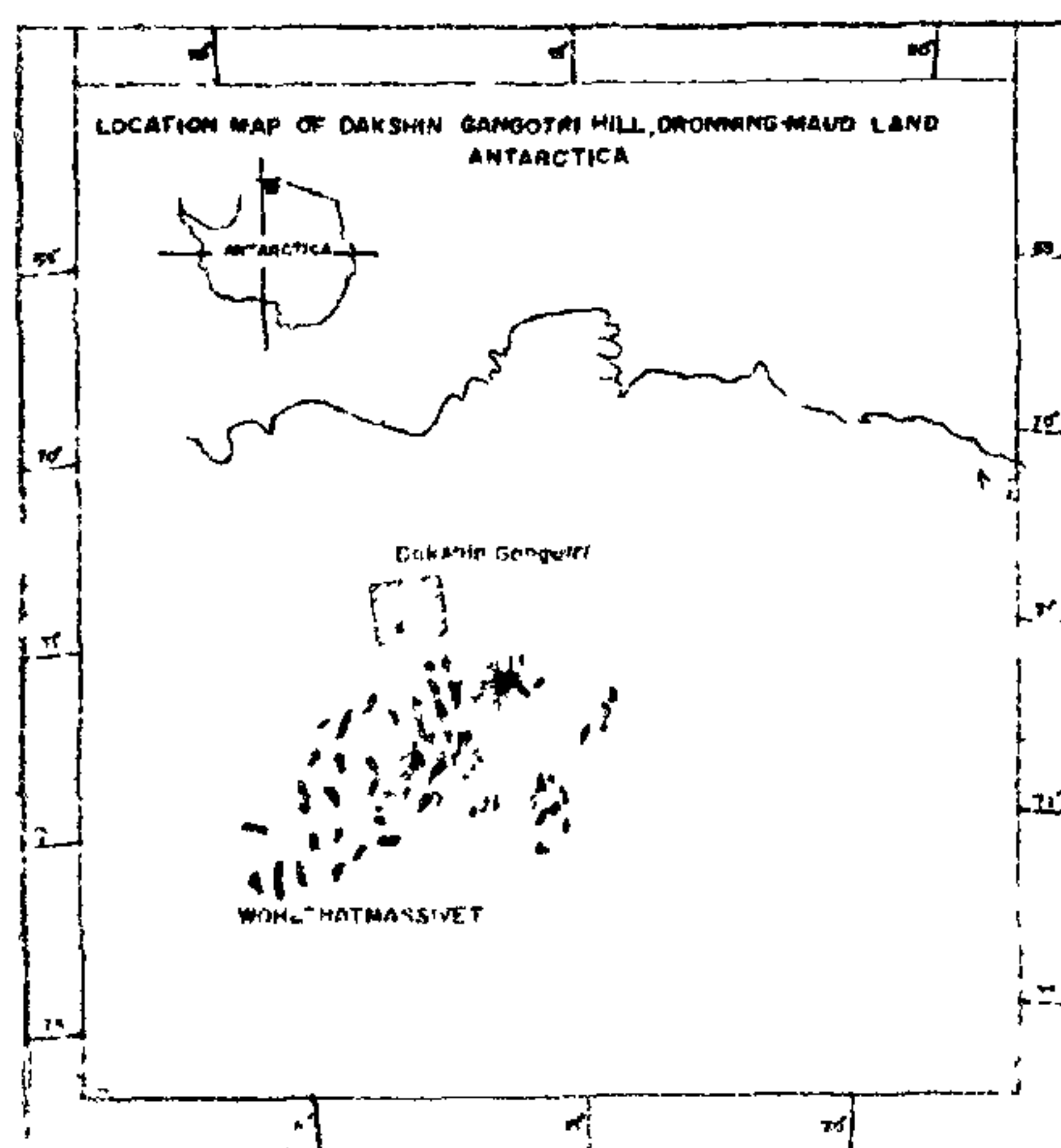


Figure 1. Location map of Dakshin Gangotri Hill, Dronning-maud land, Antarctica

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mostly of East Antarctica, and a mobile belt of West Antarctica. These two geological provinces are separated from each other by a lengthy fault block represented, topographically, by the trans-Antarctic mountain chain. Indian base research station lies in the East Antarctic province around 70° S Lat, 12° E Long (figure 1).

About 100 km south of the Indian Base Research Station lies a 16 km long hill range with a mean width of one km, abruptly rising to a height of about 100 m, from the flat expanse of continental ice. This hill range has been named Dakshin Gangotri range by the 2nd expedition after the automatic weather station established by the first expedition.

Geological Survey team who formed a part of the 11th Indian Expedition carried out geological mapping of this hill wherein the following stratigraphic sequence was established:

A. Augen gneiss with aplites: This is a coarse grained greyish coloured quartzo-felspathic gneiss, showing well developed foliation and augens of feldspar. The foliation trends monthly NW-SE with southerly dips ranging from 20°–35°. This unit forms the base of the stratigraphic sequence and is followed by

B. Rusty banded gneiss: The rock is a well foliated, rusty coloured gneiss, composed of biotite and feldspar. Garnet appears to be metamorphic index mineral which shows characteristic snow ball structure. Conspicuous feature observed in this rock unit is the presence of amphibolite bands and calc gneiss bands.

C. Garnet biotite gneiss: The rock is well foliated gneiss showing relict compositional banding. Metabasites intrude the rock in the form of sills and dykes. In addition to quartz and feldspar the rock is composed of garnet and biotite.

D. Banded gneiss: This meta-sedimentary unit forms the southern most limit of the Dakshin Gangotri hill range whence it gets buried under polar ice toward south. The foliation trend varies from NE SW to ENE-WSW with southerly dips.

Intrusives: Intrusives, both in the form of sills and dykes, are represented by pegmatites, dolerites, aplites and pyroxene-amphibolites. Core drilling was carried out in one of such intrusive bodies which on the microscopic examination was identified as melasyenite.

Structure: Entire sequence is exposed in the form of a synform plunging southerly and shows the following linear and planar features:

a. **Bedding:** revealed by compositional banding and colour laminae.

b. **Foliation:** revealed by growth of micaceous minerals.

c. **Axis of minor folds:** meso-scale folds shows southerly plunges of about 10°.

d. **Mineral lineation:** mineral lineation (biotite) plunges 30° towards S 75° W.

e. **Faults:** A major fault between rock unit C & D (above) was observed in the south-east part of the mapped area.

f. **Joints:** Most prominent joints observed were trending (i) E-W with northerly dips (ii) N 10° E-S 10° W with vertical dips and (iii) parallel to the strike of the rocks.

Mineralisation:

Except isolated stains of malchite and patches of limonitic gossan no indications of base metal mineralisation were noted in the area mapped.

A 300 m long zone of graphite mineralisation with an average width of 150 m was noticed near the contact of rock unit B (above) with the underlying gneisses, west of Dakshin Gangotri glacier.

Reconnaissance in the Wohlthat Mountains:

South of the Dakshin Gangotri a conspicuous mountain chain-Wohlthat Massif-rising to peaks of 3000 m. a.s.l. exists. This range is dissected into massifs by south to north trending glaciers which emanate from the inland polar ice. Geological reconnaissance of this mountain revealed the rock units forming this range to be garnet-sillimanite gneiss, oliving-basalt and charnockite.

II. GLACIOLOGICAL STUDIES

Antarctic continent has a periphery of ice-shelves some of which are permanent and extensive. One such ice-shelf is on Princess Astrid Coast of Queen Maud land on which the Indian base research station is located.

Glaciological Studies, to be continued on a long term basis, were initiated during the present expedition around the base research station and far inland upto Dakshin Gangotri. The studies covered the following heads.

a. Snow accumulation and ablation on ice shelf:

b. Micro-relief of shelf surface.

c. Experiment on artificial augmentation of ablation by using coal dust spray.

d. Thermal profiling of ice shelf upto depth of 7 m. with the help of dial thermometers and thermistors.

e. Crystal studies of firm ice.

f. Snow stratigraphy and density profiling of the shelf ice and Polar ice.

- g. Studies of ice-bbergs.
- h. Dating of shelf ice.

In addition, a contoured map of the snout of a small plateau glacier, named as Dakshin Gangotri glacier, was prepared on 1 : 1000 scale for future ice movement studies.

Development and Differentiation in Plants

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A UNIQUE feature of development in plants is the formation of new organs from the apical meristems throughout the life-span of a plant. The basic problem is to understand the mechanism underlying the differentiation of various structurally and functionally distinct cell types and organs at the right time at the right place, starting from a homogenous mass of cells. Major advances in our understanding of plant development became possible with the advent of techniques of growing plant cells and organs on a chemically-defined medium. The temporal and spatial aspects of development shall be discussed using moss protonema and corn plant as model systems.

The protonema of mosses like *Funaria hygrometrica* is simple and made up of three distinct cell types, the chloronema, the caulonema and the bud initials. These are formed in a precise and predictable sequence. The differentiation of each cell type is subject to a multiple control and depends on inoculum cell density (Johri and Desai, 1973) and the relative concentrations of cyclic AMP and phytohormones such as IAA and cytokinins. Depending on the relative amount of IAA and cyclic AMP, either caulonema or chloronema cells are formed (Handa and Johri, 1979). Further, it is clear that the process of cell differentiation involves a response by a group of 4 to 10 chloronema cells (Johri, 1978) and some kind of an organisation (e.g., cells arranged in a filament) is probably required. During this process, the size and multiplication of chloroplasts, the diameter of a cell and the plane of cell division become precisely controlled. It is not known how phytohormones or cyclic AMP regulate these complex events, but eventually there must be differential gene expression.

The analysis of cell lineages and of the mutations affecting the vegetative and reproductive morphology of corn plant have yielded important information about the development of corn plant (Steffensen, 1968; Coe and Neuffer, 1977, 1978; Johri and Coe,

1982, 1983). The clonal analysis shows that a group of embryonic cells undergo a gradual restriction of developmental potency which delimits their subsequent fate to a specific organ.

The developmental plan underlying the formation of corn plant from the zygote is highly repetitive and strongly regulative. The plane of early cell divisions in the zygote and proembryo roughly demarcate the major features of a plant, e.g., the axis of bilateral symmetry and group of nodes. The commitment at this level is only roughly conferred and is quite plastic. The specific individual nodes and ear branch are formed later during some kind of a refinement process which is analogous to the process of compartmentation. In the shoot apical meristem of mature embryo, the cells at the apical and distal ends are committed to produce respectively the tassel and the lower 5 or 6 nodes, while the cells in between represent a gradation from roughly-committed to a fully-committed states. The entire shoot apical meristem can thus be thought of as a 'developmental field' whose boundaries are represented by zones of committed cells. And by invoking a graded distribution of two hypothetical substances, it is possible to explain the regulative features of development in plants.

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Non-Covalent Interactions and Molecular Association Involving Biomolecules

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A DISTINGUISHING feature of a substantial part of the x-ray crystallographic work in our laboratory has been that it aims not only at determining the three—dimensional structure of biomolecules, but also at elucidating the non-covalent interactions involving them. These interactions, which include hydrogen bonds and ion-pair interactions, are highly directional and play a crucial role in the structure, assembly and function of proteins and other biological systems. The geometrical features of these non-covalent interactions are being elucidated, at the atomic resolution, in our laboratory through a novel approach consisting of the preparation and the x-ray analysis of crystalline complexes involving amino acids and short peptides, among themselves as well as with other biomolecules. This approach involving complexes has yielded a wealth of information and general patterns are now beginning to emerge. For example, it has become possible to identify four types of specific interactions the guanidyl group of arginine can, and does, take part in. The side chain amino group of lysine appears to be incapable of taking part in specific interactions, but form characteristic geometrical patterns in its interactions with carboxylate groups. The work on complexes has also yielded useful information regarding specific interactions involving effector molecules, particularly ascorbic acid (Vitamin C).

The amino acid molecules in most of the complexes arrange themselves in head-to-tail sequences of the type $\dots\text{NH}_3^+-\text{CHR}-\text{COO}^- \dots\text{NH}_3^+-\text{CHR}-\text{COO}^- \dots\text{NH}_3^+-\text{CHR}-\text{COO}^- \dots$, in which the α -amino and the α -carboxylate groups are brought into periodic hydrogen-bonded proximity. This arrangement is highly suggestive in the context of chemical evolution and could well have been made use of in prebiotic polymerisation. A subsequent careful examination of

the crystal structures of commonly occurring amino acids, their racemates and complexes indicated that head-to-tail sequences are an almost universal feature of amino acid aggregation in the solid state. Its evolutionary implications have indeed added a new interesting dimension to the work on complexes.

The approach involving complexes has also been made use for elucidating the structure and interactions of aspirin—like drugs which are believed to act through the inhibition of prostaglandin biosynthesis.

The work on complexes, which has been highly relevant to biological recognition mediated by non-covalent interactions, has recently led to an ambitious programme of macromolecular crystallographic studies on proteins with pronounced recognitive functions, such as lectins.

Car-3-Ene, A Versatile Resource for Important Industrial Fine Chemicals

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TURPENTINE oil represents a renewable resource for the manufacture of several important organic fine chemicals, especially for aroma and pharmaceutical industry. Almost all these products are based on the chemistry of pinenes, which are the chief constituents of the important American/European turpentines. Indian turpentine, on the other hand, which is obtained from the oleoresin of *Pinus roxburghii*, is quite low in pinene content (20-25%), the major constituent being 3-carene (55-65%). So far, the only application of carene has been its use, as a cheap solvent, for paint industry. However, in order to make the commercial exploitation of Indian turpentine economically more viable, it is necessary to find out more sophisticated uses for 3-carene. Malti-Chem Research Centre (MRC), the R & D Division of Camphor and Allied Products Limited, has put in during the past 10 years, considerable effort in this direction, resulting in several worthwhile commercially important transformations of carene.

Basically, utilization of a chiral molecule, such as (+)-3-carene can be on two lines. In the first approach, the projected use ignores the special advantage which the chirality of the molecule bestows on it. Thus, for example, carene can be converted into cymenes and thence into cresols etc. A lot of work on these lines has been carried out by different groups. However, it is obvious that, at least, at present such transformations are severely circumscribed by the usually limited total

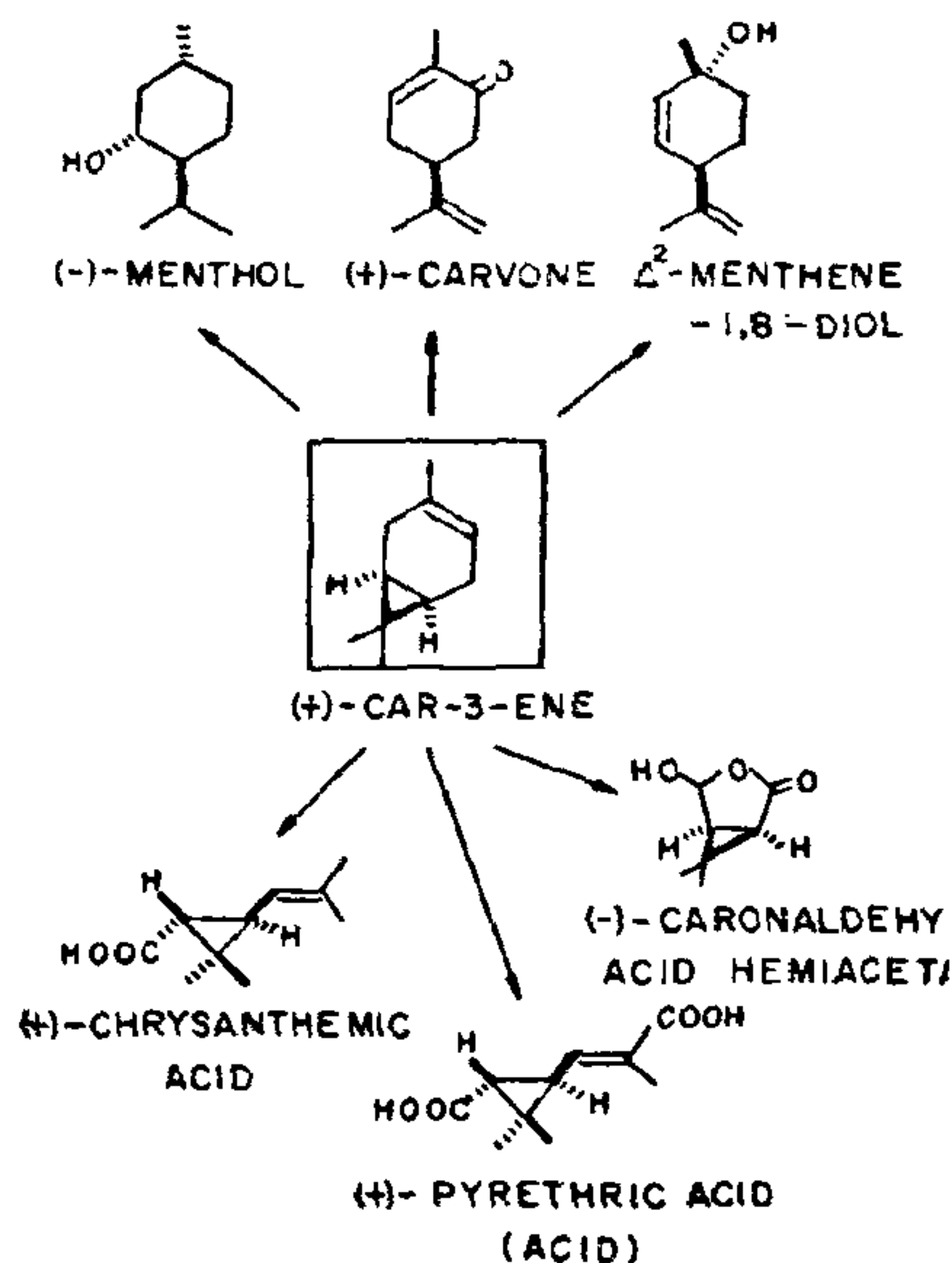


Figure 1. Some important chemicals from (+)-CAR-3-ENE.

availability of the raw material as compared to other competitive feed-stocks such as those based on petroleum. The second approach, on the other hand, envisages full exploitation of the structural and stereochemical features of this molecule. We have primarily concerned ourselves with the second approach.

Figure 1 depicts important transformations, which we have worked out at MRC. The end products shown in figure 1 are all important commercial items, and fall under the category of low-volume, high-value organic chemicals.

(-)-Menthol is an important item of commerce and is extensively used in pharmaceuticals and for flavouring purposes. It is estimated that the current world production of (-)-menthol is of the order of 3000 tonnes, and the present Indian requirements are of the order of about 300 tonnes. Though some quantity of (-)-menthol is being made in the country from the oil of *Mentha arvensis* for the past many years, the total production falls short of the present requirements. In view of this, the process developed at MRC for the manufacture of optically active natural menthol from (+)-3-carene, assumes much importance. The MRC process which was first disclosed in an International Symposium in Bulgaria in 1978, involves isomerisa-

tion of 3-carene to 2-carene, followed by pyrolysis of later to (+)-isolimonene. In the next step, isolimonene is isomerized over a special catalyst to (+)-isoterpinolene. Catalytic reduction of this gives a mixture of menthenes in which the 1,4-addition product, namely 3-menthene predominates. Precise fractionation results in a mixture of 3- and 2-menthenes, which cannot be separated further, and this mixture, as such, is converted into (-)-menthol by the usual epoxidation-rearrangement followed by hydrogenation. This process is being exploited by Camphor & Allied Products, Bareilly, for the production of (-)-menthol since October 1982.

Another important product based on 3-carene is d-carvone, which is in considerable demand, as a flavouring agent. At present, this material is available in the world market in a limited quantity due to non-availability of (-)-limonene, from which it can be synthesized. At MRC we have developed an entirely new process, starting with carene epoxide. In a special reaction, the later compound is isomerized to an unsaturated secondary alcohol, which on oxidation gives the corresponding unsaturated ketone. This ketone on pyrolysis, followed by isomerization gives d-carvone.

Of the remaining products shown in figure 1, two of these, namely (+)-*trans*-chrysanthemic acid and, (-)-caronaldehyde acid hemiacetal are important intermediates for the commercial production of certain pyrethroids, the latest class of important and highly active insecticides with extremely low mammalian toxicity. The process developed for (+)-*trans*-chrysanthemic acid is again based on carene epoxide. This oxide, on further oxidative cleavage gives a ketoacid which after esterification and exposure to peracetic acid (Baeyer-Villiger oxidation) gives an acetate ester. This on treatment with MeMgCl generates the required chrysanthemolactone, which is then converted into chrysanthemic acid. This process has been standardized on the pilot plant and is awaiting implementation. The synthesis of (-)-caronaldehyde acid hemiacetal, has been developed on a laboratory scale. This transformation involves air oxidation of carene to an unsaturated ketone, which after ozonolysis, followed by exposure to acetic anhydride and an acid gives an enolacetate. The later is readily converted into the desired compound by ozonolysis.

'Anti-Leprosy' Vaccines — The Global Scene

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IN the global contest, two vaccines containing (i) heat-killed *M. leprae* A + BCG and (ii) ICRC (killed), a cul-

tivable mycobacterium, have given promising results both in patients and healthy high risk subjects. The exact mechanism/s of action of the vaccines is/are yet to be elucidated. The available data indicate that the vaccines could be used for (i) immunotherapy, as an adjunct to chemotherapy in lepromatous patients, and (ii) immunoprophylaxis. The vaccines would soon undergo field trials. Leprosy has a very long incubation period. It would need about 5-10 years to assess immunoprophylactic efficacy of the vaccines after commencement of the trials.

The control strategies for leprosy, which continues to be a major health problem in the developing countries, are essentially aimed at¹ containment of the microbe (*M. leprae*) and (ii) enhancement of the host immunity. Leprosy patient is the major reservoir of leprosy germs and no intermediate host has been identified so far. Treatment of patients with drugs, which kill the germs, should therefore be able to break the cycle of infection. Although effective drugs are available, drug resistance is occurring at an alarmingly high rate. There are other practical problems in use of drugs in prevention of the disease. Global attempts are therefore being made to develop a vaccine which acts by enhancing host immunity.

Scientific basis for development of microbial vaccines was established by Pasteur more than a hundred years ago. In general, attenuated causative organisms that have lost pathogenicity but retained antigens involved in 'protective' immunity are ideally suited for vaccine preparation. Large quantities of the organisms are required if the vaccine is to be used in man. Although the leprosy germs (*M. leprae*) are yet to be cultivated, sufficient quantities are now available from Armadillo, in which the bacilli grow profusely. There is considerable antigenic cross reactivity between *M. leprae* and other mycobacteria, many of which are non-pathogenic to man. An alternate strategy would be to prepare a vaccine from a cultivable avirulent strain of mycobacterium that exhibits cross reactivity with reference to 'protective' antigens. Both the approaches have been recently used with promising results. *M. leprae* is antigenically, perhaps, the weakest mycobacterium pathogenic to man. A third approach, which is still in the experimental phase, would be to improve its antigenicity by suitable chemical treatment.

Determinants of 'protective' immunity are often difficult to be defined. Like for other intracellular parasites, cell mediated immunity (CMI) appears to be the major defence mechanism against *M. leprae*; circulating antibodies have very little role. Amongst the laboratory parameters of the CMI, the lymphocyte transformation test correlates well with hypersensitivity. Several years ago, Mitsuda demonstrated that

intradermal administration of lepromin, a particulate antigen of *M. leprae*, elicits, 3-4 weeks later, a local reaction consisting of induration. This type of lepromin (Mitsuda) reaction shows excellent correlation with the capacity of the host to handle *M. leprae* both in patients and normal healthy subjects.

Leprosy is a spectral disorder with highly infectious and progressive lepromatous (LL) and self-limiting tuberculoid (TT) varieties representing the two poles. Tissue bacillary load, which is maximum in the LL, progressively diminishes as one moves to the other end of the spectrum, being negligible in the TT. In untreated cases, the magnitude of the Mitsuda reaction is inversely proportional to tissue bacillary load. The reaction is absent in the LL but strongly positive in the TT. Epidemiological data indicate that Mitsuda negative individuals, particularly in endemic areas, run a high risk of contracting lepromatous leprosy.

Immunoprophylactic efficacy of a vaccine has to be ultimately assessed by its capacity to prevent the disease in the community. Before a vaccine is subjected to such field trials, it would be essential to show that it not only offers protection in animal models but also stimulates cell mediated immunity against the leprosy germs. Unfortunately, there is no good laboratory model of human leprosy. In the present state of our knowledge a 'candidate' vaccine should at least bring about persistent lepromin conversion in negative healthy subjects and patients. In this respect three vaccines containing (a) BCG alone, (b) heat killed *M. leprae* A + BCG and (c) ICRC (killed) need to be considered. BCG has already undergone field trials. The last two, which have given promising results in patients and healthy high risk subjects, are now ready for field trials.

BCG is known to induce Mitsuda conversion, particularly in children. It also suppresses growth of *M. leprae* in the mouse foot-pad. However, the results of the field trials in man are conflicting. It offered protection in Uganda but not in Burma. BCG is no more a popular modality for immunoprophylaxis.

Although BCG alone has not been very promising, Convit *et al*² have recently developed a vaccine containing a mixture of heat-killed *M. leprae* A (Armadillo derived) and BCG. A few years ago they had demonstrated that addition of BCG to *M. leprae* induced local response consistent with high immunity. Thus, in patients of indeterminate leprosy, intracutaneous administration of *M. leprae* alone produced only a poor granuloma and the bacillary clearance was tardy. A totally different picture was seen when a mixture of *M. leprae* A + BCG was given. Granuloma was not only well formed but even the mycobacteria were quickly cleared. Subsequently, it was shown that

TABLE I

Comparative effects of anti-leprosy vaccines**

	Convit <i>et al</i> (Venezuela)	Deo <i>et al</i> (India)
Composition	<i>M. leprae</i> A (Heat killed) + BCG	ICRC (killed by γ -irradiation)
Volume of each dose	0.5 ml	0.1 ml.
<i>Effects in Patients</i>		
1. Immune Conversion	SPA (48 hr)	Mitsuda (3-4 weeks)
BB/LL (Active)	38% (Multiple Injections-259)*	58% in LL (one shot 71)*
BL/LL (Inactive)	63% (Multiple Injections-113)	90% in BB/BL (one shot-11)
Indeterminate	97% (multiple Injections-32)	-
Contacts	100% (Single shot mostly-25)	92% (one shot-12)
2. Reactions		
i) ENL (only in LL)	In a number of cases	30%
ii) Reversal reaction	In 27% of active BL/LL	In 12% LL
3. Fresh Neurological lesions	Rare, easily controllable	Rare, easily controllable
4. Changes in morphology of lesions	Up-grading of the lesion and clearance of <i>M. leprae</i> in a number of patients	Up-grading of the lesion and clearance of <i>M. leprae</i> in a number of patients

SPA : Skin Reaction to Soluble Protein Antigen

*Figures in parentheses denote number of patients/contacts who participated in the study.

**Based on the material submitted for WHO-IMMLEP Meeting on Immuno-epidemiology of Leprosy, New Delhi, India, February, 1983.

the mixture was able to bring about lepromin conversion in negative patients when each organism given alone, even repeatedly, failed to do so. Convit *et al* have now tried the vaccine in a variety of Mitsuda-negative patients and their lepromin (Mitsuda) negative house-hold contacts. The results are depicted in table I.

ICRC bacillus is a cultivable slow growing mycobacterium that has been repeatedly grown from human leproma for the last 25 years. The bacilli exhibit antigenic cross reactivity with *M. leprae* specially with reference to CMI both in man and laboratory animals. A vaccine, which contains ICRC killed by gamma irradiation, has been tried in a number of patients of lepromatous leprosy and their Mitsuda-negative house-hold contacts for the last 4 years. The results are shown and compared with those obtained by Convit *et al* in table I.

Both the vaccines have induced comparable changes. Lepromin conversion is observed in a number of patients associated with upgrading of lesions as evident by morphologic changes. In some, even a reversal reaction has been observed. Lepromin-negative contacts exhibit a very high conversion rate (90-100%). Surprisingly, reactions have been minimal and easily controllable. This should be an asset for field trials.

The mechanism of action of the vaccines is not yet fully understood. Induction of immune response is a complex process requiring participation of antigens and different types of cells. However, interaction of the former with appropriate clones of lymphocytes plays the central role. It is believed that binding of an antigen to a specific site on the membrane of immune reactive cells initiates the immune response. It could be visualized that individual variations in the responses to a specific antigen could be a consequence of differing avidity of cells for the antigen. Based on these assumptions a new hypothesis, which could explain susceptibility to forms of leprosy as well as the mechanism of action of the vaccines, is proposed in figure 1.

Both in patients and healthy volunteers ICRC in a Mitsuda type antigen of ICRC bacilli, and lepromin induce comparable skin reactions. Except that inductions produced by the former are larger. The data suggest that ICRC is antigenically stronger than *M. leprae*.

In mice, a mixture of *M. lepraemurium* + BCG produces better immunity as compared to that induced when either is given alone. This appears to be the mode of action of Convit's vaccine in which BCG could act as an adjuvant. The precise mechanism underlying enhanced immunity induced by mixed vaccines is not

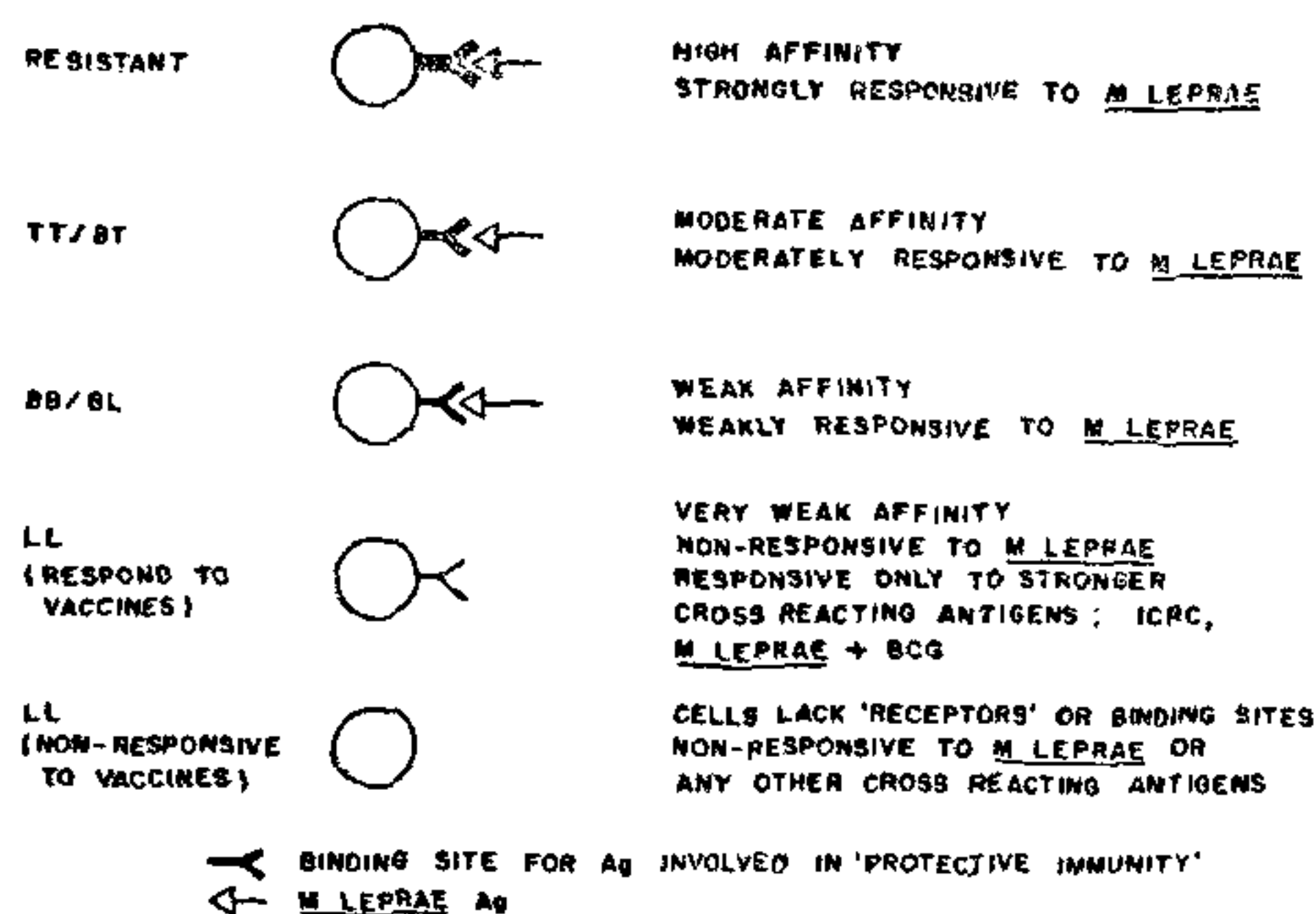


Figure 1. Schematic illustration of the hypothesis.

fully understood. Several mechanisms might be visualized including activation of macrophages which could act by improving immunogenicity of antigens.

Importance of co-operation of sub-sets of T-cells (helper, suppressor) in regulation of immune response is now well recognised. The exact mechanism/s and the type of cell/s responsible for the specific energy to *M. leprae* antigens in lepromatous patients are not yet fully established. Recently, it has been reported that lepromatous T-cells when cultivated in the presence of *M. leprae* do not exhibit blast transformation and fail to generate interleukin (IL₂) which is essential for clonal expansion of antigenically activated T-lymphocytes. Further, the non-responsiveness could

be corrected if the T-cells are cultivated in the presence of *M. leprae* and T-cell conditioned medium that contains IL₂. These experiments suggest that the immune defect in lepromatous leprosy is mainly due to impairment of generation of IL₂. As suggested by Bloom, the vaccines, particularly the ICRC 'anti-leprosy' vaccine, could act by stimulating specific clones of lymphocytes that generate the T-cell growth factor/s.

Irrespective of the mechanism of action, once cells with very weak affinity are primed with stronger immunogens (ICRC or mixed vaccine), memory cells, that are capable of responding to weaker antigens (*M. leprae*), are produced. This may explain persistence of lepromin reactivity observed by both the groups. In patients continued antigenic stimulus is provided by bacilli present in tissues, while residents of endemic areas are continuously exposed to *M. leprae* present in the environment.

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