Interfacing biological, physicochemical and engineering sciences—IMTECH

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A successful biotechnology industry hinges on integration of research in modern biology with inputs from the physicochemical and engineering sciences and an instinct for recognizing commercial possibilities of laboratory work.

Technologies based on the synthetic or degradative activities of living organisms have been intimately associated with the development of human societies all over the world. Most of these processes were established through empirical experience stretching over generations. Organized industrial ventures based on biological activities are, however, mostly twentieth-century phenomena. Especially in the last four decades or so, rapid advances in various branches of the life sciences have led to deep insights into the basic mechanisms regulating many of these processes and have provided powerful tools for modifying the metabolism of the concerned organisms to maximize desired activities. The techniques of creating desirable combinations of genes have been refined to such an extent that, in theory at least, it is possible now to design organisms for specific purposes. Closer at hand are the capabilities provided by the recombinant-DNA techniques for the production of a large array of biologicals at scales and costs considered impossible only a decade ago. Concurrent with these advances in basic biology came the realities of dwindling supplies of traditional raw materials and energy sources. These factors have spurred intense efforts towards efficient exploitation of various biological processes for the production of industrially important commodities in the developed countries. The spectrum of this renewed interest covers bulk industrial chemicals, recovery of metals from low-grade ores or even sea water, coping with industrial pollution of our environment, the traditional antibiotics and pharmaceuticals, and a whole new range of medically important diagnostics and therapeutics. These activities are grouped under the generic name biotechnology, which results from the amalgamation of the biological sciences with the physicochemical and engineering sciences. Microbial technology forms a subset of activities under biotechnology in which the properties of prokaryotic or eukaryotic microorganisms are exploited for industrial purposes. A successful microbial technology today is likely to involve the foresight of a microbial physiologist, the skills of a gene manipulator, the imagination of a mathematical modeller, and the dexterity of a chemical engineer.

Biotechnology in India

The potential of microbial technology in national economy has been amply realized in the developed countries by both industry and government. Thus, in these countries, microbial technology has become an area of furious activity, encompassing generation of venture capital, formulation of regulatory frameworks, and intense R&D efforts. In glaring contrast, the Indian scene in the area of microbial technology is definitely dismal. Other than an outmoded ethanol industry thriving more on stringent protection of the vital raw material, cane molasses, and price controls of the end product than on its inherent vigour, the impact of microbial technology on the Indian economy has been virtually nil. Attempts were made in the fifties and sixties in both the public and private sectors to establish fermentation-based industries through technology imports. Unfortunately the momentum generated by the end of the sixties was allowed to be dissipated in the seventies through regulatory constraints and myopic policies imposed by the government. As a fallout of these acts of omission or commission, we face the nineties with the stark reality that our self-sufficiency in the production of adequately pure quantities of even the very first antibiotic, penicillin, is often questioned. Although we have a very active drugs and pharmaceuticals industry that excels in formulations and, in some cases, production of bulk synthetic drugs, it has not yet been possible to establish a solid foundation for fermentative production of various drugs and industrial chemicals in either the private or the public sector. A thorough analysis of this sector of the Indian pharmaceutical industry published in 1984 (ref. 1) by the National Council of Applied Economic Research describes the situation, perhaps euphemistically, as 'a varied picture, containing a few bright patches amidst several dark spots'. The primary cause of this failure can be traced to the inherent complexity and multidisciplinary nature of microbial technologies, for which a corps of highly motivated and innovative individuals with intensive training in physical, biological and engineering sciences is required for providing the necessary inputs. Since independence, we have created a plethora of institutions of higher learning, sometimes with the best of intentions, often out of political expediency. It turned out that adequate sustenance of most of such institutions is totally beyond our fiscal capabilities or will. Many of these substandard institutions continue to churn out poorly trained graduates who can hardly be expected to make successes of complex technological enterprises. The better-supported of such institutions, on the other hand, turned out to be clearing-houses for export of the best-trained of our students to fuel the biotechnology revolution elsewhere. Scattered efforts have been made in various universities and publicly supported R&D institutions with minimal

and often half-hearted support to provide an R&D base for microbial technology. It is not surprising that these efforts could not muster the critical mass necessary for success in such complex endeavours involving multiple disciplines. Some successes on a laboratory scale were reported. However, few of these could be exploited commercially. Inadequate scale-up facilities, indifferent and often vacillating regulatory stances of the government, and near absence of appropriate interfacing counterparts in industry contributed to this dismal scenario, and entrepreneurs largely kept away from this important area that has been transforming the developed world.

Modern and well-equipped, with pilot-plant facility

Winds of change

In the early eighties, the major funding agencies began to show signs of the realization that, in order to generate competence, it is essential to fund basic research, especially in various areas of modern biology, and that attention should be given to the creation of centres with adequate infrastructure. The National Biotechnology Board (NBTB) was constituted to plan, monitor and spearhead the national thrust in this area. In 1986 this board evolved into a full-fledged department under the Ministry of Science and Technology. The Department of Biotechnology (DBT) along with the older agencies, primarily the Department of Science and Technology (DST) and the Council of Scientific and Industrial Research (CSIR), installed enlightened funding policies to strengthen existing institutions and established new institutions for specific purposes, so that, in the less than ten years since these winds of change started blowing, we are beginning to see results from a number of institutions in terms of findings that could provide the basis for commercial exploitation. Two major new institutions, namely the National Institute of Immunology (NII, under DBT) in New Delhi and the Centre for Cellular and Molecular Biology (CCMB, under CSIR) in Hyderabad, came into full bloom. Furthermore, in about a dozen institutions around the country, a basic level of infrastructure and competence in the area of modern biology has now been created that is likely to mitigate the acute shortage of trained manpower in this area.

CSIR realized the potential of biotechnology for solving some of the national problems in the areas of food, fuel and health care. It appointed a committee of eminent scientists, technologists and industrial entrepreneurs headed by M. L. Dhar to study and recommend actions necessary to augment and sustain the development of a biologicals industry in India. The Dhar committee recommended the establishment of a centre where R&D work in biological sciences would be intimately integrated with requisite inputs from physicochemical and engineering sciences with a view to developing viable technologies. The recommendations of the Dhar committee were accepted by CSIR, and in 1983 plans were set in motion to establish the Institute of Microbial Technology (IMTECH) at Chandigarh.

IMTECH charter

With considerable foresight, the Dhar committee drew up a charter for IMTECH which emphasized that this institution should have a very strong multidisciplinary scientific base on which sound technologies of the future would be built. The avowed objectives of IMTECH were enunciated as follows.

– To provide an integrated research, development and design base for microbial technology

– To undertake basic and applied research and development programmes in established and newly emerging areas of relevant biotechnology including genetic engineering

– To optimize the existing microbial processes currently available and in use in the country

– To develop and maintain gene-pool resources and genetic stocks of microbial cultures and other cell lines; this could also serve as a reference centre to assist other centres

– To establish facilities for biochemical engineering, instrumentation development including microprocessor systems, a computer centre, and development of mathematical models for process parameters

– To establish facilities for design of process equipment and bioreactors

– To impart training in microbiology, microbial technology and biochemical engineering

– To conduct training and refresher courses for researchers and technologists

– To establish documentation and information retrieval and dissemination facilities and a data bank to meet the needs of the institute

– To establish and maintain effective linkages with industry and educational institutions

– To develop capabilities for producing design and engineering packages for industrial plants

– To accept contractual work in under-

taking research and development for biotechnology of national importance

Start-up laboratory, permanent campus

Early in the planning of IMTECH it was realized that success of an institution of this magnitude depends very much on the creativity and skills of individual scientists and support personnel. It was decided that such individuals be gathered even while the permanent buildings for the institute were being built. V. C. Vora was persuaded to take on this responsibility. In 1984 a start-up laboratory was established in an industrial shed (about 10,000 sq ft) and was equipped adequately so that talented scientists could be attracted to form nuclei of capabilities in different disciplines deemed essential for realization of IMTECH's concepts This start-up laboratory was able to attract a small group of dedicated, competent and fairly young scientists. Most of this group opted for IMTECH after many productive years in leading institutions abroad.

In September 1989 the start-up laboratory was closed and IMTECH activities were shifted to the partly built permanent campus. Construction of the permanent campus began in July 1986 on a 46-acre site in Sector 39, Chandigarh. The complex consists of six buildings with a total covered area of 360,000 sq ft. which includes a main laboratory block (185,000 sq ft.), library (16,000 sq.ft), an animal house (24,000 sq ft), a fermentation pilot plant (50,000 sq ft), workshop and stores (40,000 sq. ft.) and a cafeteria (12,000 sq ft). The permanent campus will also include a guest house, student hostel, recreational facilities and staff housing

National facilities

The heartening development of IMTECH at the start-up laboratory inspired the confidence of DBT. This confidence is reflected in the charge entrusted to IMTECH by DBT for creation of three major national facilities

Biochemical Engineering Research and Process Development Centre (BERPDC)

Optimization of fermentation parameters, design of upstream and downstream processes, and their control are essential parts of an integrated approach for translating the concepts of new biology into biotechnology. BERPDC has been established at IMTECH to bridge the gap between laboratory findings and an industrially viable fermentation process For this purpose, a fully computerized pilot plant with a capacity of 1500 litres is being installed along with the requisite facilities. These facilities, jointly supported by DBT and CSIR, will be made available to industries, universities and other R&D institutions for evaluation of fermentation processes developed indigenously or proposed to be imported In the permanent campus of IMTECH, BERPDC will be located in a three-storied building with about 50,000 sq ft. of space.

Microbial Type Culture Collection (MTCC)

A well-maintained collection of authentic strains of microbes is essential for basic and applied research for development of microbial technologies. To cater to this need a national facility has been established at IMTECH with support from DBT and CSIR It will serve as a depository of microbes (bacteria, yeast, fungi and mycoplasma), including patented cultures, genetically manipulated microorganisms, and gene banks in various vectors. It will act as an agency for the distribution of authentic strains for research and industrial use. Modern facilities for identification and preservation are being set up. Documentation and inventory-control aspects have been computerized. Services for supply and deposition of microorganisms as well as identification of some groups of microbes have been started MTCC has also started providing short-term training courses on isolation, preservation and identification of microorganisms. Research programmes have been initiated for identification and potential use of microorganisms isolated from specialized ecological niches and for development of new and more efficient methods of culture preservation. Active liaison has been established with major international institutions of this nature, such as ATCC, NCTC and WCFC. In the permanent campus of IMTECH, an area of about 18,000 sq.ft. has been allocated for MTCC.

Distributed Information Centre on Enzyme Engineering, Immobilized Biocatalysts, Microbial Fermentation and Bioprocess Engineering (DIC)

To fulfil the need to have better and quick access to information-generating and subsequent, user institutions in the area of biotechnology, DBT decided to set up a Biotechnology Information System (BTIS) at national level. This aims to interlink the specialized centres through a national bioinformation network. The main beneficiaries would be researchers involved in R&D and manufacturing activities in biotechnology BTIS is to be a networking and database management organization in six identified areas involving specialized centres to be known as Distributed Information Centres (DICs), of which IMTECH is one. IMTECH has been chosen to house a DIC on enzyme engineering, immobilized biocatalysts, microbial fermentation and bioprocess engineering.

Academic activities, liaison with industry

IMTECH scientists make conscious efforts to create an atmosphere for close cooperation with universities and other institutions for both development of interactive programmes and personnel training. Thus IMTECH is recognized by several universities as a centre for research leading to the Ph D degree. A post-M Sc diploma course in biotechnology has been offered in collaboration with Panjab University in Chandigarh since 1989 IMTECH continues to provide summer training to M Sc biotechnology students from various universities upon request. Intensive short-term training courses are organized periodically in various aspects of biotechnology. Courses have been offered so far in genetic manipulations, separation techniques, molecular genetics of yeast, molecular genetics of *Streptomyces*, and selective isolation and identification of actinomycetes

Continual intensive efforts to evolve R&D programmes in consultation with and under sponsorship by industrial concerns have resulted in memorandums of understanding with the Oil and

Natural Gas Commission (ONGC), Indian Drugs and Pharmaceuticals (IDPL), Themis Chemicals, Ranbaxy Laboratories, Unichem Laboratories and Vittal Mallya Scientific Research Foundation.

R&D programmes, achievements

In formulating R&D programmes possible end-users are kept in mind. More important, however, is the fact that, in each project, novel and creative approaches are incorporated so that the projects do not degenerate into mere trouble-shooting exercises for existing technologies Most of these projects deal with application of the new tools of molecular biology and genetic engineering to develop new technologies or improve upon the existing processes vital to our national needs.

Keeping these considerations in view, the following research projects were initiated at various times as scientists with relevant competence joined IMTECH.

– Industrial ethanol fermentation: construction of improved strains by genetic manipulation and process optimization
– Rifamycin fermentation: genetic and biochemical approaches for improved process development
– Isolation and cloning of plasminogen activators from animal cells and cloning of plasminogen activator-coding sequence in *E coli*
– Molecular approach to control of rotavirus (a diarrhoea virus) pathogenesis
– Selective delivery of antileishmanial drugs to macrophages
– Novel petroleum exploration strategies: a feasibility study for developing genetic and immunological approaches for detection of microflora diagnostic of oil-bearing formations
– Development of computer software for molecular biology
– Regulation of the immune system: an approach towards developing B-cell- and T-cell-specific immunomodulators
– Construction of vectors for stable maintenance and expression of cloned foreign genes in yeast

It is rather early for tangible end-results of immediate utility in most of these new endeavours. However, significant advances have been made in several projects, as detailed below

– An osmotolerant and ethanol-tolerant strain of yeast has been developed that is capable of producing 12–16% (v/v) ethanol from molasses containing up to 30% (w/v) sugar initially. The strain has proved its utility in up to 4000-litre fermentor trials and is now being evaluated by an industry-associated R&D organization for commercial-scale use in its associate distilleries. The potential impact of the IMTECH strain on the ethanol industry in India can be gauged from the fact that the strains currently used by most of the 200 distilleries produce a maximum of 6–8% (v/v) ethanol from a feedstock containing a maximum initial sugar concentration of about 15% (w/v).
– A new method for enzymic conversion of rifamycin B to rifamycin S with near-100% efficiency has been developed. The currently used chemical method for production of this key intermediate in rifampicin production has markedly lower yields[2–4]. The know-how has been transferred to IDPL for commercial use.
– A simplified process for purification of urokinase from urine, based on antiurokinase monoclonal antibodies, has been developed. The process also eliminates the necessity of collecting large volumes of urine in liquid form through the use of disposable columns of an adsorbent that can be attached to public urinals.
– A new targeting rationale was established for selective delivery of drugs to macrophages, using the exquisite specificity and high efficiency of the process of endocytosis mediated by receptors present exclusively on cells of the macrophage lineage. This rationale provides a generalized method for manipulating the metabolic activity of macrophages for a variety of purposes and is likely to find application in the therapy of diseases in which macrophages are involved, such as leishmaniasis, tuberculosis, leprosy, rheumatoid arthritis, as well as some neoplastic diseases[5–8].
– In an interesting series of experiments employing temperature sensitivity of the expression of a reporter gene downstream of a yeast promoter, it was shown for the first time that the yeast promoter shows differential activity in *E. coli*[9].
– A database management system for recombinant-DNA clones and hosts for use in IBM-compatible personal computers was developed[10].

Elusive receptor

As alluded to before, the initiatives taken in the eighties have resulted in an awareness in India of the myriad ways in which biotechnology is going to affect our lives in this country. There are now in India about a dozen centres with the competence to take up the rather sophisticated operations necessary for commercialization of biotechnology. At least one modern and well-equipped centre has been created where laboratory-level processes can be scaled up to pilot-plant status. What is desperately needed is a 'receptor'—a receptor that is tuned to the commercial possibilities of a laboratory finding and the financial risks involved, a receptor that can look beyond the next five or even ten years for the return in terms of cash flow. Such receptors, in my opinion, were, and continue to be, available in most developed countries in the persons of advanced R&D staff in industrial concerns who can recognize the importance of the apparently idle curiosities of an absent-minded scientist in a university or research institution and tie him/her up by more or less open-ended agreements of first right of refusals backed by reasonable financial support to carry on. In contrast, what is the situation in India? A gulf of mutual distrust, if not outright disdain, separates the scientists and the industrial entrepreneurs. Much of this is due to the fact that the importance of having strong in-house R&D units is not widely recognized by the entrepreneurs. The insularity of scientists to the needs of industrial entrepreneurs also did not help matters. Lately some changes in the dismal scenario for the better are discernible. Several industrial houses seem to be taking an interest in the biotech capabilities being established in India. One must realize that the skills required for product development are quite different from that possessed by scientists working in universities or research institutions. A scientific discovery can lead to a usable product only through the mediation of an industrial R&D unit. Therefore one of the prime requirements for biotech development in our country is the establishment and manning of industrial R&D units with imaginative people with their antennas up to detect product possibilities. If this is done, much of the miasma of distrust

that separates the scientists from the entrepreneurs would be dissipated.

The other requirement has to do with the matter of financial risks in getting into biotech. In this, given the notorious lack of venturesome equity culture in the Indian psyche, we have no alternative but to evolve mechanisms of risk sharing between private entrepreneurs and financial institutions or the government.

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Basic and biomedical product-oriented research—the National Institute of Immunology

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Five biomedical products have been licensed to industry in less than five years of work.

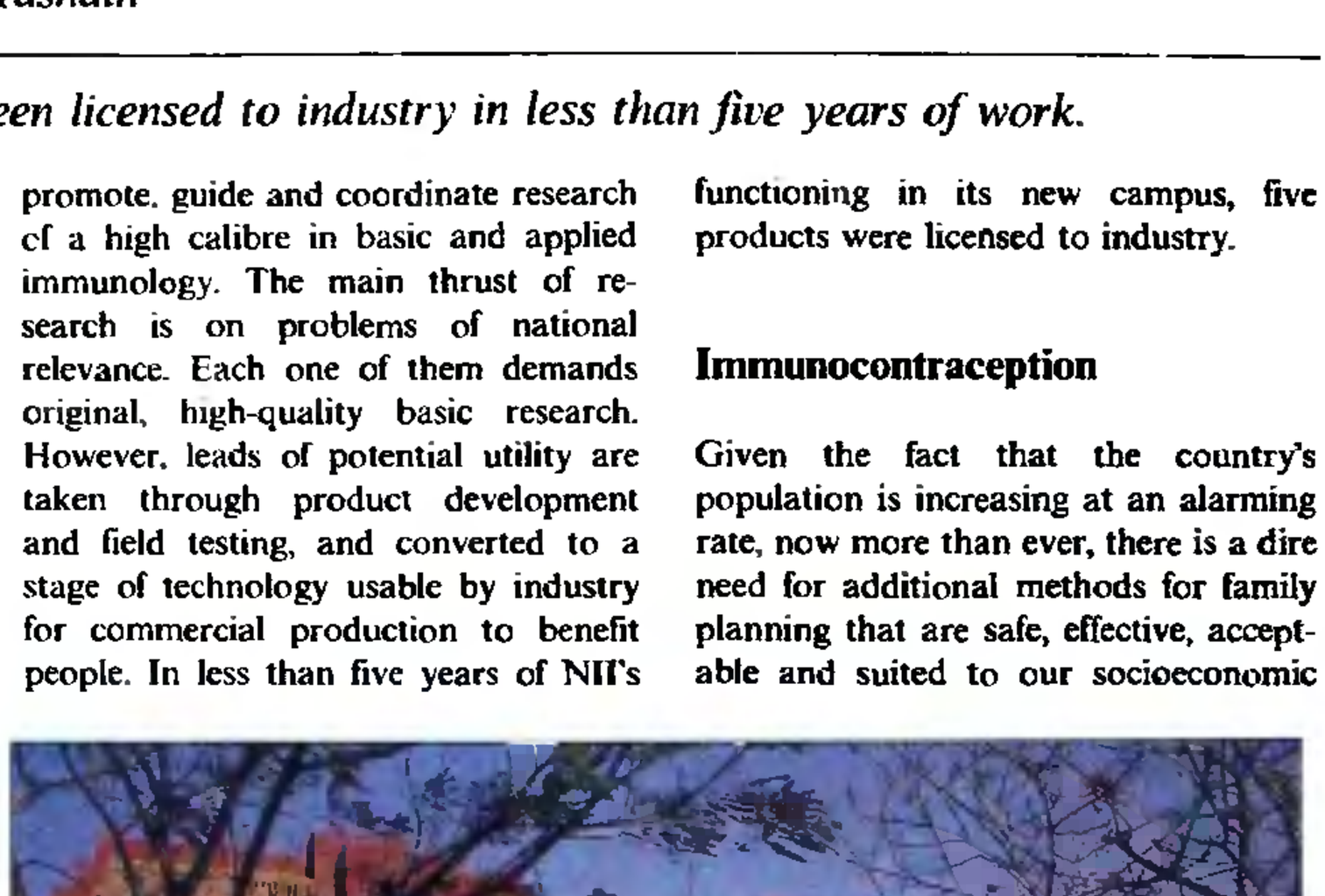
What started out some years ago around a working nucleus, an ICMR-WHO (Indian Council of Medical Research and World Health Organization) Immunology Centre in the Biochemistry Department of the All India Institute of Medical Sciences (AIIMS), and a barren patch of intractable land is today a full-fledged research institution with a firm commitment to research and development in frontier areas of the life sciences. With over 14 laboratories, 45 scientists, 45 Ph D students, and technical and administrative staff, the National Institute of Immunology (NII) in New Delhi has become a regional and international centre of excellence, with students and trainees coming from all over India and Iran, Afghanistan, Bangladesh, Sri Lanka, Nigeria, France and Brazil to work and to learn. The institute is designated as a Collaborating Centre of the WHO for Research and Training in Immunology for India and South-East Asia, a Centre for International Network for Molecular Cellular Biology of UNESCO and a Centre for Asian Network for Biotechnology Applied to Animal Production and Health of the UN's Food and Agriculture Organization (FAO).

Aims and objectives

NII was established to undertake, aid, promote, guide and coordinate research of a high calibre in basic and applied immunology. The main thrust of research is on problems of national relevance. Each one of them demands original, high-quality basic research. However, leads of potential utility are taken through product development and field testing, and converted to a stage of technology usable by industry for commercial production to benefit people. In less than five years of NII's functioning in its new campus, five products were licensed to industry.

Immunocontraception

Given the fact that the country's population is increasing at an alarming rate, now more than ever, there is a dire need for additional methods for family planning that are safe, effective, acceptable and suited to our socioeconomic



High-calibre research in basic and applied immunology