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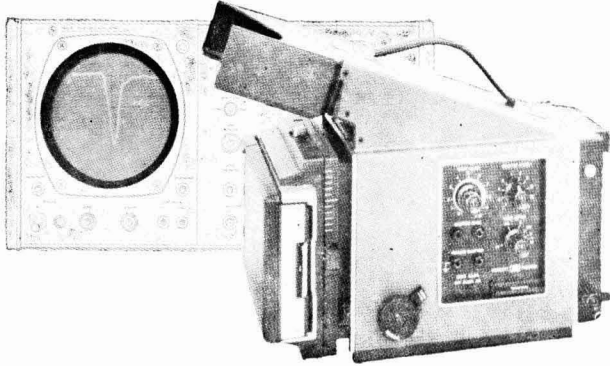
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CONTENTS

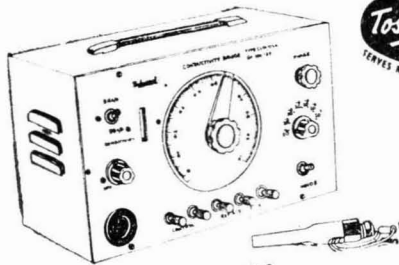
A Few Thoughts on Applied Science in India	1
Forms of Water in Biologic Systems	7
M. S. NARASINGA RAO			
Pharmaceuticals & Drugs Research Committee, CSIR: Progress Report for 1965	10
Role of Structure on Functions of Biological Processes A Symposium in Biophysics	15
N. N. SAHA			
Conductimetric Titrations of Charge Transfer Complexes in Solution: A Review	19
F. GUTMANN			
Natural Asymmetry, Isomerism & Pharmacological Action	29
C. C. PFEIFFER & E. H. JENNEY			
Mechanism of Infection of Legume Roots by <i>Rhizobium</i>	34
N. S. SUBBA RAO			
Reviews	38
Microwave Spectroscopy of Gases; Introduction to Electrical Circuit Analysis; Handbook of Differential Thermal Analysis; Energetics in Metallurgical Phenomena: Vol. II; Mass Spectrometry; Introduction to Molecular Orbital Theory; Kurzes Lehrbuch der Physikalischen Chemie; Organic Chemistry; Fatty Acids — Industry, Technology and Research in India; Progress in Biophysics and Molecular Biology: Vol. 16; Essentials of Palynology; Insect Sex Attractants; Energy — Its Production, Conversion and Use in the Service of Man			
Notes & News	43
Super-plasma with laser beam; Two-dimensional electron gas on semiconductor surface; Gigartinine: A new amino acid; Fruit storage at subatmospheric pressures; Microbial production of food from coal; A method for extracting mucoproteins from epithelial tissues; A cold box for biochemical work; Studies on communication and dissemination of scientific information; World scientific information system; <i>Automatic Documentation and Mathematical Linguistics; Concrete; Comments on Nuclear and Particle Physics</i> ; British Scientific Instrument Research Association; Forthcoming International Scientific Conferences, 1967			

For Index to Advertisers, see page A21

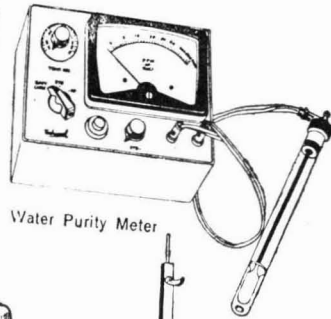
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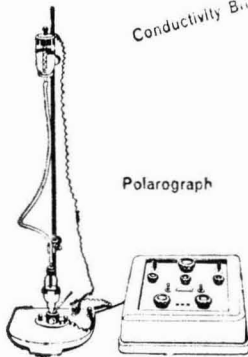
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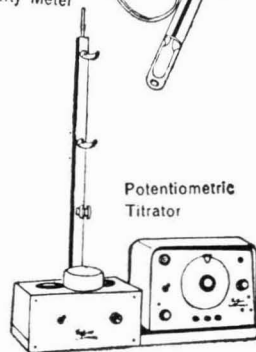
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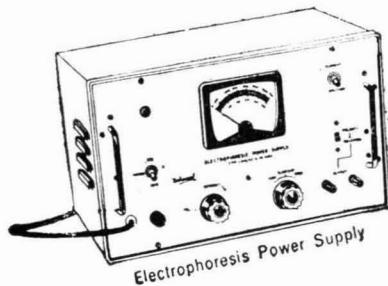
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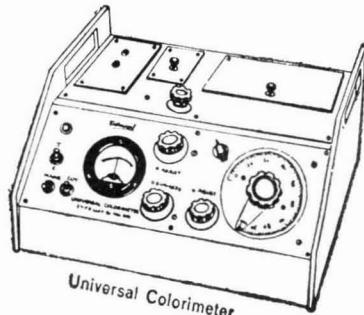
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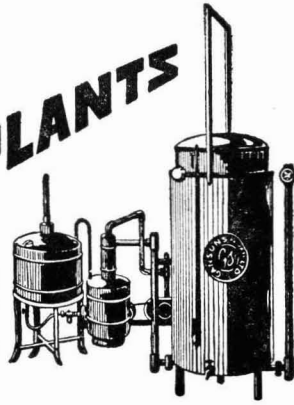
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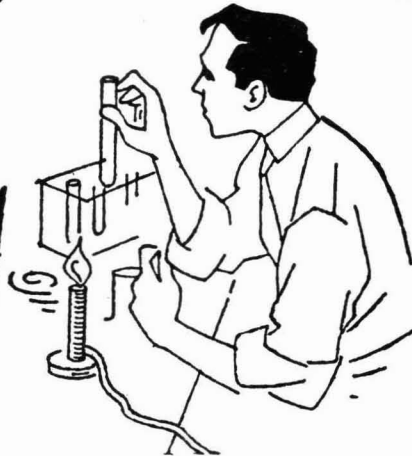
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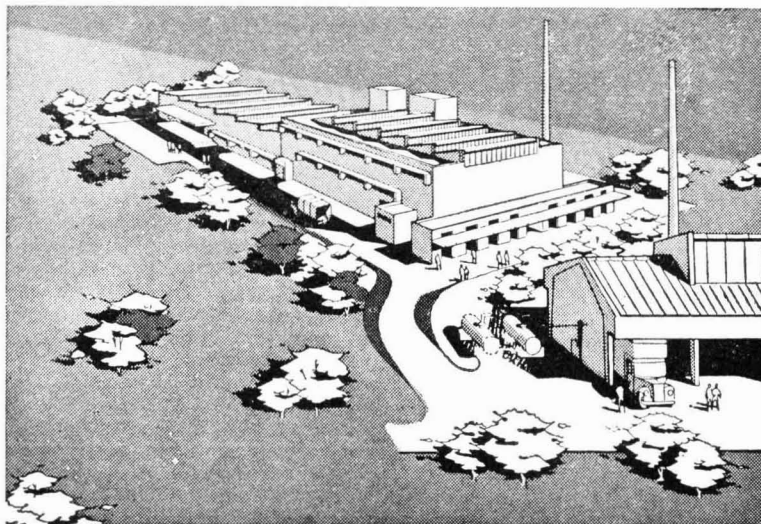
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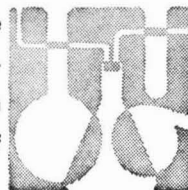
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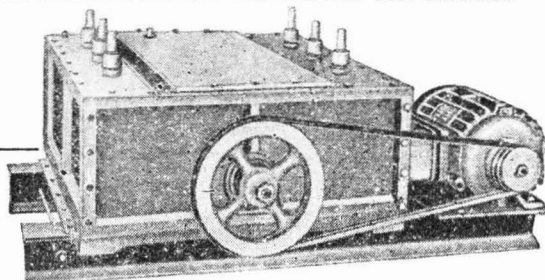
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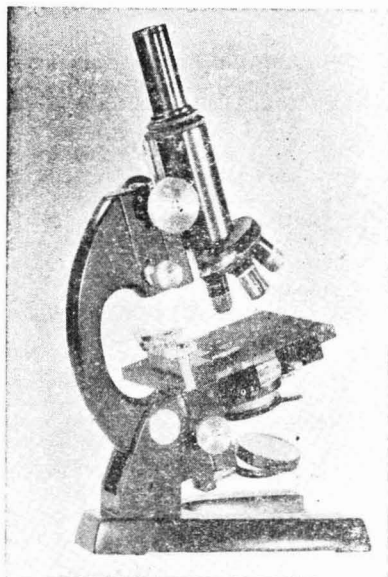
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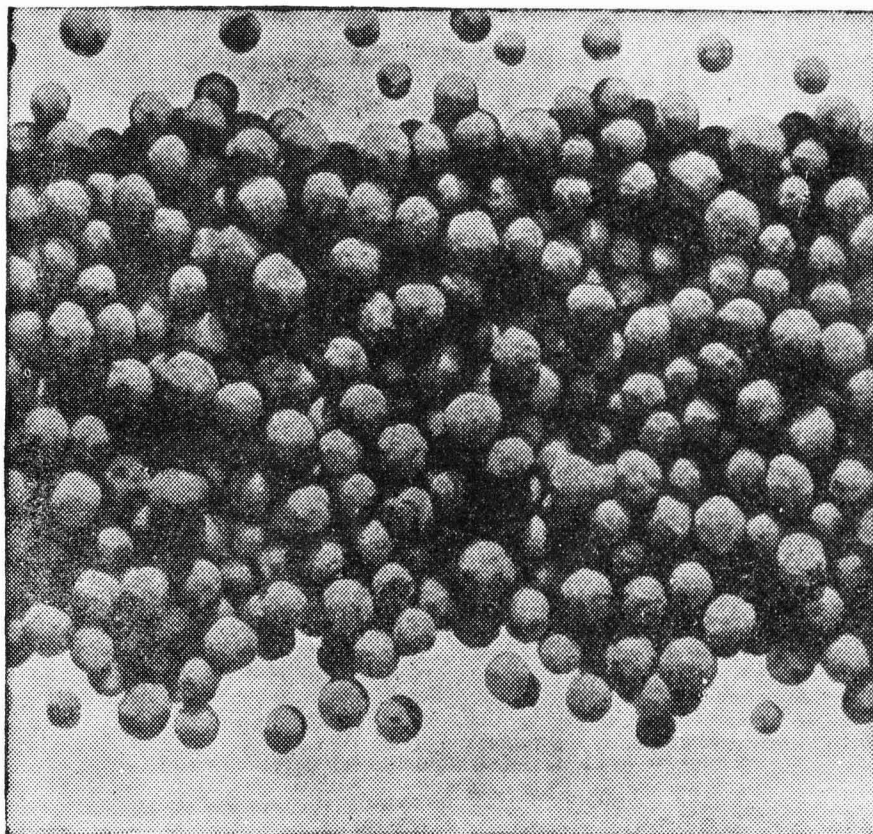
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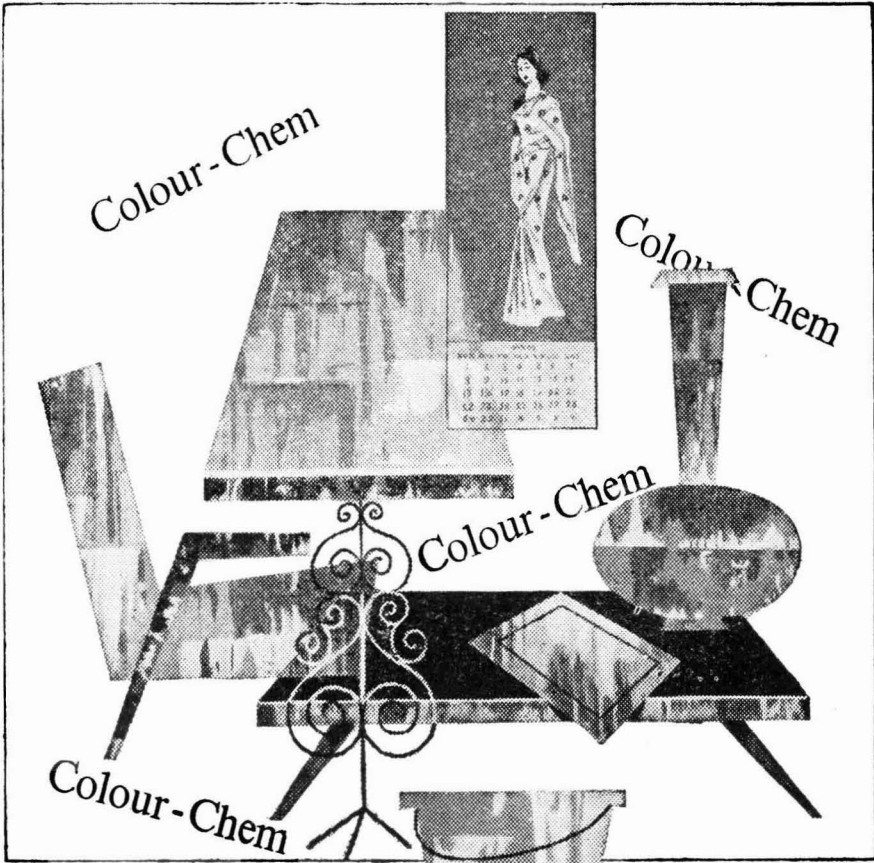


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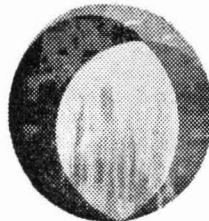
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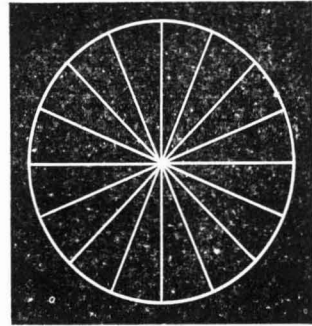
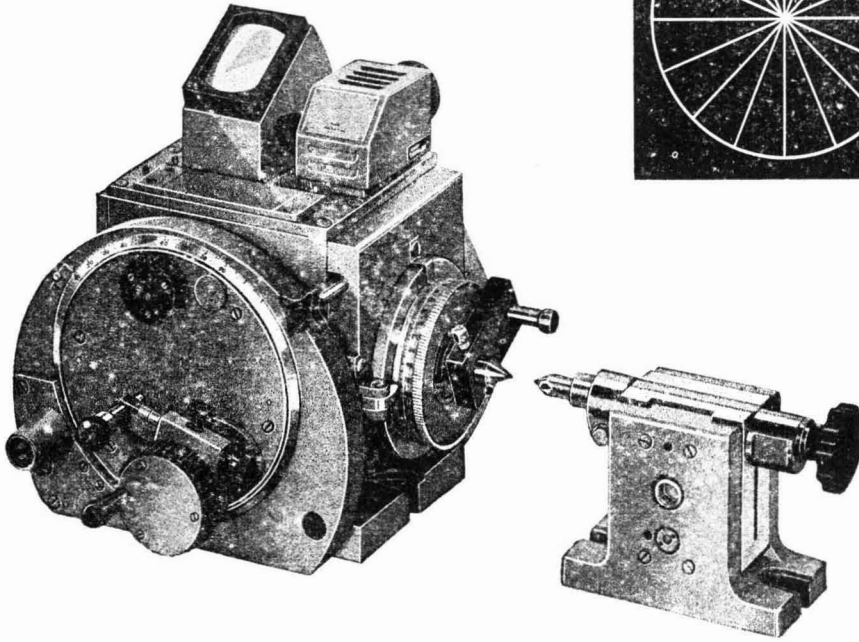
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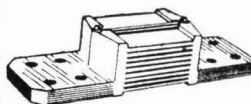
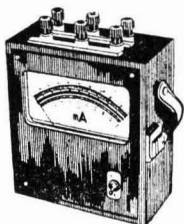
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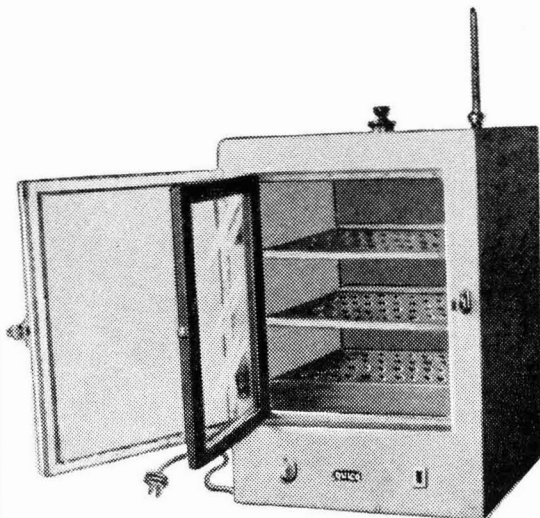
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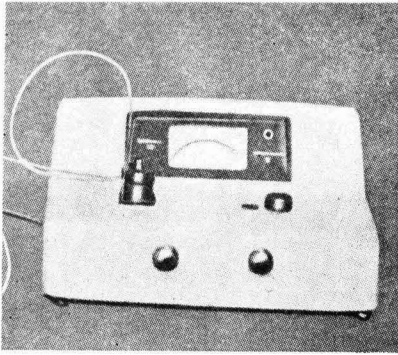
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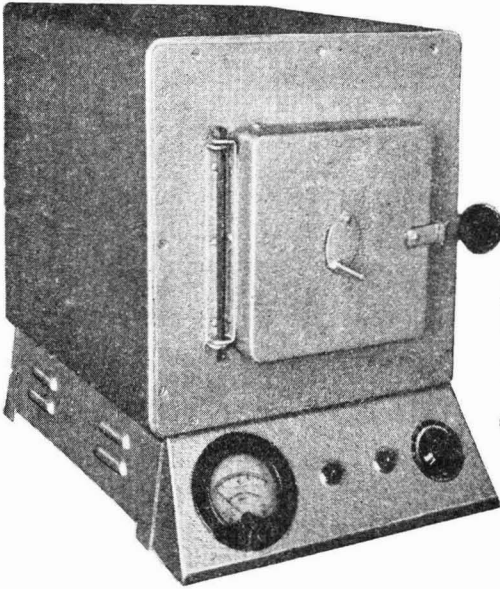
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INDEX TO ADVERTISERS

ARJUNA ELECTRONICS PRIVATE LTD., HYDERABAD ...	A32	MARTIN & HARRIS (PRIVATE) LTD., BOMBAY ...	A36
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GORDHANDAS DESAI PRIVATE LTD., BOMBAY ...	A35	SCIENTIFIC INSTRUMENT Co. LTD., ALLAHABAD	A2, 34
INDIA SCIENTIFIC TRADERS, BOMBAY ...	A24	SCIENTIFIC SALES SYNDICATE, BOMBAY ...	A8
INDIAN BOTANICAL SOCIETY, MADRAS ...	A25	S. H. KELKAR & Co. (PRIVATE) LTD., BOMBAY	A27
INTERNATIONAL AGENCIES, BOMBAY ...	A12	TEMPO INDUSTRIAL CORPORATION, BOMBAY ...	A22
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K. SCIENTIFIC INDUSTRIES, CALCUTTA ...	A17	TOWA OPTICS (INDIA) PRIVATE LTD., DELHI ...	A28
LABORATORY FURNISHERS, BOMBAY ...	A27	UNIQUE TRADING CORPORATION, BOMBAY ...	A20
LEIPZIG FAIR AGENCY, BOMBAY ...	A24	VEB CARL ZEISS, JENA ...	A16, 29
		ZOOLOGICAL SOCIETY OF INDIA, CALCUTTA ...	A9

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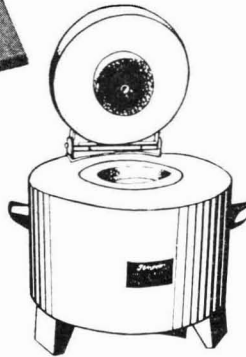


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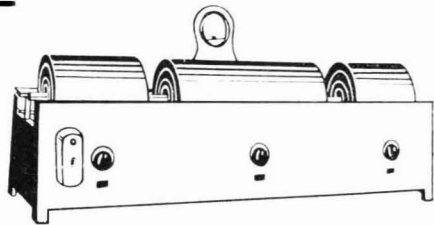
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A Few Thoughts on Applied Science in India*

AFTER the Second World War and in the wake of Indian Independence, the world has witnessed a pageant of national liberation. During the last two decades, most peoples of the world have thrown off foreign yoke and attained political freedom. But liberty has brought with it the great and exacting responsibility of ensuring freedom from gross physical want — food, clothing, shelter, sanitation, etc.

Economically, the world is divided today into two groups of nations — the affluent and the indigent; the latter includes most of the newly liberated nations, often called New Nations; some call them the hundred dollar per capita income group as against the thousand dollar group of rich nations. The economic disparity between the two groups is widening because the developed nations are growing far more rapidly than the New Nations which are being further pulled down by sheer weight of numbers — an inevitable consequence of economic backwardness. Historians agree that this difference is not due to any physical or intellectual superiority of the people of the advanced nations, but is essentially a historical consequence of the fruits of the Industrial Revolution and organized application of science to industry. If that be correct, and facts seem to support this thesis, why cannot the problems of the New Nations be also solved by the application of science? Yes, this can be done; but in this process there are many difficulties which have to be faced and dealt with.

Most developing nations have a fair share of natural resources. These, if properly developed, will go a long way in improving their economic condition. The task before the New Nations is, therefore, to develop their resources and to acquire the skills necessary for this purpose.

Science in National Development

Some eminent Indian men of science had recognized the place of science in national development and had given serious thought to this matter much before independence. The National Planning Committee constituted mainly on the persuasion of Prof. M. N. Saha, by Netaji Subhash Chandra Bose, with Pandit Jawaharlal Nehru as Chairman and consisting of eminent scientists, economists and others, deliberated on the subject of national development, and gave science a significant place in their scheme of things. Soon after independence, Prime Minister Nehru, with his unflinching faith in science, declared:

“... It is science alone that can solve the problems of hunger and poverty, of insanitation

and illiteracy, of superstition and deadening custom and tradition, of vast resources running to waste, of a rich country inhabited by starving people. ... Who indeed could afford to ignore science today? At every turn we have to seek its aid ... The future belongs to science and to those who make friends with science ...”

After about a decade, during which great efforts were made for rapid development of science in India, this faith became the basis of Science Policy, when in 1958, the Indian Parliament adopted the historic Scientific Policy Resolution which recognized the vital role of science in national development and committed the government to take measures for the rapid growth of science.

Few intellectual activities in the country have received the massive support from the state that science has. Some idea of the state's concern for science will be revealed by the figures of total expenditure. In 1947, when we attained independence, expenditure on scientific research was not even a crore (10 million) of rupees. Today, it is more than 50 crores per annum.

Increasing state patronage has caused public expectations from science soar high. People, who have long been suffering from various wants and had to put up with scarcity of food, clothing, shelter, not to speak of other daily needs, for centuries, have been led to expect quick results from science and scientific development in the country. It is not infrequent to see comparisons being made with the developed nations, who have had the benefit of scientific advancement for a longer time. It may be commonplace to say that political freedom only whets the appetite for economic prospects. The people of the newly liberated countries, most of whom have adopted a democratic form of government and who have given all encouragement they could, in money and in other ways, to scientific development, may feel that time has come to ask “Has science helped to meet their needs?” In developing nations, time is the essence and the urgency of fast development by the application of science needs no special emphasis. It must be realized that the interest of government and the people and the massive government support which science is receiving is more for the material benefits that flow from science rather than for science itself. If the people fail to get the benefits and if they get the feeling that science has not given them a satisfactory and quick answer to their needs and their economic ills continue, the failure may even induce them to work for a change, even though that change may not ultimately be for the better.

The people of India have accepted science as the key to achieving economic progress, thanks to Pandit Nehru; but as he himself said so often, science cannot grow unless society as a whole develops the

*Twenty-eighth Acharya Jagadish Chandra Bose Memorial Lecture delivered by Dr Atma Ram, Director-General, Scientific & Industrial Research, in Calcutta on 30 November 1966.

scientific and rational approach to solving social, political and economic problems. No democratic government, however progressive, can allocate a sizeable slice of public funds to an activity unless it attracts public awareness of its needs. It is not necessary that everyone should become a scientist to appreciate the vital role of science, but it is essential that there should be a proper understanding of what science can do and how and under what conditions it can show results. This is particularly important because, though science and technology have revolutionized the lives of people in the advanced countries, their use by the New Nations is not easy and is beset with great difficulties, social, economic and political.

In a country where the vast numbers of people are yet to attain literacy, the responsibility of taking decisions on behalf of the people rests largely on the intelligentsia. In his rejoinder to the official attitude of British rulers to Indian National Congress that the

“educated community as an infinitesimal minority had no right or claim to represent the view of India”,

rightly did Sir Ramesh Mitter say:

“the educated community represented the brain and conscience of the country and are the legitimate spokesmen of the illiterate masses, the natural custodian of their intelligence. But to hold otherwise is to presuppose that a foreign administrator in the service of the Government knows more about the needs of the masses than their educated countrymen. It is true in all ages that those who think must govern those who toil and would it be that the natural order of things was reversed in this unfortunate country?”

So, public awareness is generated and sustained by the opinion of the few. There is, thus, need for correct appreciation of the role of science in economic development at decision centres. If the views expressed sometimes in responsible quarters are to be considered symptomatic, it would appear that this understanding has not adequately developed; otherwise, in the face of Indian industrial research rightly claiming several impressive achievements, it is difficult to explain the general feeling, more or less undisguised—‘so little from so much?’ We should not forget the pithy saying of the Greek philosopher,

“Science grows knowledge, opinion grows ignorance”.

Let us, therefore, examine the causes of the misunderstanding.

Scientists feel that men of affairs, administrators and industrialists do not appreciate their contribution in the true perspective. Industrialists feel that Indian scientists are mainly working in an academic mood and are not realists. So a situation has developed in which there is inadequate rapport between scientists on the one hand and administrators and industrialists on the other. It may well become a serious impediment to progress.

To some extent, this lack of appreciation of each other's role is primarily due to lack of awareness of the mechanics of science in relation to industry. The scientist may not have paid any serious atten-

tion to make the people aware of his specific role. The industry too has not made much effort to understand where science comes in, what it can do and what it alone cannot achieve.

Relationship between Science and Technology

The fact that many of the economically strong countries are also in the forefront of science has led to the belief that eminence in science would automatically ensure eminence in industry. Judged by the number of Nobel Laureates in science in relation to the population, Britain, perhaps, is still on the top in science. While there can be no question about Britain being pre-eminent in science, she is economically not in too happy a position. As against this, Japan, for instance, may not claim as much eminence in basic science, but in economy she is in a much happier position than Britain.

There is considerable misunderstanding between the role of science and the role of technology. When a great breakthrough is achieved—sputnik, man in space, rocket on the moon, photographing the other side of the moon, telstar for communication, to mention just a few—it is all associated with science and on such occasions one often hears “Let us do more science”. It is not recognized that all these spectacular achievements will just not be possible without the development of appropriate technology and the availability of means of putting that technology to use. All these achievements are no doubt spectacular and endow the nations responsible for them with high prestige, but they are tremendously expensive and involved. It is important to realize that howsoever much a country may spend on science, howsoever eminent her scientists may be, whatever international recognition they may achieve, it will not ensure the impact of science on industry without technology coming into the picture in a large measure. It is not fully appreciated that to translate laboratory results to saleable products is both laborious and expensive. I might refer in passing to the new thinking in the Royal Society of London, fellowship of which carries high prestige in the scientific world. The Society has recently decided to increase the number of fellows from applied and technological subjects and one more Royal Medal has been instituted specifically for recognizing contributions to applied sciences.

It is well known that for a long time science and technology developed independently. As a matter of fact, considerable part of productive technology, then known as industrial arts, was not based on scientific knowledge. It was only about the middle of the nineteenth century that science and technology began to interact and energize each other. It is of utmost importance, therefore, that the New Nations understood the distinction and relationship between science and technology; otherwise, they are likely to devote their limited resources to developing science and neglecting technology or developing technology and neglecting science. Science can show the possibility of something to happen, it cannot by itself make it happen. It needs technology to achieve what may be indicated possible. It is technology backed by strong science that helps produce material things which India

badly needs. Science produces knowledge, technology helps produce wealth.

To quote J. Herbert Hollomon, US Assistant Secretary of Commerce for Science and Technology, from his 34th Edward Orton Jr Lecture:

"It is extremely rare that devastating discoveries occur and when they do, the front of science becomes the front of engineering. Because the circumstances are so rare and so dramatic, we tend to believe that new discoveries in science lead directly to technology and new products but that is hardly the case."

I am stressing the obvious, because sometimes the zest for greater scientific research can make the decision makers forget the distinction that I have pointed out and this can lead to strange results. Over-emphasis on science may create centres of highly developed activity in an underdeveloped country and call for tremendous efforts to prevent such centres from degenerating. Also, underdeveloped science in underdeveloped countries may lead to difficult and trying situations. For, people of underdeveloped nations failing to get quick material benefits from science may lose faith in it. This will indeed be a calamity.

I have placed my views on the distinction between science and technology, and their respective roles in an economy like India's, perhaps, could be summed up in the words of Arnold Toynbee, who has beautifully expressed it:

"Science and technology may be conceived as a pair of dancers, both of them know their steps and have an ear for the rhythm of the music. If the partner who has been leading chooses to change parts and to follow instead, there is, perhaps, no reason to expect that he will dance less correctly than before."

Science and technology are interdependent and one is necessary for the growth of the other.

Having related the role of science and technology and their interaction, I may mention some of the difficulties of applied science and industrial research in the environment obtaining in a developing country like India. It is necessary to appreciate that there is fundamental difference in the organization of industrial research in the socialistic countries, the USSR and the East European and West European countries and USA. I feel that most of the New Nations do not strictly fall in either of these two groups and constitute a group of their own. For example, India which has chosen a democratic form of elected government has also adopted socialism as its political ideal and in actual practice a mixed economy prevails.

Research, Development and Production

In the socialistic countries, scientific research, industrial research—basic and applied—production and distribution are all government activities. So, in many of the large production establishments one witnesses research, development, production, all under state auspices as if under the same roof. In the western countries, most industrial research is carried out by industry itself. Development of products and processes is done in research laboratories of manufacturing firms and, perhaps,

this is the best dispensation for such work. The major part of modern technology has, therefore, emanated from manufacturing establishments. However, it must not be presumed that introduction of innovations by manufacturing firms in the form of new products, processes and improvements is a simple affair. It is well known that, even though functioning under the same organization, there is considerable resistance from the shop floor to ideas from research laboratories and it is only a well-informed and progressive management, backed by competent technical advisers that can overcome such resistance.

In India, most industrial research activity is concentrated in government-sponsored research establishments. Except for a very few manufacturing organizations, it is only lately that industry has shown some interest in carrying out research and development and that too is done by cooperative research associations. Their functions are somewhat different from research laboratories of individual firms. Even public sector industrial establishments in most cases have not yet established their own research laboratories.

Both in the socialistic pattern and the West European system of industrial research, research and production are integrated. In India and other developing countries, such integration is yet to develop. This lack of integration of research and production has resulted in mutual criticism and recrimination. This situation has been well summed up by Steven Dedijer, who made a first hand objective study of Indian industrial research during his several visits to this country. He says:

"It is too much to expect from industrial research laboratories not only to plant the research seed of a product, to make it grow into a tree, to harvest the fruits but also to chew them and feed them into the mouths of industrial companies*."

Such expectations from scientist have, in my opinion, produced not only physical but psychological isolation of science and industry. As I mentioned, process and product development and improvements in production should essentially be carried out by research laboratories of manufacturing firms or at least well integrated to them, as only then it will be easy to evaluate economic results of development and have the knowledge and ready resources of the market to try out innovations.

What should then be the function of scientists and technologists in a developing country like India? In my opinion, the scientists and technologists should help in solving the vital problem of the country, namely how to put to maximum advantage the limited resources of the country within the shortest possible time for the benefit of the people for ensuring that every man in the country gets his essential needs like food, clothing and shelter and other necessities. If we fail to be of help in this task, I am afraid, we would be weakening our claim for public support for our activities.

*Address delivered at the Shri Ram Institute for Industrial Research on 13 June 1964.

Fundamental Research

When I say this, I should not be taken as minimizing the importance of fundamental research. It is essential that we should establish the highest traditions of intellectual enquiry in our universities and research institutions; but our primary aim of such activities should be training of scientists and technologists and developing what I may like to term 'scientific capabilities'. Without a large number of scientists and technologists in a variety of disciplines possessing the requisite capabilities, it will not be possible for us to deal with the problems I have mentioned. In this context, basic research is fully justified. Also, if we need first rate men for basic research, there is as much need for first rate men for applied work and that is possible only if we have first rate men capable of understanding, creating and imparting knowledge. Science cannot be planted in traditional societies based on primitive thinking. It can grow only on a base of good education and needs people who can absorb, adapt and use it.

New Nations need not run the race for Olympic Medals of science purely for its own sake. They just cannot afford it; science has become during the last quarter of a century too costly an activity. I do not see any purpose of being classed in the category of 'also ran' because people will turn round and ask, 'at what cost?' Even while undertaking fundamental research, I hold the view that it can and should be made relevant to the environment of the country and needs of the people. *We must aim at excellence in relevant fields and not be busy in establishing relevance of excellent fields.* For example, tropical diseases and medicines present a field of great relevance to India. In spite of several valuable results to the credit of Indian scientists large areas of it still remain unexplored and should receive our urgent attention. With certain amount of thinking, it is possible to reorient scientific research in our universities and research institutions carrying out fundamental research without making any inroads on their freedom. Production orientation of our science education would be as exciting and rewarding.

If we accept the thesis that the immediate and great task of Indian scientists is to assist in the expeditious, judicious and efficient development of national resources to provide for the material needs of the people, is it not incumbent on the scientists and technologists to indulge in some introspection? We should first ask ourselves, "Are we doing the right thing for our people?" There is much knowledge available, scientific and technological, waiting to be applied to Indian conditions. It is a question of choice what should have priority—creation of knowledge, no doubt important, or quick application of what is known. Unless we orient ourselves to be of help in creating wealth out of our resources, how shall we finance fundamental research, and from where? The experience of Britain and Japan will help us to make this choice.

Research Can Help in Many Ways

There are various ways in which scientific research helps in developing resources, promoting growth

and accelerating progress of industries, viz. (i) systematic investigation of raw materials of mineral and plant origin, their evaluation, beneficiation, etc.; (ii) understanding of scientific principles underlying technological operations leading to improved quality, reduced cost of production and higher productivity; (iii) creation of knowledge leading to new products and processes; (iv) development of new products and processes and improvement of existing ones; in other words, development of know-how; and (v) training of skills necessary to bring into being new industries and manage them.

In a developing country authentic information on resources of raw materials and their proper evaluation is one of the first essential things. Even critics of research laboratories do not deny that valuable knowledge has been produced by Indian scientists in this direction and has been of immense value in many projects. This has not been confined to cataloguing routine information but has led to indicate how low grade materials could be beneficiated to suit quality production and to provide basic information for determining what industries could be established in the country. It has saved the country injudicious depletion of critical resources on uses which could be satisfied by low grade materials and has helped to plan conservation of raw materials. While it is hard to work out the money value of such investigations, they have been most valuable.

When we come to the more difficult area of developing know-how and putting to use science, basic and applied, for the development of products and processes, we meet with many difficulties. The sequence of operations of translating research results into industrial production has not been fully comprehended in India. Lately there has been a greater appreciation of the difficulties involved, but there is still a tendency to blame each other for any mishaps which may occur.

The sequence of operations is somewhat like this: (i) research—basic and applied; (ii) pilot plant; (iii) development; (iv) engineering, namely design, fabrication, erection of plant and equipment; (v) production; and (vi) marketing.

At one end is the scientist doing research absorbed in understanding things and at the other, the industrialist intent on production and profits; but there are many steps in between. Unless we go through this chain of operations, no research is likely to mature into actual production. Many ideas and results of research may fall by the wayside in traversing this sequence. Even if a research result is established to be technically feasible, the ultimate product may not be economically attractive. Establishing economic feasibility of a technically good product may sometimes involve operations much larger than pilot plant, besides intense marketability studies. These are all difficult, costly and slow processes with a considerable element of unpredictability, yet there is no alternative but to go through them. Development is far more costly than research and this is not often appreciated.

Indigenous technology has, therefore, to put up in this country an extremely hard struggle against the more advanced technology of developed countries. I often ask myself the question what

chance has indigenous technology to survive before the more sophisticated, financially strong know-how from the advanced countries of the world? If I deal with the many problems connected with this question, I may be provoking many controversies; but I feel that the time has come when we should take a national view of these problems and take decisions which I know will be very hard and may upset many pet theories.

Borrowing Technology

I would now like to refer to the question of borrowed technology. Let me make it clear that personally I am not against borrowing or purchasing technology. I also do not subscribe to the view that there is no necessity to carry out research and development in India as there is a technological supermarket to which we can go shopping and buy all that we need. We require well-directed sustained research to adapt borrowed technologies to suit our conditions, that is, adaptation research and this requires very competent and imaginative handling. Our approach should be pragmatic and we should do what is best in each case. Technology should be borrowed only when suitable local technology is not available.

In developing countries like India, time is the essence. Therefore, if a technology can be purchased from others without compromising national interests and crippling local efforts, there is no reason to take a doctrinaire view. It reduces the time factor for development of such technology anew. In the present context of world development, complete technological independence is unthinkable. Every country has certain expertise and knowledge which others do not possess and practically all countries borrow technology to a greater or lesser extent. USA, UK, France, Germany and other countries of Western Europe, which are industrially or technologically highly developed, have been exchanging technology quite frequently. Perhaps with the exception of USA, no other country has a positive technological balance of payment (Table 1). In the face of this, it is difficult to believe that India which has to make a great leeway in the industrial field can afford to isolate herself. It will be pertinent to point out that Japan's massive industrialization was based essentially on the use of borrowed technology; but it is also relevant to understand the methodology followed by Japan in achieving this result. Once a few factories were set up, Japan not only mastered the technology but even left behind the pioneers from whom she borrowed.

While, by and large, industrialization in India is taking place on borrowed technology, have we devoted the attention needed to grow on this technology a structure which will project Indian technology to the forefront? As was very poignantly mentioned by Dr Homi J. Bhabha, even in fields in which industries were existing for half a century, when the question of expansion of plants and setting up new ones arose, the country again resorted to foreign collaboration. Why this is so needs hard thinking. *There is no harm in borrowing to end borrowing, but it is harmful to borrow to continue borrowing* which is happening in many cases.

TABLE 1 — ESTIMATED TECHNOLOGICAL BALANCE OF PAYMENTS*

(Figures expressed in \$ millions)

	Receipts	Payments	Balance
Transactions with all countries, all industries			
USA, 1961†	577	63	+514
France, 1962	40	107	-67
Germany, 1963	50	135	-85
Transactions with USA only, all industries			
France, 1962	11	53	-42
Germany, 1963	10	52	-42
United Kingdom, 1961	17	86	-69
Western Europe (including others), 1961	45	251	-206
Transactions of particular industries with all countries			
Germany (1963)			
Chemicals‡	19.3	33.8	-14.5
Electrical machinery	10.7	29.0	-18.3
Steel, machinery, vehicles	14.2	45.2	-31.0
France (1960)			
Chemicals‡	10.3	14.0	-3.7
Electrical machinery	1.7	12.6	-10.9
Machinery	0.2	4.1	-3.9
USA (1956)			
Chemicals‡	34.1	10.7	+23.4
Electrical machinery	21.0	0.7	+20.3
Machinery	28.2	1.3	+26.9
Vehicles	16.6	2.3	+14.3
Transactions of particular industries with USA only			
Germany (1963)			
Chemicals‡	7.5	13.5	-6.0
Electrical machinery	0.9	13.5	-12.6
Steel, machinery, vehicles	2.5	16.2	-14.1

*New Scientist, 16 December 1965, p. 818.

†The unadjusted 1961 figures were \$ 707 millions (receipts) and \$ 81 millions (payments). These figures include some non-technical payments. On the unadjusted basis the 1962 figures were \$ 807 millions (receipts) and \$ 104 millions (payments).

‡Including petroleum products.

Selection of Technology

I might also mention a few words on the selection of technology suited to our needs. I doubt if adequate thought is being given to the question of relevance when we borrow technology. I think we have reached the stage when we should scan and choose technology which fits into our needs. In doing so, various aspects have to be taken into consideration. In certain industries like steel making and other forms of metallurgy, basic chemicals, defence equipment and the like, the country must have the very best and economy of scale must be ensured. So also for products meant for export to international markets. But there is a vast number of other industries which produce goods for internal consumption; these could be used as the means for providing employment to vast numbers.

Gandhiji used to say, "*It is not mass production but production by the masses that would do the trick*". Mass production techniques although superior may not be suitable to Indian conditions in all cases. Superiority and suitability are not necessarily synonymous. These techniques are capital intensive

and labour short. Conditions in India are just the opposite; capital short, labour affluent. She is particularly short of foreign exchange. India has to substitute a good deal of monetary capital by human capital. She has to develop technologies or adapt known technologies with the important objective of finding employment for nearly 500 million people. There appears to be considerable scope for adapting even known capital intensive technologies by modifying them to suit labour intensive situations. There is the example of Japan. Even highly sophisticated industries like electronics have been built up on labour intensive basis in that country. As a matter of fact, in electronics, Japan has stolen a march over most of the countries of the world. This, in my opinion, is an outstanding example of starting with borrowed technology and adapting it to suit totally different situations and solving problems like those we face today in our country. In adapting technologies such considerations as modernization or prestige should have no place. Contrary to general belief, Japan has shown that non-automatic technologies are not necessarily primitive, nor that sophistication need necessarily be automatic. It is possible to develop intermediate technologies which could be used to produce even sophisticated products without adopting completely automatic processes.

A judicious choice is, therefore, necessary in selecting technologies from abroad. This itself necessitates more basic and applied research in the country to deal with problems arising out of adaptation of technologies. By merely travelling along the dazzling path of modern technology we will run into difficulties and become more dependent on others. *The transition of traditional economy to industrial economy can be achieved better and possibly quicker through an evolutionary rather than a revolutionary process.*

Technological Competence

Here is a challenge to Indian scientists, technologists and economists — how to use the vast storehouse of knowledge of modern science to develop resources expeditiously and efficiently under conditions and with objectives different from those in which modern science and technology have so far functioned. We can be pioneers in this field and on the success of finding an answer will depend our future and our stability. In this, *I feel that the problem should be looked at not from the point of view of technological independence or dependence but from the point of view of technological competence.* We have to develop a new competence suited to our environment and needs. Merely transplantation of technology cannot produce this competence. How

or why is it that in spite of the use of up-to-date borrowed technology many of our industries are not efficiently, functioning and are not able to stand international competition? By and large, our industrial set-up is enjoying a sheltered market, and, therefore, a sellers' market. Were such protections to disappear, how many of our industries will be able to stand by themselves? This is mainly because we have failed to develop technological competence relevant to our needs.

Lack of Competition

What I have said about the protected state of industries and the sellers' market is relevant from another point of view also. Throughout the history of development of modern technology, the underlying motivation has been compulsion born out of competition. This is largely lacking in this country. So, indifference towards research is understandable. It is not necessary as some advocate to import goods for creating an element of competition to indigenous manufacture. My view is that competition can be provided within the country itself. This is also necessary in relation to our export trade. Competition may, perhaps, do a lot of good by making industry more mindful of the role of science and technology for their own benefit. This will create demands on research and give more opportunities and incentives for industrial research. It will also help our scientists to engage themselves in activities on *felt needs rather than felt fashions*; they will no longer remain isolated and will take full part in the active channelization of science to industry.

It is paradoxical that in a developing country like India, which provides such tremendous opportunities both to industrialists and scientists, they should be working in isolation and at cross-purposes. If science and technology hold the key to national progress, why is it that Indian scientists and technologists have not still been able to come into their own and so many of our able scientists and technologists have to look elsewhere for their opportunities?

I have expressed my views, 'which are not essentially those of the great organization which I have had the privilege to serve right from its inception, on applied science in relation to the needs of a developing country like India in the hope of assisting in a proper assessment of some important aspects involved in the application of science and technology. The earlier there is a general awareness of these considerations the better it will be. We can then get down to the job in an atmosphere of cooperation between the scientist, the technologist, the administrator and the industrialist. All of us have a responsibility in this regard as it vitally affects the very future of our country.

Forms of Water in Biologic Systems*

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WATER is such a familiar liquid that its properties at first do not strike us as unusual.

However, compared to substances of the same electronic structure water is anomalous in almost all its properties. These unique properties have important consequences for biological systems which are composed mostly of water. To cite an obvious example, because of the high specific heat and latent heat of vaporization of water, oceans which cover about 71 per cent of earth's surface are capable of absorbing the vast energy received from the sun and regulating the temperature on and around the earth. The high electrical reactivity of water molecule endows it with the ability to form ordered structures around macromolecules, such as proteins and nucleic acids, and this property has a bearing on the transport of electrolytes and non-electrolytes between the cells and the fluids which bathe them. Thus water is not a mere filling agent in biological systems; it is 'the matrix of life'.

Because of its ability to explain several biological functions, the concept of structured-water is attracting wide attention. Three conferences, two in 1964 and one in 1965, have discussed the role of water in biological systems: Symposia of the Society for Experimental Biology, No. XIX, *The state and movement of water in living organisms*, Cambridge University Press (1965); a conference jointly organized by the New York Academy of Sciences, the National Aeronautics & Space Administration and the Office of Naval Research, *Forms of water in biologic systems*, *Ann. N.Y. Acad. Sci.*, **125** (1965), 249-772; a seminar by the Federation of American Societies for Experimental Biology, *Fedn Proc. Fedn Am. Socs. exp. Biol.*, **25** (3) (Pt I) (1966). The conference of the New York Academy was interdisciplinary in character and research workers in such diverse fields as electrical engineering, cellular biophysics and marine sciences shared a common platform. The proceedings have appropriately been dedicated to Prof. J. D. Bernal who has pioneered research on the structure of liquid water.

Structure of Water Molecule

We do not as yet have the full story of the structure of liquid water, more so that of water in biological systems. However, we know that liquid water has a highly associated structure due to extensive hydrogen-bonding. A H_2O molecule is tetrahedrally bonded to four neighbour H_2O molecules. Since in the water molecule the number of hydrogen bond donors is exactly balanced by the number of acceptors, two protons and two lone pair electrons in oxygen, a four-coordinated cross-linked structure of exceptional strength and stability is possible.

*Based on the proceedings of a conference, *Forms of water in biologic systems*, held by the New York Academy of Sciences in October 1964 and published in *Ann. N.Y. Acad. Sci.*, **125** (1965), 249-772.

According to Frank-Wen concept, water has a mixed structure. It consists of 'clusters' and nonhydrogen-bonded, monomeric H_2O molecules. The formation of hydrogen-bonded dimer of two H_2O molecules facilitates the formation of additional hydrogen bonds and thus 'clusters' are formed by a cooperative phenomenon. Just as the formation of 'clusters' is cooperative so is their breaking. Thus 'clusters' are being formed and broken down at a high frequency and hence the concept of 'flickering clusters'. The size and life of clusters depend on temperature and on the nature of solutes present in aqueous solution. Substances such as salts act as structure-breakers whereas hydrocarbons act as structure-stabilizers.

Using Frank-Wen concept with minor modifications, Scheraga has calculated the cluster size as a function of temperature for both H_2O and D_2O using a Boltzmann distribution function. The cluster size decreases with increase in temperature. The calculated values are compatible with the viscosity of water and the conductivity of aqueous solutions of electrolytes. They explain the density maxima observed at 4° and 10.2° with H_2O and D_2O respectively. Further, the calculated values of free energy, enthalpy and entropy agree with the experimental data.

It is common knowledge that the solubility of hydrocarbons in water is low. The process of solution involves a decrease in entropy and hence the reaction is not favoured. This negative entropy arises out of the fact that the water molecules in a hydrocarbon solution are more ordered. According to Scheraga, a near spherical cluster in pure water tends to have a convex surface. The surface water molecules can take part in four hydrogen bonds and also interact by van der Waal forces with the hydrocarbon molecule as a fifth neighbour. In effect a partial cage is formed around the hydrocarbon. This penta-coordination of water molecule lowers the energy of the tetrahydrogen-bonded molecule. Because of this lowering of energy, more H_2O molecules will be in the ordered structure than in pure water. The introduction of aliphatic and aromatic hydrocarbons into pure water, therefore, makes the structure of the latter more ordered.

The effects of solute and solvent, however, seem to be mutual, as the results of Felix Frank would suggest. In dilute solutions of alcohol, which contain both a polar group and a hydrophobic tail, the alcohol molecules are incorporated into water structure in a manner similar to hydrocarbon solutions. In alcohol-rich solutions, on the other hand, water breaks up the alcohol aggregates and water-centred association may take place.

Physical Properties and Structure of Water

In a number of physical properties, water and aqueous solutions show thermal anomalies. They

seem to occur around 15°, 30°, 45° and 60° and may reflect transitions in water structure. Several biological phenomena such as growth of bacteria, rate of cell division, oxygen uptake, etc., also show abrupt changes with temperature. Drost-Hansen suggests in his paper that there may be a relation between these thermal anomalies of water and the existence of the abrupt changes in biological phenomena, although it is not clear by what mechanism this would occur.

In recent years the concept of hydrophobic bonds stabilizing the helical conformation of proteins has gained importance. Hydrophobic bond formation can be considered as the reversal of solution of hydrocarbons in water. The thermodynamic data would of course have opposite sign. These data can be used to calculate the contribution of hydrophobic bonds to the stability of protein structure. From such calculations Scheraga has shown that, for example, the thermal transition curve of poly-L-alanine is in accord with experimental data.

If water consists of 'clusters' and if hydrocarbons stabilize this structure, it may be expected that many properties of an aqueous solution would be altered by hydrocarbons. The results of Fenichel and Horowitz show that in the diffusion of hydrocarbons of small size in formamide, the frictional coefficient depends on the hydrogen-bonding ability between the solute and solvent. In water, no such correlation exists. The explanation of the results appears to be that the diffusion in 'clusters' may be 'solid-like' whereas in nonhydrogen-bonded water it may be 'gas-like'. In 'gas-like' diffusion there is an inverse relation between size and diffusion of the solute whereas there will be a higher order dependence on size in 'solid-like' diffusion. It is possible that in the aqueous medium inside a living cell the diffusional properties of solutes may be different from those in pure water. In a similar way it may be expected that proton conductivity in water containing hydrocarbons may be more like in ice. Such measurements in ice show that conductivity is affected not only by electrolytes but by nonionic substances such as glycerol and sugar also. In biologic structures proton conductivity may, therefore, depend on the local environment.

Protein molecules contain a number of hydrophobic groups and should, therefore, be capable of making water structure around them more ordered. A number of experimental results provide evidence for this postulate. From dielectric relaxation and electrical conductivity at various frequencies of hemoglobin solutions, Schwan concludes that bound water has a structure intermediate between those of normal water and ice. Grant comes to a similar conclusion from his study of the dielectric relaxation of egg albumin and bovine serum albumin. The NMR study of Berendsen and Michelsen on collagen, silk fibroin, keratin and DNA indicates that in collagen water molecules adhere to each other and to collagen macromolecules and form chains in the fibre direction. The rotations of water molecules in such chains are restricted and yield the observed interaction of proton spins. The molecular lifetimes of such a structure are five orders of magnitude longer than in liquid state. In the case of the other macro-

molecules, the water molecules are held in a direction perpendicular to the fibre axis. These water molecules have a rapid rotation but the rotations are non-isotropic. This anisotropy increases with humidity. Perhaps at low humidity isolated side chains of the macromolecule hydrate and with increasing humidity water bridges are formed.

Structure of Water in Biological Systems

Recent advances in the technique of electron microscopy seem to make this a powerful tool for studying the structure of water in biological systems in the native state. One of the drawbacks of this technique so far had been that the specimens could be studied only in high vacuum and thus in a dry state. The design of vacuum-tight microchambers and microscopy at low temperature to 'freeze' any existing motion make possible the study of hydrated biological systems. Fernández-Morán discusses in his paper the application of this ingenious technique and presents data on the formation of noble gas hydrate microcrystals between lipid layers of a lipoprotein when the noble gas is applied under pressure and at low temperatures.

A wide variety of substances with different chemical structures act as anesthetic agents. Perhaps all of them act by a similar mechanism. Gases like xenon and argon, which are used as anesthetics, have no ability to form chemical bonds, either covalent or ionic. They form hydrates with water giving rise to a clathrate or cage structure. It is believed that these substances act as anesthetics by affecting water structure around proteins. They may increase the amount of structured-water and thereby lower the conductance of nerve membrane. These microcrystals would also lower the energy of electric oscillations in the brain and damp the activity.

Role of Water in Cellular Transport

The mechanism of cellular transport of electrolytes and non-electrolytes has been a subject of continuing research and two mutually exclusive points of view have been advanced. It is commonly observed that K^+ concentration inside the cell is high and Na^+ concentration is low compared to their concentration in the fluid bathing the cell. Similar unequal distribution of other cations, sugars and amino acids has also been observed. According to one school of thought this is due to the fact that the cell membrane acts as a permeability barrier which limits the entry and exit of substances. There are specific binding sites which are assumed to be located on the plasma membrane of the cell; the transport of solutes across the membrane is assumed to involve 'pores' or 'channels' possessing binding sites. In the case of active transport, i.e. transport against a thermodynamic gradient, metabolic energy is used to operate membrane 'pumps'. Thus the transport of Na^+ from the cell interior to the external medium and that of K^+ into the cell interior, against a gradient, are due to a coupled Na^+K^+ pump which converts metabolic energy into osmotic work. Pumps for the transport of other solutes have also been postulated. According to this mechanism of cellular transport, the selectivity arises out of specific binding sites and the energy for active transport

comes from the metabolic energy. Water of the cell interior has no primary role in the transport process. The water and cations inside the cell are 'free' as in an aqueous solution. A small portion of the cations and water are bound to the macromolecules and are unavailable for transport. However, the macromolecules do not show any selectivity in binding cations or non-electrolytes.

The mechanism postulated by the other school of thought attributes a primary role to water of the cell interior. The structure of this water is different from that of ordinary liquid water and is highly ordered. It is capable of excluding certain cations and solutes from the biological macromolecules which have fixed sites available for binding the solutes. The macromolecules are also ordered in a specific architecture. Negative and positive groups on the macromolecules bind the counterions and there appears to be selectivity in binding. Thus K^+ is selectively used as the counterions for the negative sites whereas Na^+ is excluded from the structure. Metabolic energy is required primarily for the formation of the ordered structure and is not involved in the flow of the solute.

There is considerable experimental evidence to support either of the mechanisms postulated. Leaf in his paper cites results which indicate the existence of a permeability barrier on the mucosal surface of toad bladder. This barrier is highly permeable to water, urea and sodium but effectively blocks the entry of thiourea. The application of amphotericin to the mucosal surface removes the outer diffusion barrier, leaving the deeper porous barrier intact, and increases the permeability of sodium, potassium and thiourea.

From a variety of experimental techniques it has been concluded that water in the vicinity of biopolymers has a more-ordered structure than in liquid water. This (bound) water is capable of excluding solutes from this ordered structure. The results which Ling cites with a model substance like ion-exchange resins show that the water imbibed in the matrix of the resin can exclude a variety of substances such as glucose, sucrose, etc., and that their concentration in the interior water is lower than that in the surrounding aqueous medium. Similar results seem to obtain with frog muscle, rat diaphragm muscle and rat adrenal gland in the distribution of xylose, galactose and sucrose. According to Ling the postulate that metabolic energy can operate the membrane pump is also untenable. His calculations indicate that the energy needed to operate the pumps for three cations (Na^+ , Ca^{2+} and Mg^{2+}) alone would be about 3.5 times the total metabolic energy available per unit time. Water of the cell interior seems to play an important role. The rotation of hydrated Na^+ , for example, in the ordered water would be largely restricted and hence the entropy of the system would be lower. This large negative entropy would offset any favourable enthalpy value which would favour accumulation of Na^+ in the cell and would thus exclude Na^+ from the cell interior.

It is perhaps only natural that in dealing with such a complicated biological system as a cell, no single postulated mechanism will be able to explain all the observed facts. As Hecter points out a

pluralistic approach incorporating some ideas of both the postulated mechanisms may be valid.

Biological Membranes and Structure of Water

There is definitive evidence for the existence of an energized Na^+K^+ pump in the plasma membrane of erythrocyte ghosts. ATP is the source of energy and per molecule of ATP utilized the efflux of $3Na^+$ and the influx of $2K^+$ occur. A membrane-bound enzyme — a Mg^{2+} ATPase requiring both Na^+ and K^+ for activity — has been shown to be an integral part of the pump. The existence of similar membrane-bound ATPases in other cells such as liver, intestine, etc., has been widely accepted. It is possible that more than one type of enzymic mechanism may be involved in the coupled Na^+K^+ pump.

Plant and animal cells contain a profusion of membrane systems and these membranes separate the cell interior into segregated compartments. The membranes are lipoprotein in character and have a uniform structure consisting of lipid layers sandwiched between protein layers. Although there is uniformity in structure, the membranes are functionally diverse because of the differences in the associated enzymes. These enzymes are responsible for the directional transport of solutes. An attempt has been made by Fernández-Morán and Green to correlate structure with function in mitochondrial membrane systems.

Selective binding sites may be located on the membrane systems. There can be two types of binding sites; one type involved in the transport of solute across the membrane and the other selectively binding the solute without any transport. Both the types may be operative in K^+ accumulation and Na^+ exclusion.

If a system of membranes separates the cell interior into compartments then water inside the cell will find itself in different environments and there will not be uniformity in water structure. Whereas the water in between organelles is mobile and likely to have liquid water structure, water associated with the membranes will have an ordered structure. This ordered structure might act as a molecular sieve and be responsible for selective permeability of solutes. This provides also an architectural frame whereby a local perturbation can be propagated. The marked permeability induced by excitation could be the consequence of the 'melting' of water structure in special regions. There does not appear to be much doubt regarding the structural role of water in membrane systems. Mitochondrial suspensions when treated with dry zeolite disrupt; membrane-bound cytochrome oxidase is solubilized and the lipids become separable from the residual membrane fraction. However, wet zeolite causes no such disruption. The membrane disruption is attributed to the avidity of zeolite for water. Hecter rightly points out that there is a hazard involved in extrapolating results from one cell type to another. The proportion of membrane componentry to the total mass of the cell differs among various cell types. The water structure could also be, therefore, different.

From the foregoing it is apparent that the concept of structured-water is capable of explaining several biological functions. In an article of this length

it has not been possible for the reviewer to discuss all the twenty-eight papers presented at the conference. Some papers which the reviewer could not easily fit into a coordinated discussion have not been

touched upon. However, research workers in the area of biology, to whichever discipline they may belong, will find a wealth of information in the proceedings of the conference.

Pharmaceuticals & Drugs Research Committee, CSIR: Progress Report for 1965

DURING the year 1965 the Pharmaceuticals & Drugs Research Committee sanctioned the continuance of 27 research schemes and 6 research units. Some of the highlights of the work done during the period are summarized below.

Fifteen medicinal plants have been investigated under various schemes. It has been shown that the extracts of *Aconitum spicatum* Stapf and *Cassia fistula* Linn. possess powerful antipyretic and analgesic activity with a wide margin and safety and have been recommended as safe therapeutic agents, while extracts of *Terminalia bellirica* Roxb. and *Emblica officinalis* Gaertn. have been shown to possess promising hypotensive activity. An increase in serum aldolase activity in patients has been shown to be of diagnostic value for tetanus. Bengal gram steep liquor has been shown to be a good substitute for imported corn steep liquor in fermentation broths. A number of compounds have been synthesized for screening as hypoglycaemic, antifilarial, anti-spasmodic, antiviral and antifertility agents, but none of the compounds was found to possess significant activity.

With a view to finding new drugs in the country, the Committee has set up one clinical testing unit at Bombay and several new drugs have been clinically tested in this unit. Three compounds were found to possess promising activities: N-1-(4-methylphenyl)-2-phenylethylpropionamide having tranquillizing activity has been selected for clinical trial, the second compound, after subacute toxicity studies, has been put to limited clinical trials on volunteers and patients of anxiety neurosis, and the third, a camphor derivative, 2-ethylamino-3-phenyl-nor-camphan hydrochloride, showed interesting antifatigue activity. The unit has also screened 300 compounds of different chemical series received from different laboratories.

The Committee has also undertaken the task of organizing biological screening facilities so that screening of compounds synthesized or isolated from plant materials in various research laboratories can be carried out. During the year 83 compounds have been screened under the programme.

Fifteen original research papers have been published embodying the work carried under the various research schemes.

Pharmacological Investigations

Screening and development of drugs in prevention and therapy of thrombosis and atherosclerosis — A number of coumarins, which included dicoumarol analogues

having a phenyl or a substituted phenyl ring on the bridge methylene, complex 4-phenylcoumarins and coumarino- α -pyrones, have been screened for their anticoagulant activity in order to test the hemiacetal hypothesis. The results do not fit into a clear structure-activity relationship pattern. The slow activity of some *ortho*-substituted compounds in which there is considerable distortion of the original shape demonstrates the importance of the molecular shape. Anticoagulant activity can be enhanced or decreased by substitution at various positions. It may be inferred that coumarin anticoagulant act by competition with vitamin K for a specific enzyme.

Since the pig is considered to be a suitable model for the study of the human type of atherosclerosis, detailed biochemical, haematological and histopathological investigations were carried out on pigs in relation to the development of spontaneous atherosclerosis. With advancing age of the pig, rise in the contents of free and total serum cholesterol, serum triglyceride, aortic mucopolysaccharide, β -lipoprotein and the cholesterol concentration of the latter was observed, but there was decrease in the serum magnesium, α/β -lipoprotein concentration ratio, ESR and coagulation time. Blood sugar level and platelet clumping time, however, did not show any change. These changes were remarkably significant between the ages 2½ and 3 years. Histopathologically, three different types of lesions were observed in the aorta: fatty streaks in the age group 36-48 months (3 per cent), fibrous plaques at 49-60 months (22 per cent), and complicated lesions at 60-70 months (R. B. Arora, All India Institute of Medical Sciences, New Delhi).

Pharmacological investigation of some synthetic compounds — About 300 synthetic compounds of various chemical series were screened for CNS activity. 2-Methyl-3-(3,4-dichlorophenylethyl)-4(3H)-quinazolinone and 2-methyl-3-(*o*-chlorophenylethyl)-4(3H)-quinazolinone showed promising CNS depressant and antitussive properties. A number of phenothiazine derivatives has shown interesting anti-inflammatory activity in preliminary screening. A number of triazine, triazole, benzylamine derivatives and anthranilic acid derivatives have been screened for diuretic activity in rats. Further work is in progress.

Clinical drug trial of some new drugs — Anabolic activity of methandiomone was studied in underweight healthy subjects. Results are not encouraging. Patients with chronic tonsillitis were treated with sulphamethyl pyrimidine or sulphamethoxy pyridiazine; the level of the latter in blood and tissues

was found to be higher than that of the former. In lobar pneumonia lincosyline, a new antibiotic, showed promising results (U. K. Sheth, Seth G.S. Medical College, Bombay 12).

Studies in adrenergic blockade—In a study of the antagonism of the α -adrenergic blockade produced by ergotamine, phenoxybenzamine and phentolamine, it has been found that pronethalol competitively reverses their blocking action in dogs and rabbits. It is suggested that the antagonism by pronethalol is brought about by a competitive displacement of the more potent blocking agent from the receptor site which as a result becomes more easily accessible to the catecholamines.

The mechanism of adrenergic nerve blocking action of bretylium, guanethidine and reserpine has been investigated in rats, using the pressor effect of physostigmine. It has been found that dexamphetamine and methylamphetamine reverse the blocking action of guanethidine and bretylium but not that of reserpine, thus showing that the blocking action of the former two are similar while that of reserpine is quite different.

In an extension of this work it has been shown that certain indirectly acting sympathomimetic drugs like dexamphetamine, methylamphetamine, morphoramine and monoamine oxidase inhibitors like phenylisopropylhydrazine can effectively reverse the action of guanethidine.

It has been found that osthol (7-methoxy-8-isopentyl-coumarin) isolated from the roots of *Prangos pabularia* Lindl., in addition to its analeptic activity, also causes a rise in arterial blood pressure. This pressor response can be blocked by α -blockers, thus suggesting that the pressor effect of osthol is mediated, at least in part, through a release of nor-adrenaline from tissue stress.

The effect of various drugs on phenylquinone-induced writhing in mice has been studied. No correlation between antiwrithing response and analgesic activity has been found. By administration of ganglion blocking agents it has been shown that the ganglionic synapse is not involved in protecting the writhing response (O. D. Gulati, Medical College, Baroda).

The effect of microbiological leaching of low grade sulphide containing uranium ore—The purification by microbiological leaching of low grade sulphide ores containing uranium has been investigated. Uranium containing soil samples were incubated on a number of organic and inorganic media. Waksman medium [KH_2PO_4 , MgSO_4 , $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{SO}_4$, $\text{Na}_2\text{S}_2\text{O}_3$ or S] was found most suitable for this purpose. An organism was isolated from uranium containing ores which changed the pH of the medium from 5.8 to 1.0. Bulk culture of this organism has now been prepared with a view to testing its efficiency in the leaching of uranium (S. S. Bhatnagar, Caius Research Laboratory, St Xavier's College, Bombay).

Biochemical and immunological investigation of the toxin of Clostridia—The study of the mechanism of action of tetanus toxin showed that it was able to uncouple the oxidative phosphorylation of brain mitochondria of the susceptible rat but not of the resistant pigeon. When the pigeon brain mitochondria were disrupted by freezing and thawing,

the phosphorus/oxygen (P/O) ratio was lowered in the presence of the toxin, indicating that the mitochondrial membrane of pigeon brain is impermeable to tetanus toxin. This may be one of the reasons for the high resistance offered by the pigeon. The toxin had no effect on the P/O ratio of liver and muscle mitochondria of rats and pigeons. It has also been shown that the uncoupling of oxidative phosphorylation of rat brain mitochondria takes place only in the DPN-requiring steps, since the toxin did not have a significant effect on phosphorylation when succinate and ascorbate were used as substrates. The toxin had no effect on the swelling properties of rat brain and liver mitochondria.

In a study of the fixation of tetanus toxin by subcellular fractions obtained from the brain tissue homogenates of the rat and pigeon, it was found that the absorption capacity of pigeon brain homogenate was 20 per cent less than that of rat brain homogenate. Homogenates prepared from rat and pigeon liver tissue did not absorb toxin. The highest adsorption of toxin was on the mitochondrial fraction. There was a fairly close correlation between the adsorption of tetanus toxin by subcellular fractions and the ganglioside content of the latter. When gangliosides were made insoluble with CaCl_2 , these fractions could bind about 75 per cent of the added toxin, but since the binding is loose the toxin is released *in vivo*. There was no change in the brain ganglioside content in rats injected with tetanus toxin which indicated that gangliosides are not degraded after they are bound to tetanus toxin.

Determination of enzyme activity in 14 cases of tetanus showed the absence of detectable amount of creative phosphokinase (CPK) in the serum. Aldolase activity was found to be very high in some fatal cases. It closely followed the clinical course of the disease and was related to spasms and rigidity. There was no rise in the serum aldolase activity in 3 cases of suspected tetanus but which were later not found to be so. Serum CPK was not altered in rats injected with tetanus toxin. These results show that the leakage of aldolase may be due to a change in permeability of cell membrane caused by localized anoxia coupled with muscular and respiratory spasms and rigidity (H. I. Jhala, Haffkine Institute, Bombay).

Studies on Indian Medicinal Plants

Chemical and pharmacological investigation of cardiovascular drugs used in indigenous system with particular reference to Unani medicine—The following plants were investigated under this scheme: (a) *Terminalia bellirica* Roxb. (bahera), (b) *Emblica officinalis* Gaertn. (amla), and (c) *Withania coagulans* Dunal (akri).

Alcoholic extract of the seeds of *T. bellirica* Roxb. produced a full blood pressure, with peristaltic movement of intestine. Some varieties of the seeds showed antitussive action. A colourless crystalline substance, m.p. 185°, has been isolated. Alcoholic extract of the fruits of *T. bellirica* Roxb. caused sudden fall in blood pressure with slow recovery and increase in rate and amplitude of the respiratory movements. The aqueous extract produced a biliary stimulation and a fall in blood pressure.

Alcoholic extract of the fruits of *E. officinalis* produced a slight initial fall of blood pressure followed by rapid recovery to normal. It had also some antispasmodic action. Extract of *W. coagulans* caused a marked fall in blood pressure of short duration (H. H. Siddiqui, Institute of History of Medicine, Delhi).

Investigation of indigenous medicinal plants—From *Cimicifuge foetida* two crystalline glycosides, m.p. 255° (C₁₅H₂₃O₅) and 305° (C₂₀H₃₂O₅), have been isolated. These did not give the usual tests for triterpenes. It has been shown that sugar obtained on hydrolysis is not glucose, fructose or galactose. Other plants investigated and the products isolated in this scheme are: *Premna latifolia* Roxb., β -sitosterol; and *Anthocephalus cadamba* Miq., a few crystalline alkaloids (A. R. Kidwai, Aligarh Muslim University, Aligarh).

Search for compounds having estrogenic properties from indigenous plants—Crude extracts of the following plants have been screened for estrogenic activity with negative results: *Symplocos racemosa* Roxb., *Uraria lagopodioides* Desv., *Caesalpinia crista* Linn., *Randia dumetorum* Lam. and *Morinda Coreia* Buch-Ham.

Chemical investigation of *S. racemosa* resulted in the isolation of β -sitosterol, a crystalline phenolic alkaloid, C₁₇H₁₇NO₄, m.p. 204-5°, [α]_D+60°, and a mixture of amorphous, non-phenolic alkaloids. The UV spectrum of the crystalline alkaloid showed λ_{max} at 282 and 307 m μ . Its hydrochloride showed promising hypotensive property (U. P. Basu, Bengal Immunity Research Institute, Calcutta).

Cardioactive principles from certain species of Leguminosae—The chemical investigation of *Clitoria ternatea* Linn. gave the following compounds:

Substance A—Brownish crystals, m.p. 212-14°, [α]_D+6.0 (methanol), acetate, m.p. 206-8°, [α]_D 0.55 (methanol); the IR spectrum indicated the presence of COOH and 1,2,4-trisubstituted benzene; UV spectrum, λ_{max}^{EtOH} 210 and 290 m μ and λ_{max} 202 and 335 m μ in 0.002N EtONa-ethanol.

Substance G—Yellow needles, m.p. 201°, [α]_D+61.25. Its IR spectrum showed associated OH and characteristic ν -pyrone bands; UV spectrum, λ_{max} 265 and 350 m μ in EtOH and at 275 and 400 m μ in 0.002N NaOEt.

Substance J—A glycoside colourless needles, m.p. 140°, [α]_D+172.0, contains chlorine. It did not have any absorption in the UV region; acetate (crystalline), m.p. 87-88°, [α]_D+135.35, does not contain chlorine.

Substance M—Colourless needles, m.p. 210°, [α]_D+172.0, contains nitrogen. IR spectrum showed bands for NH (secondary amide). It did not absorb in UV and appears to be an aliphatic secondary amide.

Substance B—Amorphous, contains OH groups. It is sensitive to acid and alkali (M. P. Khare, Lucknow University, Lucknow).

Synthesis of Potential Biologically Active Compounds

New antispasmodics in the isoquinoline series—N,N-bis-(3-ethyl-1-isoquinolymethyl)- α -methyl- β -phenylethylamine has been synthesized for screening

as an antispasmodic (U. P. Basu, Bengal Immunity Research Institute, Calcutta).

Antidiabetic drugs—In view of the interesting biological properties associated with some symmetrical triazoles, the following compounds have been synthesized for screening as antidiabetic agents: 5-benzenesulphonamido-3-phenyl-1,2,4-triazole, 5-*p*-methoxybenzenesulphonamido-3-phenyl-1,2,4-triazole, 1,2-diphenyl-5-anilino-1,3,4-triazole, 1,2-diphenyl-5-anilino-1,3,4-triazole, 1-*m*-chlorophenyl-2-phenyl-5-*m*-chloroaniline-1,3,4-triazole and 1-cyclohexyl-2-phenyl-5-cyclohexylamino-1,3,4-triazole.

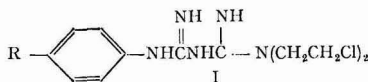
Phenylacetic acid and cyclopropane-carboxylic acid show hypoglycaemic activity when administered orally in guinea-pigs and rabbits. In order to study whether the acyl residue enhanced the hypoglycaemic activity of biguanides, the following compounds were synthesized: N'-(2-thiazolyl)-biguanide hydrochloride, N'-(5-methyl-2-thiazolyl)-biguanide hydrochloride and N'-(5-ethyl-2-thiazolyl)-biguanide hydrochloride. These compounds await biological testing and the work is in progress (U. P. Basu, Bengal Immunity Research Institute, Calcutta).

Microfilaricides—In a search for new microfilaricides which may be better than hetrazan, some biguanidino-piperazines have been synthesized and screened. Of these, 1-methyl, 1-carbethoxy and 1-diethylcarbamoil-4-biguanidino-piperazine hydrochloride showed antifilarial activity in *L. carinii* infected albino rats while the biguanidino-piperazines had no activity. Some other piperazine derivatives synthesized include 1-(N-piperidinocarbonyl)-piperazine and 1-4-bis-(N-piperidinocarbonyl)-piperazine (U. P. Basu, Bengal Immunity Research Institute, Calcutta).

Potential anticancer steroid analogues—In a continuing study of azasteroids of the cholestane series, the synthesis of 7- α -aza- β -homocholest-5-ene-3- β -ol-7-one starting from 3- β -acetoxycholest-5-ene-7-one has been carried out in three steps.

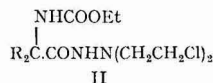
In the androstane series, 3,17- α -diazo-A,D-bis-homoandrost-4 α -ene-4,17-dione has been prepared from which compounds of potential therapeutic interest are intended to be synthesized (Harkishan Singh, Panjab University, Chandigarh).

Amino acids and peptide derivatives as possible antitumour compounds—In continuation of the previous work or arylbiguanido mustards of the type (I), eleven new compounds of this series have been synthesized.

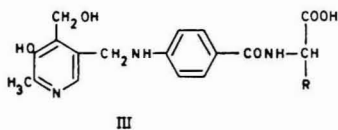


where R = CH₃, C₂H₅, OCH₃, OC₂H₅, -OH, COOH, SO₃H, NHCOCH₃, NO₂, Cl and Br.

The earlier work on mustard derivatives of N-carbethoxy amino acid hydrazides of type (II) has been extended to seven new amino acids.



The synthesis of analogues of folic acid of type (III) has also been carried out.

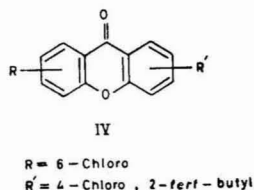


Isopropylideneopyridoxine was converted to its chloro derivative which on condensation with ethyl *p*-aminobenzoate followed by hydrolysis of the resulting ester and condensation with different amino acids using *N,N'*-dicyclohexylcarbodiimide gave the desired compounds. Ten compounds in this series have been prepared (A. B. Sen, Lucknow University, Lucknow).

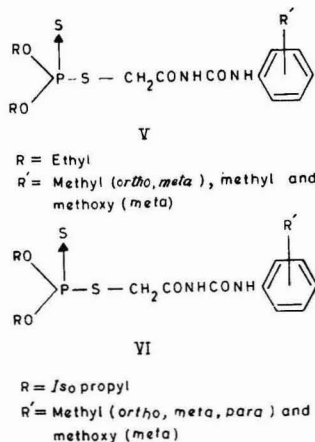
Synthesis of some potential anticonvulsants and hypnotics and studies of their biochemical mechanism of action — Several quaternary ammonium compounds of 2,3-disubstituted quinazolones have been synthesized to investigate their antiacetylcholinesterase and monoamine oxidase inhibitory properties and their action on the central nervous system. In the case of 2-methyl-3-(2'-)pyridyl-4-quinazolones (OZ-2') the enzyme inhibition activity was dependent upon the number of carbon atoms in the alkyl chain at the pyridinium nitrogen atom, whereas no such effect was observed with 2-methyl-3(4')-pyridyl-4-quinazolone (OZ-4') compounds. This difference in the behaviour of the two series appears to be due to the close vicinity of the positive nitrogen atom quinazolone nucleus in the OZ-2' series, which would interfere with charge availability. In an attempt to reduce the toxicity of the monoamine oxidase inhibitors several quinazolones have been synthesized incorporating in their structure known hydrazide and non-hydrazide monoamine oxidase inhibitors. It is hoped that the quinazolone moiety having central nervous activity would transport these compounds to the brain where the active monoamine oxidase inhibitor would then be liberated to produce the desired effects in low concentrations and thereby reduce their toxicity. The *in vivo* and *in vitro* effects of these compounds are being investigated. In addition several quinazolone hydrazides have been synthesized and tested for their monoamine oxidase inhibitory properties. It was observed that a hydrazide without any substituent at 6-position of the quinazolone nucleus was found to have little inhibitory effect. Substitution at position-6 significantly increased monoamine oxidase inhibitory properties; maximum inhibition was observed with iodo-substituted derivative. The pattern of enzyme inhibition is in no way related to electronegativity of the substituents. These results support the assumption of the existence of one or more additional sites for the enzyme in addition to the primary site acting through the amino moiety. Further substitution at position-8 of the quinazolone nucleus decreased the monoamine oxidase inhibitory property, although the degree of inhibition was greater than that observed with compounds having no substituent at either 6- or 8-position. It is presumed that substituents at 6- and 8-positions may compete for the active site of the enzyme for such inactivation. Experiments were also conducted to determine *in vivo* monoamine oxidase inhibitory properties by (i) dihydroxyphenylalanine (DOPA)

response test and (ii) reserpine reversal test. Though substitution effects were observed, the *in vivo* effects of these inhibitors were in no way found to correspond with their *in vitro* enzyme inhibitory properties. Furthermore, anticonvulsant property of some quinazolone hydrazides was also investigated to find out if there was any correlation between *in vitro* enzyme inhibitory properties and their pharmacological effects. Unsubstituted hydrazide was devoid of any such anticonvulsant property. All the other quinazolone hydrazides having substituents at position-6 possessed anticonvulsant property when tested against metrazol threshold test. Finally, effects of quinazolone hydrazides were investigated on pressor responses to epinephrine and norepinephrine on blood pressure of cats and also tryptamine responses on isolated rat uterus. In all the cases these responses were found to be potentiated. Since such potentiation of responses in no way parallel the enzyme inhibitory properties of these newer monoamine oxidase inhibitors, it seems unlikely that monoamine oxidase may be solely responsible for these effects. Attempts are being made to establish their mechanism of action (S. S. Parmar, K.G. Medical College, Lucknow).

Synthesis of compounds allied to pyrethrins and their biochemical mechanism of action — Certain alkyl-chloroxanthanes and O,O-dialkyl-dithiophosphoric acids have been synthesized for screening as insecticidal agents. In the alkyl-chloroxanthones series the compounds represented by general structure (IV) were prepared.

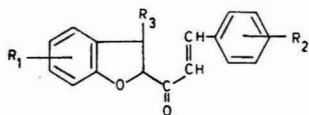


In O,O-dialkyl-dithiophosphoric acid series two types (V) and (VI) of compounds were prepared (S. S. Parmar, K.G. Medical College, Lucknow).



Preparation and screening of 3,4,5-trimethoxybenzene derivatives in the search for more potent therapeutic agents — In a study of trimethoxybenzene derivatives as CNS agents, ω -nitro-2,4,5-trimethoxystyrene, dibromo-2,4,5-trimethoxynitrostyrene and dibromo-2,4,5-aminostyrene have been synthesized and screened. They show CNS depressant activity and also effect the CAR (P. C. Dandiya, S.M.S. Medical College, Jaipur).

Synthesis of possible antifertility agents — Several 2-cinnamyl-3-alkylbenzofurans (VII) have been synthesized for screening as antifertility agents and for oestrogenic activity. None of the compounds have so far been tested (S. S. Tewari, Lucknow University, Lucknow).



VII

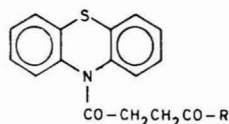
- $R_1 = 7\text{-Cl}, 5\text{-Cl}$
 $R_2 = 4\text{'-OH}, 3\text{'-OCH}_3$
 $R_3 = \text{CH}_3$

Synthesis of new CNS active drugs: Structure-activity relationship of antiemetics, analgesics, etc. — The antiemetic activity of a number of compounds having the general structure (VIII) were tested against apomorphine-induced vomiting with negative results.

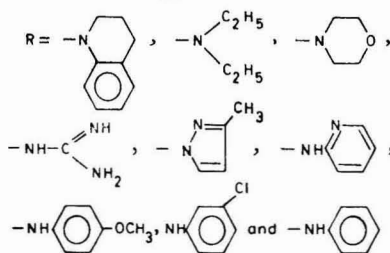
In a search for new antiemetic agents which may be better than the phenothiazines, a number of known drugs have been tested against apomorphine-induced emesis in dogs and their relative potency recorded.

The results indicate that acetophenazine and heleriperidol may be better therapeutic agents and chlorpromazine as an antiemetic agent.

In an attempt to elucidate the mechanism of action of JB-516, the effect of administering JB-516 (2-8 mg./kg.) has been studied on the spontaneous activity of the intact cortex and the isolated cortex and on the evoked after discharges of the isolated cortex in adult mongrel dogs. It has been found that the intravein administration of JB-516 induced low voltage fast wave activity both in the intact and



VIII



isolated cortical preparations. Also the threshold of the after discharges was lowered and the duration increased. This stimulant effect persisted from 45 to 60 min. The initial stimulant effect was followed by a depressant effect both in the intact and isolated cortex. It, therefore, appears that JB-516 exerts initially a stimulant effect at the cortical level which is followed by depression of the cortex (K. P. Bhargava, K.G. Medical College, Lucknow).

Studies in Antibiotics

Microbiological transformation of cortexolone to hydrocortisone and prednisolone — In a search for substitutes for corn steep liquor, steep liquors obtained from several Indian graminaceous and leguminous seeds such as *Pennisetum typhoides* Stapf & Hubb. (bajra), *Sorghum* spp. (red and white jawar), *Setaria* sp., *Eleusine* sp., *Cicer arietinum* Linn. (Bengal gram), *Phaseolus aureus* Roxb. (green gram), *P. mungo* Linn. (urd), *Vigna sinensis* Savi ex Hassk. (cow peas, big and small), *Cyamopsis* (guar) and *Melilotus* sp. (clover) were investigated. The time of steeping was studied. It has been found that *C. arietinum* Linn. steep liquor is superior to corn steep liquor and steeping for 72 hr was able to extract most of the nitrogenous material (P. G. Rao, Birla College of Science, Pilani).

NITYA NAND

Role of Structure on Functions of Biological Processes: A Symposium in Biophysics

N. N. SAHA

Secretary, Indian Biophysical Society, Calcutta 9

A SYMPOSIUM in biophysics, sponsored by the Indian Biophysical Society, was held at the Saha Institute of Nuclear Physics (SINP), Calcutta, in April 1966. While inviting suggestions regarding the topics to be discussed during the symposium, prospective participants wanted to know whether it would be possible to present a composite picture of up-to-date developments in biophysical researches. Biophysics is a vast discipline and any attempt to cover all aspects of biophysics and its ramifications in a brief symposium would result in superficial treatment of the topics. As modern biophysical research is oriented predominantly towards the clarification and understanding of structure and functions of biological processes, emphasis was given to the subject of the role of structure at various levels of all organization in elucidating the functions of various biological phenomena. This emphasis as well as the question of time restricted the range of topics.

During the last two decades, researches in biophysics, particularly in molecular biology, have advanced with such rapidity that we have now at our disposal enough experimental data to make a study of the correlation between the structures and functions of biological processes. Indeed, much has been done in this direction, but much more remains to be done. The Indian Biophysical Society provides a forum where biophysicists may discuss their problems in the light of the latest developments in the subject and formulate plans for the development of biophysical research on modern lines in this country.

Prof. B. D. Nag Chawdhury, Director, SINP, welcoming the participants in the symposium, said that the borders between the sciences are being gradually erased and it is in this larger view that the meeting of the biophysicists, molecular biologists, medical men and biologists had been brought about by the Indian Biophysical Society. He hoped that this will be the beginning of a long dialogue between scientists concerned with various subject disciplines.

Dr D. M. Bose, President of the Indian Biophysical Society, inaugurating the symposium, said that since the study of biophysics is being taken up only recently in this country, probably a historical approach to the development of general physiology, of which biophysics is an offshoot, would be proper in an introductory seminar such as this. Dr Bose gave an account as to how he himself became introduced to the subject, through the biophysical researches of Sir J. C. Bose on plant responses. As Director of Bose Institute, he became interested in the plant physiological investigations of Sir J. C. Bose with two types of plants having motile organs, viz. *Mimosa pudica* and *Desmodium gyrans*. These were

selected as prototype plants with which the phenomenon of contractility, conductivity and rhythmicity could be studied as plant analogue. Study of the long periods of rhythmic pulsations of the small side leaflets of *D. gyrans* raised in his mind the question of the source of energy of these mechanical movements. Further investigations revealed the photosynthetic basis of carbohydrate production in plant leaflets and their subsequent dismutation to water and carbon dioxide, releasing the energy for mechanical pulsations. Later, Dr Bose's attention was drawn to biochemical processes underlying growth, movement and of responses following excitation; such studies pointed to the unit cell as the seat of all metabolic changes underlying the different forms of plant activities. Thus, beginning from the study of an organism as a whole, attention became more and more directed to the level of cellular activities. The final step in the process was the study of the structure and functions of large biological molecules, the proteins, the enzymes, and lastly the cell nucleus with the chromosomes and the genes which are the carriers of the hereditary information from generation to generation. Finally, the synthesis of some complex biological molecules controlling cellular activities, for which the DNA molecules provide the template, introduces us to molecular biology which has become the field of wide variety of studies at present. This synoptic approach to the study of biology provided a good introduction to the study of life activities at all levels of organization, from the organism as a whole to the large biological molecules which control the processes at the molecular level.

The following account is a brief summary of the papers presented at the symposium.

Ultrastructure of Haemoglobin Molecule

Prof. N. N. Dasgupta (SINP) discussed the ultrastructure of haemoglobin as revealed by electron microscopic study. After making a critical survey of the subject, he dealt with his own findings on three haemoglobin variants, A, S and E, which were studied under an electron microscope after negative staining. The electron micrographs showed a prominent hole in the centre of the molecule and also gave some evidence of separation between the four units. The shape was in general agreement with the X-ray diffraction data.

Molecular Structure and Biological Function

Prof. N. N. Saha (SINP) dealt with various biological functions in a living system in terms of structure at different levels of organization, particularly at the molecular level. He said that the key to the understanding of functions of what we

call 'living' lies mostly in the comprehension of the structure and organization of its component parts. That the properties of a material depend to a great extent on the arrangement of atoms in its building units and also how these units are arranged in space is an established fact in nonliving systems. As an example, he mentioned two synthetic polymers, polyethylene (CH₂)_n and teflon (CF₂)_n. In both these polymers, the backbone is composed of carbon. In the former, hydrogen forms the side chains, whereas in the latter hydrogen is replaced by the bulkier fluorine atom. As a result the hydrocarbon chain is planar, whereas the fluorocarbon is a helix due to the steric hindrance imposed by bulkier fluorine atoms. This structural change causes a considerable alteration in the function of these two chain molecules.

As proteins form the major constituent of biological systems, Prof. Saha confined his discussion mainly to proteins and conjugated proteins. Instead of only one particular type of side chain as in teflon, protein chains have about 20 different types of side chains arranged in a variety of sequential order depending on the sequence of its 20 component amino acids. In order to have an idea of the conformation of protein chains, the amino acid sequence is an essential information and to determine the molecular structure, i.e. three-dimensional arrangement of atoms, X-ray diffraction analysis is the most powerful tool that has so far been employed profitably. The salient features and limitations of X-ray diffraction methods as employed in the study of both globular and fibrous proteins, with particular reference to the concepts of nonintegral helix, diffraction by continuous and discontinuous helix, and isomorphous replacement technique, were discussed. In this connection Prof. Saha referred to the work of his group in molecular biology and discussed in detail the structure and function of shark fin ray collagen. Though most of the physical and chemical properties of this material, viz. the wide angle X-ray pattern, amino acid composition, band-interband nature of electron micrographs, etc., are more or less similar to those of mammalian collagens, there are some marked differences. The author explained this departure in the properties of this collagen from other collagens in terms of structural differences, not at molecular level but at higher level of organization as revealed by electron microscopic and small angle X-ray study. He also discussed the structure and function of collagen in normal and diseased human bones, based on his findings. The author finally pointed out that the existing molecular structure of collagen needs a particular sequence of its constituent amino acids or, in other words, this structure conforms to only that part of the chain which has this particular sequence. But the amino acid compositions of some collagens do not provide such a sequence. The author stressed the fact that the structure of collagen to be universal must not only account for collagens from various sources but their varied functional properties as well. It is with this end in view that a programme of study of collagens from different phyla, both vertebrates and invertebrates, was in progress in Prof. Saha's laboratory.

Ultrastructure and Function of Viruses

Prof. S. N. Chatterjee [School of Tropical Medicine (STM), Calcutta] reviewed the present-day concepts of the ultrastructure of viruses as derived from electron microscopic, X-ray scattering and other physico-chemical studies, with special reference to the relation between function and structure of virus. This was followed by an account of the investigations carried out in his laboratory on the morphological and functional aspects of two viruses, Chikungunya virus and cholera bacteriophage.

Electron microscope studies on the Chikungunya viruses infecting suckling mouse brain cells revealed that the virions are spherical in shape and have a diameter between 380 and 600 Å. The cytopathic effects caused by the viral infection are largely general in character. Interesting evidence of the mode of differentiation of the viruses in brain cells has been recorded.

Detailed morphological characterization studies of cholera phages have revealed that the phages belonging to any of the four different serologic groups are morphologically distinct. Group I phages have no tail, as confirmed by the studies on their adsorption to the respective hosts.

Biophysical Aspects of Human Red Cells

Dr J. B. Chatterjee, Director, STM, dealt with the biophysical studies on the structural and functional aspect of human red cells in health and in various disorders. The life span of red cells is being determined with more sensitive tracers, unfolding hitherto unknown knowledge relating to the contributory role of an underlying haemolytic mechanism. The function of bone marrow, the differentiation between effective and ineffective erythropoiesis, evidences of haeme deviation and of intramedullary haemolysis are now precisely ascertainable with biophysical techniques. The mechanics of absorption and utilization of haemopoietic nutrients (iron, folic acid and vitamin B₁₂) and their variation in various metabolic disorders are now precisely documented with appropriately designed techniques. The direct visualization of human haemoglobin molecule and electron microscopic and ultracentrifugal characterization of some of the normal and abnormal haemoglobins have opened up a new field of molecular biology. The biophysical parameters of the ultrastructure of red cell stroma, which still await detailed characterization, are expected to provide newer knowledge on the integrity of the red cells under conditions of physiological and pathological stresses. Still unexplored subjects include localization of the enzymatic systems, nature of arrangement of haemoglobins within the red cells and the exact mechanics of the instability of red cells in various haemolytic syndromes.

Brain, Behaviour and Memory

Dr B. Mukerji, Director, Chittaranjan National Cancer Research Centre (CNCRC), spoke on the neurological basis of brain structure and functions of individual neurons or groups or slabs of neurons in bringing about behaviour patterns and memory

storage in the brain. The brain is now regarded as a 'single' organ, for its action is that of an integrated unit. Actually, it is a bundle of structures and when these structures react with each other in a normal manner they produce the final unified effect.

Functionally, the brain can be divided into three important impulse relay centres: (i) Neocortex (the 'new' brain) is the site of the discriminative aspects of consciousness. The cortex performs perhaps the same function in the brain as the scientists and technicians in society. It operates like a computer and can predict the consequences of any given action from relevant data and provides the intellect and suggests projects, etc. (ii) Midbrain (diencephalon) consists of thalamus, hypothalamus, reticular system, limbic region, etc. These are the key structures for emotional activities and different behaviour patterns. The midbrain is the central point for autonomic nervous system and is the seat of final integration, real executive power sources of energy and drive. (iii) Hindbrain (including cerebellum) is responsible for vasomotor centre, respiratory centre, vomiting centre, etc. Cerebellum is the seat of physical balance.

As regards behaviour, Dr Mukerji said that the biological success of animals depends partly on their power to make movements in the light of signals coming from the outside world. When conditions are standardized, it is possible to get a constant response to a given signal. The perceiving organs and nervous system of the bees and the birds are able to obtain from their environment the kind of information which the navigator gets by using his sextant, compass and chronometer, and to process this information and turn it into motor programmes, which result in flight in the right direction and for the right length of time. He then discussed the organization of behaviour in the nervous system, the mechanism of control of behaviour, the perception, the learning, the complicated behaviour patterns and instincts, the evolution and spread of patterns in the cortex, and the mechanism and the chemical transmission.

As regards memory, Dr Mukerji said that an external stimulus is converted to electrical impulses in a sense organ. Interposed between this encoding process and the output, or 'read out' lies the associative mechanism. By a selection or instruction process, the brain makes decisions and records, in some as yet unknown form, information for a particular response which becomes, with time, preferred. This information is called memory. It can be compared to the biological information storage seen in immune body formation, enzyme induction and the genetic process. Short-term memory passes into the temporal lobe. But this does not involve any major change in the molecules. Long-term memory, on the other hand, results in the code information being stored in the RNA molecule and might involve a major change in protein synthesis.

Nuclear Magnetic Resonance in Biophysics

Prof. Monisha Bose (SINP) first introduced very briefly the principles of nuclear magnetic resonance (NMR) and then enumerated the parameters important for interpreting a resonance spectrum.

The applications of NMR in biophysics were reviewed, with particular emphasis on the limitations as well as the potentialities of the method for exploring the structures of biologically important molecules and their interactions. The suitability of the method for studying the mechanism of fast reactions in biophysical systems was indicated.

Replication of Double-stranded Nucleic Acids

Dr Sankar Mitra (Bose Institute) presented a review of the studies on *in vitro* replication of double-stranded nucleic acids. In proposing a double-stranded helical structure for DNA, Watson and Crick postulated that, during replication, two complementary strands separate and each then acts as a template for the synthesis of a new strand. Recent autoradiographic and genetic studies have confirmed this hypothesis and have shown that there is a sequential replication of bacterial chromosomes. Two strands of DNA are replicated simultaneously to give an overall semiconservative mode of replication. *In vitro* studies with DNA polymerase purified from bacteria, phage-infected bacteria and animal cells have shown that only the bacterial polymerases can utilize double-stranded DNA as a template for DNA synthesis. Polymerases from other sources can use only the single-stranded DNA as primer. However, even the bacterial polymerase copies only the 3'-strand of the DNA. The enzymatic mechanism by which both 3'- and 5'-strands are replicated simultaneously from the same end is not yet clear. However, the tertiary structure of DNA template may play a critical role for correct replication.

Recently, double-stranded RNA's have been isolated from plant (Wound tumor) and animal (Reo) viruses. Their double-helical structure similar to that of DNA has been established from X-ray diffraction and other physico-chemical data. The mode of replication of such RNA molecules is not known at all. Reo-virus RNA acts as template for RNA synthesis by RNA polymerase and the product appears to be partially double-stranded. However, the *in vivo* significance of such synthesis is not clear. It should be emphasized again that, so far, no biologically active nucleic acid has been synthesized *in vitro* on double-stranded helical templates.

DNA Molecule in Genetic Information Transfer

Dr R. K. Poddar (SINP) presented a paper on the relative importance of the two strands of a DNA molecule in genetic information transfer. The genetic material of bacteriophage ϕ X174 is a small piece of DNA which exists in its cell-free state as a polynucleotide strand. Upon infection of the host cell the single-stranded ϕ X DNA converts itself into the usual double-stranded form, usually referred to as the replicative form (RF), by synthesizing its complementary strand. The parental and the complementary strands of this (RF) DNA were selectively labelled with ^{32}P of the same specific activity *in vivo* and the effects of radioactive decays on the subsequent development of complete phage particles were compared in the two cases. That the single- to double-stranded transformation of the

ϕ X DNA really took place was confirmed by a parallel study of the inactivation of the infected cells by ultraviolet light. It was found that a ^{32}P decay on the parental strand was lethal, while that on the complementary strand was practically without any effect. It has been suggested that only the continuity of the parental strand of (RF) DNA was essential for its capacity to transfer genetic information.

Structure and Function of Mitochondria

Dr A. K. Ghosh (SINP) first dealt with the current concepts of mitochondrial structure. He said that these particles have an outer membrane and an inner one containing a set of infoldings known as the cristae. The geometric arrangement of cristae is believed to be the main factor controlling the swelling and contraction of mitochondria. The mitochondrial membrane contains 30-33 per cent lipid, mainly phospholipids, and 65-70 per cent proteins, 40 per cent of which are the enzymes of mitochondrial function.

The primary functions of mitochondria are the oxidation of metabolites by oxygen and the formation of high energy phosphate ATP molecule. During the oxidation process, electrons from the substrate are transferred to the molecular oxygen through an organized array of carriers, known as the respiratory chain. The agreed components of

the respiratory chain are the flavoproteins, cytochrome *b*, ($c+c_1$) and ($a+a_3$) and the controversial ones are non-haeme iron proteins, coenzyme Q, cuproprotein and vitamin K. The pioneering work of Britton Chance and his school has provided a detailed picture of the composition, sequence and dynamics of the respiratory chain. The work of Green, Okumuki, Singer, Slater, Racker and others has supported Chance's ideas.

In intact mitochondria, the oxidation and phosphorylation are tightly coupled. The phosphate to oxygen ratio for DPN-linked oxidations is 3 and that for succinate is 2. The phosphorylation sites have also been clearly demonstrated by Chance and Williams. Studies on the reaction of intact mitochondria and mitochondria in whole cells have been efficiently made by Chance and this is now a very attractive field of study so far as the respiratory and metabolic control phenomena are concerned.

Dr Ghosh described his work on whole cells of *S. carlsbergensis*. He showed that inositol deficiency produces in the yeast a new effect similar to 'crabtree effect', whereby respiration gets inhibited by glucose or ethanol. This is believed to be due to the limitation of ADP in the cells. A cross-over point has also been established between hexokinase and phospho-fructokinase activity during aerobic utilization of glucose by the inositol-deficient cells.

International Symposium on Protein Foods & Concentrates

An International Symposium on Protein Foods and Concentrates sponsored by the Council of Scientific & Industrial Research, in collaboration with the National Institutes of Health, USA, and the Association of Food Technologists, India, will be held at the Central Food Technological Research Institute, Mysore, during 27 June to 4 July 1967. To be attended by specialists in science and technology of protein foods from all over the world, the symposium will lay emphasis on processing, consumer acceptance and marketing of protein foods in the developing countries. The symposium will have seven technical sessions dealing with

(1) General aspects of protein foods; (2) Economic aspects (resources); (3) Public health and chemical aspects; (4) Amino acid production and supplementation; (5) Toxicology; (6) Protein concentrates and isolates — technology and economics of production; and (7) Consumer acceptability and marketing.

The last date for submitting abstracts of papers (in duplicate) is 31 March and full papers (in duplicate), 1 May 1967. Further details regarding the symposium can be had from Dr B. L. Amla or Dr T. N. R. Rao, Symposium Secretaries, Central Food Technological Research Institute, Mysore 2.

Conductimetric Titrations of Charge Transfer Complexes in Solution: A Review

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Charge Transfer Complexes

CHARGE transfer complexes^{1,28} are intermolecular addition compounds consequent upon the interaction of an electron donor (low ionization potential) with an electron acceptor (high electron affinity). They are often characterized by an intense, broad, new optical absorption band. Their basic theory is due to Mulliken^{1,2}. He showed that charge transfer interactions[†] within a molecular complex consisting of an electron donor D and an electron acceptor A are in the main caused by a resonance involving a transfer of charge from D to A . In the limit, complete charge transfer would occur and an ionic bond, a simple pair interaction, would result. The interaction may not be limited to two molecules but may extend farther. The ground state is thus partly ionic and may be described by a wave function Ψ_{DA} :

$$\Psi_{DA}(D,A) = a\Psi_0(D,A) + b\Psi_1(D^+A^-) \quad \dots(1)$$

where Ψ_0 represents a nonbonding and Ψ_1 a charge transfer wave function, involving the transfer of an electron from D to A . If the interaction is weak, $a \gg b$, $a \rightarrow 1$ and $b \rightarrow 0$. The ground state then is essentially nonbonding. On the other hand, if $b \gg a$, the ground state will be essentially ionic.

For the excited state, we can write a wave function Ψ_E given by

$$\Psi_E(D,A) = a^*\Psi_1(D^+A^-) - b^*\Psi_0(D,A) \quad \dots(2)$$

This is responsible for the characteristic colour of the complex, as well as for the spectral changes accompanying complex formation; these have been widely studied³⁻¹⁶. The charge transfer also, in many cases, gives rise to one or more unpaired electrons, producing an ESR signal¹⁷⁻²⁴.

It is thus seen that for weakly interacting complexes, that is for $a \gg b$, the excited state will be essentially ionic. The ionic character arises from at least a partial transfer of electrons from the donor D to the acceptor A ; partial in this context means that, on the average, the electron will spend more time in the vicinity of A than in the proximity of D .

Thus we have to distinguish between strongly interacting systems in which the ground state already has at least partially ionic character, and weakly interacting complexes in which only the excited state is ionized: (i) weakly interacting complexes, as exemplified by those between polycyclic hydrocarbons

and tetracyanoethylene; (ii) strongly interacting complexes, such as those between polycyclic aromatics and halogens; and (iii) ionic complexes, e.g. those between antimony pentachloride and polycyclic aromatics. Many intermediate interactions are also possible.

In some cases, appreciable charge transfer involving an excited state follows upon irradiation, especially in the ultraviolet²⁵⁻²⁸.

Generally, the weakly interacting complexes of the type (i) do not give an ESR signal, have high resistivities, though often exhibit photoconductivity, and show a distinct, broad, charge transfer band shifted towards longer wavelengths.

A medium to stronger interaction may cause the charge transfer band to shift to shorter wavelengths; the strong complexes of the type (ii) have low resistivity and may, or may not, produce an ESR signal. The ionic complexes of the type (iii) give strong ESR signals and low resistivities. Thus, those charge transfer complexes which are strongly interacting, i.e. those in which the ground state already is ionic, have low resistivities, while the weaker interactions lead to resistivities which are very much higher, though still lower than those of the components.

The existence of charge transfer excited states between donor and acceptor molecules is well established in solutions and also in solids. These states are produced in a one-quantum process. Thus, the anthracene-anthraquinone charge transfer band could be significant in anthracene containing anthraquinone. No charge transfer band between one anthracene molecule and another has been reported. It is calculated to lie at 3 ± 1 eV. and thus to be near the strong singlet $\pi \rightarrow \pi^*$ absorption at 3.1 eV.; the upper states, therefore, would mix²⁹. In donor-acceptor systems that show a charge transfer band, the threshold of the intrinsic photoconduction³⁰ is regularly about 0.2 or 0.3 eV. above the peak of the charge transfer band, possibly because of dissociation of an ion pair. A second and smaller threshold at lower energies (e.g. 0.7 eV.) than the first was attributed possibly to defects.

The primary biochemical event of bacterial photosynthesis probably involves a charge transfer complex. The reaction occurs in less than 20 $\mu\text{sec.}$, and the rate depends but slightly on temperature. An electron transfer involving the heme-linked imidazole group of cytochrome-C is envisaged by Chance and Devault³¹. Calvin and coworkers have also worked on similar problems in photosynthesis³²⁻³⁷.

Conductivity

The conductivity of a given system of non-interacting carriers present in concentrations n_i and carrying z_i electronic charges e , with a mobility μ_i

[†]We shall neglect here 'contact charge transfer reactions' as exemplified by $DA + A \rightleftharpoons DA^+ + A^-$. While contact charge transfer may be important for the weaker complexes, it is likely to play only a minor role in the case of the stronger complexes, particularly those involving an appreciable degree of π -electron overlap.

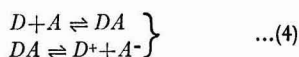
is given by

$$\sigma = e \sum_i n_i \mu_i z_i \quad \dots(3)$$

where the summation has to extend over all the i species of carriers present. Eq. (3) holds for solids as well as for liquids. The charge carriers may be electrons, positive holes (i.e. electron vacancies), or ions of either sign.

Theory

The probability of charge transfer in solution increases with increasing permittivity (ϵ) of the medium³⁹⁻⁴¹. If (ϵ) is sufficiently high, the resulting complex may dissociate into ions giving rise to appreciable ionic conductivity, according to the general scheme³⁸⁻⁴⁰



The formation of charge transfer complexes thus can be followed by measuring changes in the electrical conductivity of a solution of, say, the donor in an inert solvent of sufficiently high permittivity, consequent upon additions of a solution of the acceptor in the same solvent, or vice versa. In effect, this amounts to a conductimetric titration; the donor may be titrated with the acceptor or vice versa⁴¹.

The dielectric solvents concerned are usually of relatively low conductivity, e.g. acetonitrile, acetone, benzene or dimethylsulphoxide (DMSO). While their conductivity appears to be mainly ionic, the charge carriers are probably generated by a mechanism involving the population of localized excited states, and their subsequent migration under the influence of the applied electric field. At a suitable interface, e.g. the electrodes, the exciton then dissociates forming free ions; if these are removed from the liquid, e.g. by means of an ion exchanger⁴², at a faster rate than their generation, one would expect the conductivity to drop sharply, as has been observed⁴².

The applicability of such solid state concepts to organic liquids appears to be amply justified by the proven existence of a great many liquid organic semiconductors⁴³. Forster had demonstrated that *inter alia* benzene is a semiconductor in the liquid as well as in the solid state⁴⁴.

In the absence of any interaction, chemical or otherwise, the addition of a solution of conductivity σ_A to another solution of conductivity σ_D yields a conductivity σ given by Eq. (5).

$$\sigma = \sigma_D + \sigma_A = ze[C_D(\mu_{D^+} + \mu_D) + C_A(\mu_{D^+} + \mu_D)] \quad \dots(5)$$

where C_D and C_A stand for the concentration of the solutes A and D respectively, and the μ 's refer to the mobilities of the carriers contributed by the D and by the A solution. We shall assume that $z = 1$ for all carriers. Concentrations will be used instead of activities, and the conductivity of the solvent, or medium, itself will be neglected.

In a titration, where the volume changes upon addition of titrant, donor and acceptor are supplied to the system in concentrations C_D^0 and C_A^0 respectively, so that

$$\left. \begin{aligned} C_D &= D_0 C_D^0 / (A_0 + D_0) \\ C_A &= A_0 C_A^0 / (A_0 + D_0) \end{aligned} \right\} \quad \dots(6)$$

where D_0 and A_0 refer to the volumes of stock solutions of D and A , with concentrations C_D^0 and C_A^0 respectively, which have been supplied to the system, e.g. from a burette.

In the absence of interaction, $C_D = C_D^0$ and $C_A = C_A^0$. Since the conductivities are additive, it follows that σ , in the absence of interaction, should be linearly related to the concentration of titrant.

Consider now a case where charge transfer does occur, resulting first in the formation of a complex DA followed by its dissociation into ions D^+ and A^- , and thus giving rise to excess conductivity besides any due to the solvent and to the stock solutions themselves. Let the concentration of the complex DA be given by C_{DA} , and the concentrations of D^+ and A^- by C_{D^+} and C_{A^-} respectively. C_D and C_A stand for the concentration of unreacted donor and acceptor, as before.

We then can write the following equations for the equilibrium state of the system.

(a) Complex formation: $D+A \rightleftharpoons DA$

$$\frac{C_{DA}}{C_D C_A} = K_1 = f(\epsilon, T \dots) \quad \dots(7)$$

(b) Complex dissociation: $DA \rightleftharpoons D^++A^-$

$$\frac{C_{D^+} C_{A^-}}{C_{DA}} = K_2 = f'(\epsilon, T \dots) \quad \dots(8)$$

(c) Direct transfer and recombination: $D^++A^- \rightleftharpoons D+A$

$$\frac{C_D C_A}{C_{D^+} C_{A^-}} = K_3 = f''(\epsilon, T \dots) \quad \dots(9)$$

The solution must be electrically neutral, so that

$$C_{D^+} = C_{A^-} = n \quad \dots(10)$$

The conductivity then may be written as

$$\sigma = en(\mu^+ + \mu^-) = \sigma_0 n \quad \dots(11)$$

Thus $C_D \neq C_D^0$ and $C_A \neq C_A^0$ and in the solution there will be present carriers D^+ and A^- in concentrations C_{D^+} and C_{A^-} respectively, undissociated complex molecules DA in concentration C_{DA} , and unreacted donor and acceptor molecules in concentrations C_D and C_A respectively. Thus

$$\left. \begin{aligned} C_D &= C_D^0 - n - C_{DA} \\ C_A &= C_A^0 - n - C_{DA} \end{aligned} \right\} \quad \dots(12)$$

We require n as a function of the donor and acceptor concentrations and of the equilibrium constants K_i .

Multiplying Eqs. (7) and (8)

$$\frac{C_{D^+} C_{A^-}}{C_D C_A} = K_1 K_2 = K_3^{-1} \quad \dots(13)$$

by comparison with Eq. (9). Introducing n from Eq. (10),

$$\left. \begin{aligned} \frac{C_D C_A}{n^2} &= K_3 = (K_2 K_1)^{-1} \\ C_{DA} &= n^2 / K_2 \end{aligned} \right\} \quad \dots(14)$$

so that Eq. (12) becomes

$$\left. \begin{aligned} C_D &= C_D^0 - [n + n^2 / K_2] \\ C_A &= C_A^0 - [n + n^2 / K_2] \end{aligned} \right\} \quad \dots(15)$$

Combining Eqs. (14) and (15),

$$\begin{aligned} [C_D^0 - (n + K_2/n^2)][C_A^0 - (n + n^2/K_2)] \\ = n^2 K_3 = n^2 (K_1 K_2)^{-1} \end{aligned} \quad \dots(16)$$

The conductivity σ , which is proportional to n , thus follows a complicated, fourth power relation to added donor or acceptor. However, it is readily seen that n , and thus σ , is a maximum if $C_D^0 = C_A^0$, as in the case of a 1:1 complex. Equating dn/dC_D^0 to zero yields

$$-C_A^0 + n + n^2/K_2 = 0 \quad \dots(17)$$

Since from Eq. (15) $n + n^2/K_2 = C_D^0 - C_D$, a σ peak will appear if

$$C_D^0 - C_D = C_A^0 \quad \dots(18)$$

so that, combining Eqs. (12) and (14)

$$n + C_{DA} = C_A^0 \quad \dots(19)$$

Again, from Eq. (12)

$$n + C_{DA} = C_A + n + C_{DA} \quad \dots(20)$$

so that

$$C_A = 0; \quad C_D = 0 \quad \text{and} \quad C_D^0 = C_A^0 \quad \dots(21)$$

or in terms of the titrating solutions, given by expression (6),

$$\left. \begin{aligned} D_0 C_D^0 &= A_0 C_A^0 \\ D_0/A_0 &= C_D^0/C_A^0 \end{aligned} \right\} \quad \dots(22)$$

hold for the conductivity peak. The stoichiometry of the complex may thus be deduced from the titration volumes at the endpoint, i.e. the conductivity peak, and from the known concentrations of the stock solutions.

Let the value of C_D^0 at the conductivity peak be denoted by C_D^{00} . Since at this point C_D vanishes, from Eq. (17) we get

$$n^2 + K_2 n - K_2 C_D^{00} = 0 \quad \dots(23)$$

and the peak conductivity σ_{00} thus is, ideally, proportional to

$$K_2 \pm \sqrt{K_2^2 + 4K_2 C_D^{00}}$$

It is thus seen that σ_{00} is entirely determined by the degree of dissociation of the complex*. Since, ideally, at the conductivity peak $C_A + C_D = 0$, it is seen that at that point $K_1 \rightarrow \infty$ and $K_3 \rightarrow 0$. Thus, a well-developed, sharp conductivity peak near the donor-acceptor ratio of (1:1) indicates such a nearly ideal behaviour, with no direct recombination and with the dissociation of the complex as the rate-determining step. However, we emphasize that this applies to an idealized case. It is unlikely that the reaction would go to completion in any real system, i.e. C_A would vanish, at least within any reasonable time. Thus, in a real system, K_1 would not become infinite. It will be seen from the experimental results that the conductivity

*The quantity

$$n = \frac{K_2}{2} \left[\sqrt{1 + C_D^0/K_2} - 1 \right]$$

is always positive. For large values of K_2 , i.e. for nearly complete dissociation, expansion of the square root term yields, as to be expected, $n \cong C_D^0$.

peaks always occur slightly off the stoichiometric, say 1:1, ratio, thus indicating non-ideal behaviour. This is further supported by the time plots discussed below.

If direct recombination can be neglected, i.e. if $K_3 \rightarrow 0$, Eq. (16) reduces to

$$C_D^0 = n + n^2/K_2 \quad \dots(24)$$

for the case of added donor. Then, a linear relation between n , hence conductivity, and the concentration of titrant added should hold in the initial stages of the titration where n is relatively small, provided that K_2 is large, i.e. dissociation is nearly complete. Such a linear relation indicates negligible recombination as well as complete dissociation.

The analysis in this section assumes both donor and acceptor to be univalent, resulting in a 1:1 complex. It is immediately apparent that the same treatment also applies to the case of a bivalent donor. The endpoint of the titration as indicated by the conductivity peak will then occur at a 2:1 stoichiometric ratio instead of at a 1:1 ratio.

Conversely, therefore, the stoichiometry of the complex may be deduced from the titration volumes at the endpoint, viz. the conductivity peak, and from the known concentrations of the stock solutions. The value of the conductivity peak above a baseline connecting the conductivities of pure donor and acceptor solutions is a measure of the excess conductivity caused by the formation and subsequent dissociation of the complex.

We define a molar conductivity coefficient σ_M

$$\sigma_M \equiv \frac{1}{\alpha M} \frac{\sigma_P - \sigma_0}{\sigma_0} \quad \dots(25)$$

where M is the molar concentration of the titrant, either donor or acceptor, at the conductivity peak where $\sigma = \sigma_P$. σ_0 is the linearly interpolated conductivity background, read off a baseline joining the conductivities of pure donor and pure acceptor solutions as shown in Fig. 1. The complex may not be fully dissociated; the dissociation constant has to be allowed for, since the carrier concentration is proportional not to the concentration M of the reagents, but rather to αM , where α is the dissociation constant of the complex.

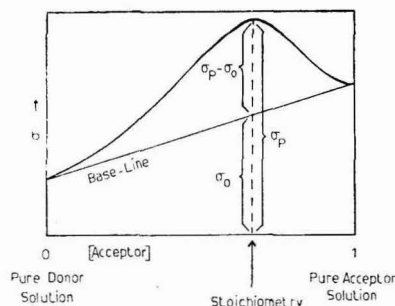


Fig. 1 — Idealized conductivity titration curve superimposed upon a baseline [The latter results in the absence of an interaction]. This figure illustrates the molar conductivity coefficient defined in Eq. (25)

Experimental Techniques

Conductivity titrations were carried out using spectral grade solvents and analytical grade donors and acceptors. The solution was stirred for several minutes after each addition and a time interval allowed for the bridge reading to become substantially constant. The solutions were thermostated but not outgassed. The bridges used were Philips type GM4249/01 and Wayne-Kerr type B-221.

Design of a Conductivity Cell

The design of the conductivity cell employed is illustrated in Fig. 2. This permits titrations to be carried out in a protective atmosphere. The cell is temperature controlled by spraying hot or cold air around it. Provision has been made for the study of conductivity changes consequent upon ultraviolet irradiation of the solution²⁵⁻²⁸. Separate current and voltage electrodes are provided allowing measurements with direct current as well as studies of the effect of superimposing direct voltages while measuring the conductivity with an alternating voltage.

The design may be considerably simplified by omitting these special features. Temperature control may be attained by means of a jacket surrounding it, through which hot or cold water is pumped. It is advisable for the stirrer to be turned off while actually taking a reading.

Choice of Electrodes

The complex formation or its dissociation appears to be susceptible to catalytic activation at the electrode surface. Results with platinized platinum

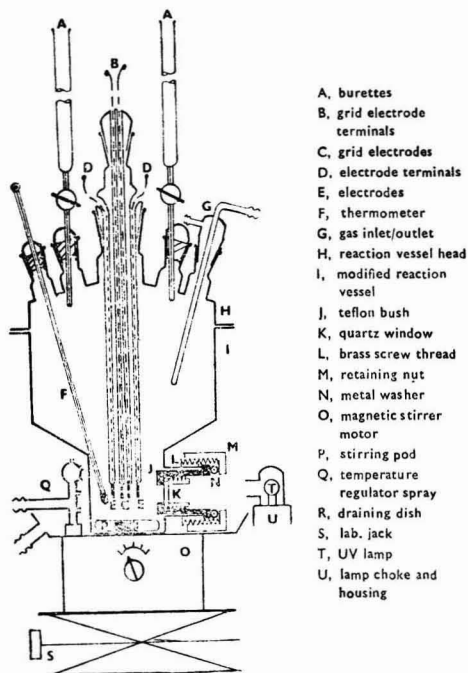


Fig. 2 — Design for a conductivity cell

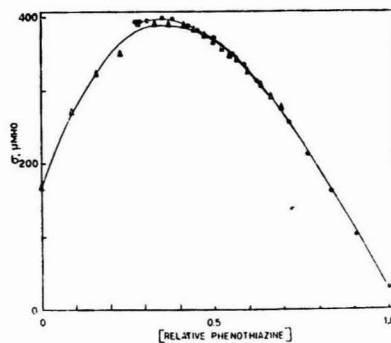


Fig. 3 — Conductivity titration of the phenothiazine : iodine system in acetonitrile [(Δ) refers to the addition of phenothiazine solution to an iodine solution, and (○) to the converse titration]

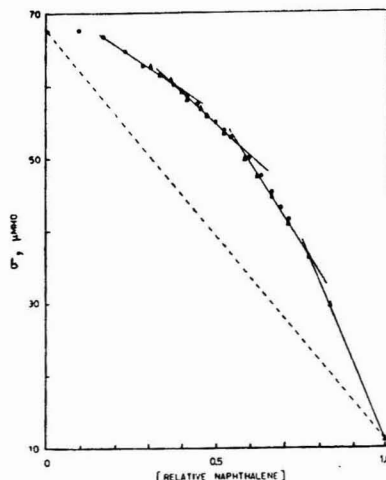


Fig. 4 — Conductivity titration of the naphthalene : iodine complex formed in acetonitrile [Points are marked for the addition of iodine solution and its converse as in Fig. 3]. Mismatch of the conductivities of the donor and acceptor solutions results in a bowed line rather than in a peak

electrodes in solutions of low permittivity are less reproducible than those in media of high permittivity and using catalytically less active electrodes, viz. gold or bright platinum. This may also be one cause of the frequently observed lack of coincidence of the titration curves when titrating a donor with an acceptor and vice versa, though the conductivity peaks remain unaffected and appear at closely identical concentrations. Space charges¹³ due to either blocking, injecting, or catalytically active electrodes result in excessively long relaxation times and time constants, in drifts and poor reproducibility, and may also cause failure of a titration curve and its converse to coincide.

Conductimetric Titration Curves

A well-developed conductivity maximum, such as that resulting from the phenothiazine-iodine in

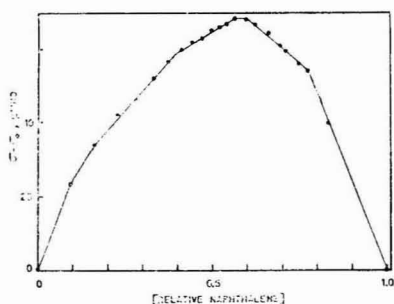


Fig. 5A — Replot of the data given in Fig. 4 [Ordinates in this case are relative to the baseline]

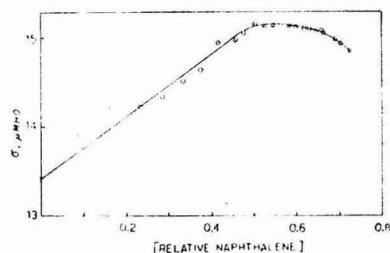


Fig. 5B — Repeat of the titration in Fig. 4 using better matched solutions [Conductivity peak is evident]

acetonitrile system, illustrated in Fig. 3, is not always obtained. In particular, a steeply sloping baseline caused by serious mismatch of the initial conductivities of the pure donor and acceptor solutions tends to yield ill-defined, or completely obscured, conductivity peaks. This is illustrated in Fig. 4 which refers to the iodine-naphthalene complex formed in acetonitrile. The decidedly nonlinear plot indicates complexing. The stoichiometry of this complex can be determined by correcting for the background conductivity obtained by linear interpolation, i.e. by plotting $\sigma - \sigma_0$ versus relative concentration. This is shown in Fig. 5A. A peak becomes evident at a stoichiometric ratio of 0.57, indicating a 1:1 complex.

Mismatch usually occurs when the initial conductivities differ by more than about 50 per cent. Thus, if the solution of iodine in acetonitrile, which is relatively well conducting, is diluted to match the donor solution to within about 30 per cent, a well-defined peak corresponding to a 1:1 complex is obtained as shown in Fig. 5B, in agreement with the result of the background correction (Fig. 5A).

Solvent Interaction Effects

Correct choice of solvent is an important factor. If its conductivity is too high, conductance changes due to complex formation may become obscured. If its permittivity is too low, the complex may fail to dissociate. If the solubility of the complex is low, the complex might precipitate, which may even lead to a reduction of the number of charge carriers

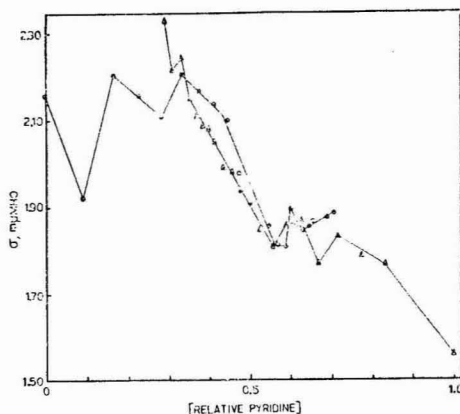


Fig. 6 — Conductivity titration of the pyridine:iodine system in carbon tetrachloride [(Δ) refers to the addition of pyridine solution, and (\circ) to the converse titration]. Permittivity of CCl_4 is too low and the complex fails to dissociate; also, CCl_4 tends to act as an acceptor

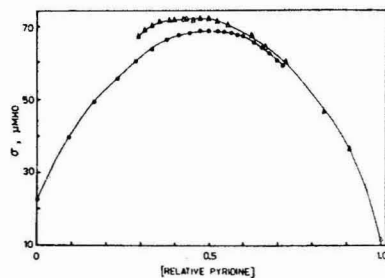


Fig. 7 — Repeat of the titration in Fig. 6 using acetonitrile as the medium [Peak previously obscured becomes clearly evident]

present in solution and cause the appearance of a conductivity minimum in the titration. The minimum exhibited in the titration of pyridine and iodine in carbon tetrachloride at a 1:1 stoichiometry may be cited as an example (Fig. 6). Pyridine and iodine are known⁴⁵ to form 1:1 complex in this solvent. When the pyridine/iodine titration is carried out in acetonitrile, a well-developed curve with a maximum indicating 1:1 complexing is obtained (Fig. 7). If the solvent has decided donor or acceptor character, then it may compete with the reagents and actively enter into the reaction. Thus, CCl_4 is known⁴⁶ to act as an electron acceptor.

Quite significant permittivity changes occur during the conductivity titrations; in fact, the conductivity peak nearly always coincides with either a minimum or maximum in the measured capacitance of the system. This matter is at present being further investigated.

Results

In the absence of complex formation and other interactions, Eq. (5) predicts a linear relation between the concentration of, say, donor added to an acceptor

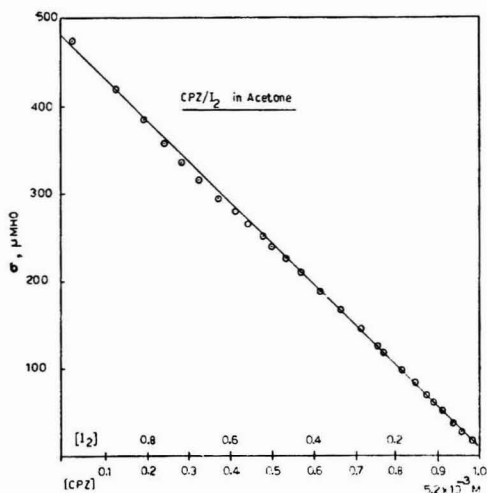


Fig. 8 — Conductivity titration of the system chlorpromazine: iodine in acetone [There is no apparent interaction and the titration curve follows the straight line as required by Eq. (5)]

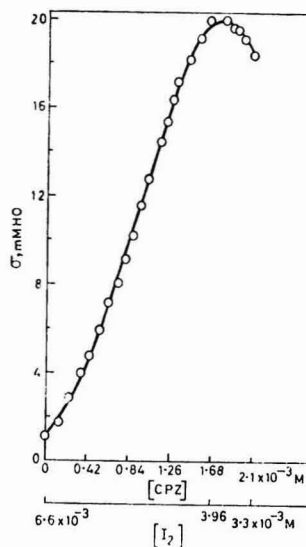


Fig. 9 — Repeat of the titration in Fig. 8 using acetonitrile as the medium [Conductivity peak becomes apparent]

solution, both in the same solvent. This is illustrated in Fig. 8 for addition of the acceptor iodine to the donor chlorpromazine (CPZ), both in acetone. Thus, no significant charge transfer complex formation takes place between CPZ and iodine in acetone which has a permittivity of 20.7.

The results of conductivity titrations of a number of charge transfer complexes, using different types of electrodes, are presented in Figs. 9-14.

Conductivity titration of the strong charge transfer complex formed between the donor CPZ and the acceptor iodine, both in acetonitrile ($\epsilon = 36$) shown in Fig. 9, indicates that a sharp peak results at a CPZ concentration of $1.68 \times 10^{-3} M$ and an I_2 concentration of $3.96 \times 10^{-3} M$. Thus, each CPZ molecule donates two electrons, yielding a 1:2 complex. This stoichiometry would be hard to explain in terms of a purely chemical reaction. It is of interest to note that CPZ is known to be capable of forming two free radicals, one by donating one electron, and the other by donating a second electron. Some peculiarities of the solid state electrical properties of CPZ have been explained in terms of these two free radicals⁴⁷. The complex observed in acetonitrile thus could well involve the doubly charged free radical.

Alternatively, it could be argued that one iodine molecule is sited atop each of the two benzene rings of the CPZ molecule.

The results of a conductivity titration of the phthalocyanine/ I_2 complex formed in DMSO are shown in Fig. 10. The two curves refer to two repeats with different electrode systems. A conductivity peak at a concentration of $4 \times 10^{-4} M$ of phthalocyanine (metal-free) and $7.6 \times 10^{-4} M$ of I_2 is indicated. Thus, again, each phthalocyanine molecule donates two electrons, requiring two iodine molecules as acceptors. The peak is somewhat obscured by the relatively high conductivity of the I_2 in DMSO solution.

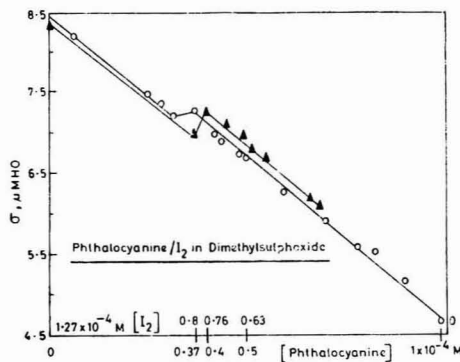


Fig. 10 — Conductivity titration of the system phthalocyanine: iodine, both in dimethylsulphoxide using Pt electrodes

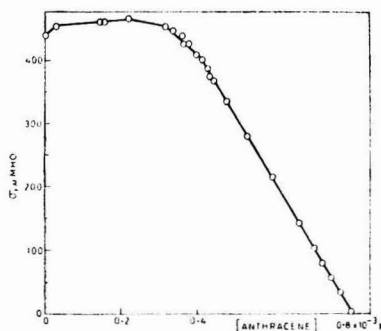


Fig. 11 — Anthracene: iodine complex, formed in acetonitrile

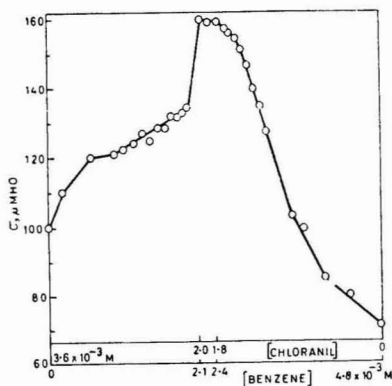


Fig. 12 — Benzene-chloranil complex, formed in methanol

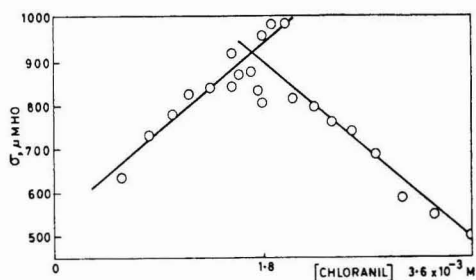


Fig. 13 — Anthracene: chloranil complex, formed in methanol using bright Pt electrodes

A conductivity titration plot of the donor anthracene with I_2 , both in acetonitrile, is given in Fig. 11. A rather broad maximum is observed, probably due to a solvent interaction; the peak obtained with the same acceptor, and the same medium but with CPZ as donor (Fig. 9), is quite sharp. The anthracene complex is seen to occur at a 1:3 ratio, each anthracene molecule thus donating three electrons. This would tend to support the alternative hypothesis presented above for the CPZ, though the anthracene complex is relatively weak, while CPZ forms a strong complex with I_2 .

The charge transfer complex between benzene and chloranil, in methanol, is studied in the plot shown in Fig. 12. Here, the peak occurs at a benzene concentration of about $2.1 \times 10^{-3} M$ and a chloranil concentration of about $2 \times 10^{-3} M$, indicating a 1:1 complex. The non-ideal shape of the titration curve is again probably due to solvent interaction, since methanol is known to be capable of acting as an electron donor and the benzene complex is weak⁴⁸.

A similar effect is thought to occur in the case of the anthracene-chloranil complex formed in methanol and illustrated in Figs. 13-15. The peak is seen to occur at a 1:1 ratio of donor and acceptor, both at a concentration of $1.8 \times 10^{-3} M$. Fig. 14 refers to the same system though with different electrodes. The two branches shown represent the results of

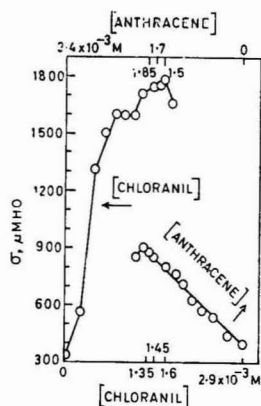


Fig. 14 — Repeat of the titration displayed in Fig. 13, using Au electrodes [The arrows identify the titrants; the endpoint is seen to result from titrating anthracene with chloranil or vice versa]

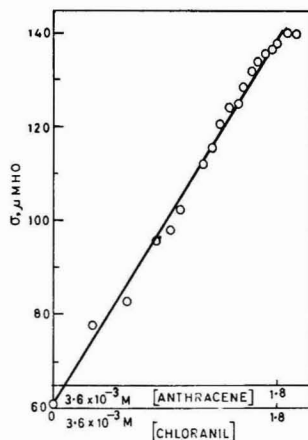


Fig. 15 — Same anthracene-chloranil complex titration, using bright Pt electrodes

two titrations, one adding the donor anthracene to a chloranil solution and the other, upper, curve relating to the addition of the acceptor chloranil to an anthracene solution. In both cases, the peak is at very nearly identical concentrations. There is some evidence of a subsidiary peak in the latter titration.

The very much higher conductance values indicated for this experiment, as compared to the conductances obtained upon adding anthracene, again indicate solvent competition, the donor methanol complexing with the acceptor chloranil.

Fig. 15 is a repeat of the same study, with a different electrode system. Again, the conductivity peaks at a 1:1 stoichiometry.

The linearity predicted by Eq. (24) for the beginning of the titration curves is seen to hold in many cases, though there is usually a degree of

TABLE 1 — STOICHIOMETRIES AND σ_M VALUES FOR A NUMBER OF COMPLEXES

Donor	Acceptor	Solvent	Apparent stoichiometry	σ_M	Reference
Anthracene	Chloranil	Methanol	1:1	1200	43
do	I ₂	Acetonitrile	1:3	2200	43, 69, 70
Benzene	Chloranil	CCl ₄	1:1	150	43
do	do	Methanol	1:1	390	43
do	I ₂	Acetonitrile	1:2	480	43, 71
Naphthalene	I ₂	do	1:1	240	43
Phenothiazine	Chloranil	do	1:1	280	—
do	I ₂	do	1:2	2100	—
Phthalocyanine (metal-free)	I ₂	Dimethylsulphoxide	1:2	870	—
Tetracene	I ₂	Acetonitrile	1:3	1540	43
Pyridine	I ₂	do	1:1	350	—

nonlinearity evident for the first one or two ml. of titrant.

Since this relation was derived on the assumption that direct recombination is negligible, and dissociation nearly complete, it appears that, generally, this is the case at least to a first approximation.

Values of σ_M for a number of complexes are listed in Table 1, together with the stoichiometries as determined from the position of σ_p . The dissociation constant α has been assumed to be unity.

Iodine is a well-known³ σ -acceptor, i.e. the interaction involves σ -electron orbitals. If the donor has lone pair electrons, as in the case of chlorpromazine or phenothiazine, which readily form free radicals, then the interaction is expected to involve an excited state⁴⁵. In addition a π -interaction should also be expected⁴⁶ in the case of very strong electron donors possessing extensive π -electron systems. In fact, stoichiometries different from unity have been observed, together with evidence for the formation of several complexes having different stoichiometries⁴⁹. It then may become difficult to resolve these complexes conductimetrically; the peak in such cases indicates a statistical average.

Thus, in the case of tetracene, there may be contributions also from 1:4; 1:2 and 1:1 complexes which thus far have not been resolved; their statistical average then yields a 1:3 stoichiometry from the conductivity plot.

Discussion

On the present model, the conductivity is caused by the free ions produced by the dissociation of the complex ion pairs; a mechanism closely similar to that proposed by Bässler and Riehl⁵⁰ in order to account for the conductivity of pure organic liquids. One would then expect that these ions be solvated⁵¹, causing a relatively low ionic mobility and thus a lowering of the conductivity peak in strongly solvating media.

Solvation effects^{52,53} are of special importance in solvents like DMSO, in which the cations are nearly always solvated⁵⁴.

Szwarc⁵⁵ has shown that dissociation in organic, complexing solvents may be followed by association

leading to the formation of dimers and even higher order entities. The overall reaction may be quite complex.

Multiple ions may be formed upon increasing dilution going hand in hand with a relatively large change in the effective permittivity of the resulting medium and causing a drop in conductivity upon dilution. An increase in the value of the association constant with decreasing permittivity (ϵ) is well known⁵⁶ and quantitatively expressed by the Denison and Ramsey⁵⁷ relation or by an equation developed by Fuoss and Kraus⁵⁸; in addition, one would expect that the lowering of (ϵ) would tend to repress the dissociation of the complex.

Janz and Danyluk⁵⁹ proposed the following empirical equation for the equivalent conductivity, Λ of "systems in which the interactions give rise to solute-solvent species of molecular nature (*cf.* compounds) that ultimately contribute to the conductivity through additional ionic entities":

$$\Lambda = A + BM^{-1} \quad \dots(26)$$

here A and B are constants and M the molar concentration of the solute.

One would expect conductivity theory^{60,61} to apply to the complex solutions at any one given complexing ratio. Fig. 16 shows plots of the equivalent conductivity of the 1:1 complex between anthracene and chloranil in methanol and in DMSO against (complex concentration)¹ upon dilution with an inert solvent, CCl₄. Substantially straight lines, though of reverse slopes, result, i.e. the equivalent conductivity drops with increasing dilution. Deviations from the straight lines are more pronounced in the high permittivity solvent DMSO where the equivalent conductivity is less than expected. One might be tempted to ascribe this to a competition between the solvent, DMSO, and the diluent, CCl₄, in that the latter enters into a non-dissociating side reaction. In fact, CCl₄ is capable⁶² of forming weak charge transfer complexes with aromatic hydrocarbons, though the actual transfer of the electron in such weak charge transfer complexes has been questioned⁶³. However, initial experiments showed that no measurable complex formation took place in CCl₄; the conductivity remained always only just within the range of the bridge. Therefore, since the diluent

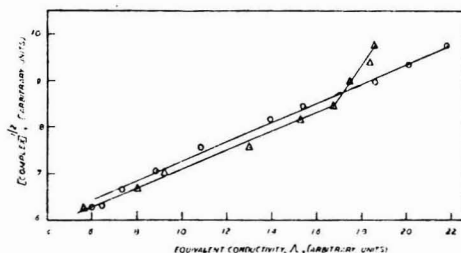


Fig. 16 — Dilution curve for the 1:1 anthracene:chloranil complex, formed in methanol (O) and in dimethylsulphoxide (Δ) [The diluent was carbon tetrachloride. After addition of donor to acceptor, 24 hr were allowed to elapse for methanol as the medium, and 28 hr in the case of DMSO, before commencing the dilution experiment. Au electrodes]

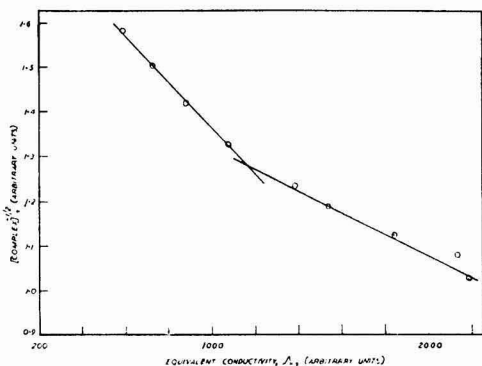


Fig. 17 — Janz-Danyluk⁵⁹ plot from Eq. (26) for the anthracene:chloranil complex, formed in methanol and diluted with carbon tetrachloride [Au electrodes. The data are the same as those used in Fig. 16]

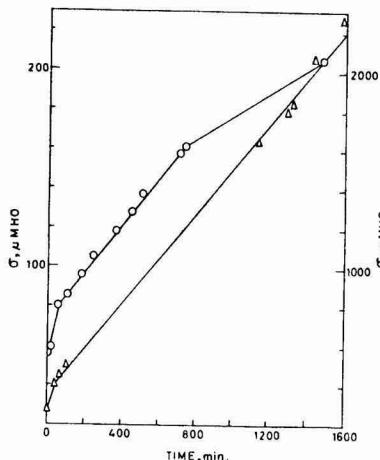


Fig. 18 — Time versus conductance for the anthracene:chloranil complex [(Δ) refers to methanol as the medium and (O) to DMSO as solvent. The time scales commence at the time of combination of the reactants. A 1:1 stoichiometry was employed. Au electrodes]

CCl_4 is inert, it appears that part of the conductivity is associated with a complex formation involving methanol, which itself is known to be an electron donor.

The reverse slope may again be caused by a solvent interaction, i.e. by an association of the complex ions with the solvent, leading to the formation of multiple ions, e.g. triple ions. A reverse slope has also been reported by Janz and Danyluk⁵⁹: the equivalent conductivity of HBr solutions in acetonitrile showed a minimum in its concentration dependence, exhibiting a region where further dilution caused a drop in the equivalent conductivity.

Breaks in the straight line representing Eq. (26) were thought likely to be associated with unilateral triple-ion formation. We have tested our hypothesis by the Janz-Danyluk plot shown in Fig. 17 for the

anthracene-chloranil complex in methanol, following dilution with CCl_4 . It is seen that Eq. (26) is indeed obeyed and that, as in Janz and Danyluk's original cases, a sharp break occurs.

The conductivity of the 1:1 complex rises linearly, though slowly, with time as seen from Fig. 18. This indicates that the reaction involves a high degree of adsorption at the electrodes and is catalytically activated, and of zeroth order in the initial stages. The reaction, in that respect, appears to be largely independent of the nature of the medium in which the complex is formed.

The coefficient $1/\sigma_0 d\sigma/dt$ appears to be independent of the nature of the solvent, though more work on this is needed.

It is considered unlikely, though not impossible, that the complex dissociation is the slow process; the dissociation should be a bulk rather than a surface effect, while the complex formation may well involve a surface reaction.

The presence of an ion pair should give rise to an ESR signal^{61,64-68}; thus the formation of the phenothiazine-iodine charge transfer complex in acetonitrile was found to increase the spin concentration from a value of about 10^{14} cm^{-3} for the donor to about 10^{18} cm^{-3} for the complex. A very large σ_M value has been obtained for this titration (Table 1). Similarly, the formation of a charge transfer complex between chlorpromazine and iodine in acetonitrile causes the appearance of an ESR signal associated with a spin concentration of about 10^{17} cm^{-3} , while neither donor nor acceptor solutions separately give a signal, unless irradiated⁶². Neither an ESR signal nor a conductivity peak could be obtained for pyrene and iodine in acetonitrile. The same holds for the pyridine-iodine complex in carbon tetrachloride as the solvent. In acetonitrile a very faint signal indicating a spin concentration of 10^{13} cm^{-3} was observed and a small σ_M was obtained.

Summary

The formation of charge transfer complexes can be followed by conductimetric titration. Either a donor solution is titrated with a solution of an acceptor, or vice versa, both using the same solvent. A theory is presented requiring the conductivity to pass through a maximum when donor and acceptor are present in the solution in the stoichiometry required for the formation of a charge transfer complex. Conductimetric titrations yielding conductivity peaks in agreement with the theory are reported for a number of complexes involving different donors, acceptors as well as solvents. Experimental techniques and solvent interaction effects are discussed. The value of the conductivity peak is expressed quantitatively in terms of a molar conductivity coefficient

$$\sigma_M = \frac{1}{\alpha M} \frac{\sigma_P - \sigma_0}{\sigma_0}$$

where σ_P is the measured peak conductance, and σ_0 the linearly interpolated background conductance at the concentration ratio of the peak, i.e. that conductance which would be obtained in the absence of any interaction, from merely mixing the donor and acceptor solutions. M stands for the molar

concentration of the titrant at the stoichiometry of the complex, viz. at the conductance peak, and α is the dissociation constant of the complex. Some ESR results are also presented, and discussed in relation to the σ_M values. Dilution of the 1:1 anthracene-chloranil complex formed in dimethylsulphoxide with the inert solvent carbon tetrachloride yields linear plots between equivalent conductivity and (complex concentration)^{1/2}, but of reverse slope; the equivalent conductivity drops with increasing dilution. The Janz-Danyluk equation is accurately obeyed, though with a sharp break; thus a solvent interaction involving ionic association is suspected, at least in dimethylsulphoxide.

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Natural Asymmetry, Isomerism & Pharmacological Action

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ANY discussion of optical isomerism brings to mind the general problem of asymmetry in nature. An estimate of 8.6 per cent left-handedness among the adult male population of the United States has been made by Karpinos and Grossman¹, and the newer schools are providing about 10 per cent left-handed writing chairs to accommodate this number of left-handed students. The percentage of potentially left-handed people may be even higher if one includes those ambidextrous individuals who have been trained to write with their right hand. In monkeys and chimpanzees, where hand preference is present, this preference appears to be equally divided between the left and right hands². Some clinicians believe that the whorl of scalp hair growth may correspond to the handedness of the individual: whorl counter-clockwise in left-handed and clockwise in right-handed individuals^{3,4}. Left-handedness in mental patients has been estimated to be as high as 30 per cent. But handedness is only an interesting sidelight on asymmetry in nature.

Asymmetry in Plants

Right- and left-handed spiral formations are common in nature and art. In nature, the right-handed spiral appears to be more prevalent. The right-handed screw is duplicated in nature by a majority of the twining vines. Twining plants wind round in a definite direction according to the species of the plant. The hop and honeysuckle, for instance, invariably grow upwards in left-hand twists; the majority follow the example of the *Convolvulus* (morning glory, bind weed) and exhibit right-hand twists⁵. Tendrils, on the other hand, often exhibit a spiral in one direction, then a portion of straight growth, followed by a spiral in the opposite direction. This order may be repeated several times⁶. Trees frequently exhibit spiralling of the trunk. Wentworth⁷ found that out of 400 coniferous trees with noticeable twist, 384 had right-hand twisting, 13 had very slight left-hand twists and 3 showed very strong left-hand twisting. Fir cones, on the other hand, are diadromic⁸, i.e. they have spirals running both left and right. Even at the cellular level, asymmetries exist in that the spiral twist appears to be universally characteristic of plant chromosomes, with the right-hand twist more common⁹.

Asymmetry in Animals

The tendency to spiral formation is also present in man and animals. Of more than 60,000 species of snails, most shells exhibit right-handed spiralling, only a few are left-handed. Fiddler crabs and whelks are in general right-handed, the left-handed whelk being stunted and feeble compared with the right-handed whelk. According to Thompson⁸, left-handed

whelk used to be the most common form, but they now occur as a rare abnormality.

The spiralling of horns of sheep and goats is usually right-handed for the right horn and left-handed for the left horn, i.e. homonymous spiralling. In contrast is the heteronymous condition in which the right horn spirals left and the left horn spirals right. The latter type of spiralling is rare and has been found among wild goats in Kurdistan¹⁰. The narwhal has but a single tusk which is always a left-handed spiral⁵. The unicorn's tusk, patterned after that of the narwhal, is also left-handed and the unicorn occupies a position on the left side of the mythical throne¹¹.

Spirality is also observed in the growth of wool fibres, but the direction of rotation varies in the different staples, the number one way or the other being about equal, showing it to be a matter of indifference¹². The organs of man, viz. heart, intestine, muscle fibres, gall bladder, all show a predominant tendency toward dextral spiralling¹³. In contrast to this is the spiral of the umbilical cord which is always left-handed⁵.

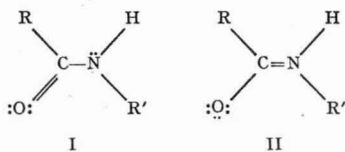
Geophysical Asymmetry

Gravitational forces are adequate explanations for the shift in the twist of waterspouts and tornadoes in the northern versus the southern hemispheres and also for the change in the direction of the whorl of water down the bath-tub drain in Sidney, Australia, or San Francisco, California. Tornadoes circle round the centre in a counter-clockwise direction in the northern hemisphere, therefore, a left-handed whorl, and the reverse is true in the southern hemisphere¹¹. The fact that trees of the northern hemisphere have a preponderance of right-hand spiralling may be a result of gravitational forces, or the direction of the prevailing winds. Gravitational forces are probably not the prime factor in optical isomerism since the plants of the southern hemisphere have not been shown to have a preponderance of D-alkaloids when compared to the indigenous plants which produce L-alkaloids in the northern hemisphere, nor has the suggestion that the Australian aborigine has D-amino acids in his body proteins been substantiated. So the mystery of the natural origin of L-amino acids and D-sugars still exists.

The heliotropic effect of the sun cannot be a factor since this again would be influenced by the distance above or below the equator. At least the effect of heliotropism and gravitational forces has not been adequately investigated to provide positive data or exclude them as possible trophic factors.

Possible Origin of Molecular Asymmetry

Pauling¹⁴ suggested that the simple amides which compose proteins are naturally skewed by resonance



factors. Thus, in the simple amide, $\text{O}=\text{C}$ form (I) resonates with $\text{C}=\text{N}$ form (II), and from quantum mechanics the ratio of (I) to (II) is 60 to 40 per cent respectively. A 40 per cent contribution of the structure (II) would contribute planarity to the molecule and corresponding asymmetry. Fox *et al.*¹⁵ suggest that in the oldest forms of life, such as that of algae, a predominance of D-amino acid oxidase occurs and that some of the microorganisms that are relatively low in the phylogenetic scale are rich in D-amino acids, as evidenced by the D-amino acid content of many antibiotics such as tyrothricin. The occurrence of D-amino acid oxidase in the blood of mammals may be a vestigial or protective phenomenon designed to eliminate the occasional D-amino acid. The authors suggest that ammonium hydrogen D-malate might have spontaneously crystallized from a DL solution leaving a primitive, natural nutrient rich in the L forms of aspartic acid and alanine. Thereafter the natural Darwinian selection would take over and enzymes with the L configuration would have a selective advantage.

Asymmetry at the Molecular Level

Receptor Specificity

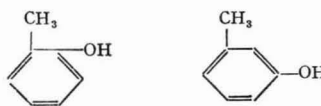
Several years ago Pfeiffer *et al.*¹⁶ generalized on the geometric conformation of D and L drugs and came to the conclusion that the isomeric ratio of drugs was a measure of their geometrical conformation to receptors¹⁶. At that time data on 14 common drugs were collated into a straight line semi-logarithmic graph. The asymptote to the Y axis was unexplored because the L-isomer of lysergic acid diethylamide had not been tested at high doses. Since then, Pfeiffer *et al.*¹⁶ have found that 10,000 μg . (10 mg.) of L-LSD₂₅ is without hallucinatory activity in normal human subjects. If one takes 25 μg . of D-LSD₂₅ as the recognizable dose, then the isomeric ratio for this drug is more than 400. LSD₂₅ by this criterion has a high degree of specificity for receptors in the central nervous system.

Transport across Membranal Barriers

Wilbrandt (personal communication, 1955) has found that L-arabinose and D-xylose pass the membrane of the red blood cell while their antipodes do not. Pletscher¹⁷ claims that D-amino acids do not pass the blood-brain barrier while L-amino acids do. This observation has been corroborated by Udenfriend¹⁸.

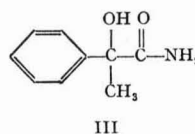
Types of Asymmetry

Simple structural isomers — For example, two of the many possible isomers of the simple empirical formula $\text{C}_7\text{H}_8\text{O}$ are *ortho*- and *meta*-cresol:



Optical isomerism — The dissimilar crystals of tartaric acid as described by Pasteur¹⁹ in 1845 and chemically identified by Van't Hoff²⁰ in 1874 offer an excellent example of this type of isomerism. The physical characteristics of various forms of tartaric acid are given in Table 1.

DL salt formation raises the melting point and decreases water solubility in that the racemate acts as a compound of higher molecular weight — probably due to hydrogen bonding. Toxicity data on the various isomers of tartaric acid are controversial, but a similar compound, atrolactamide (III), has been adequately studied by Pfeiffer *et al.*²¹ in 1954.



The optically active isomers of atrolactamide (mol. wt 144) were prepared essentially by the method of McKenzie and Smith²². Atrolactic acid was resolved by means of D- and L-isomethadone salts. The D and L acids were then esterified and reacted with ammonia to give D- and L-atrolactamide (Table 2).

The melting point and solubility data indicate that the racemate is a DL compound. Examination of the infrared spectra of the enantiomorphs and the racemate in chloroform solution show identical

TABLE 1 — PHYSICAL CHARACTERISTICS OF VARIOUS OPTICAL ISOMERS OF TARTARIC ACID

	Tartaric acid			
	L	D	Meso	DL
m.p., °C.	170	170	140	206
Solubility in water g./100 ml.	139 (20°)	139 (20°)	125 (15°)	20.6 (20°)
Optical rotation	-12°	+12°	Inactive	Inactive

TABLE 2 — PHYSICAL CHARACTERISTICS AND PHYSIOLOGICAL ACTIVITY OF OPTICAL ISOMERS OF ATROLACTAMIDE

	Atrolactamide (III)		
	DL	D	L
Optical rotation	Inactive	+13.5°	-13.1°
m.p., °C.	101-102	62	62
Solubility in water at 25°C., g./100 ml.	6	50	50
LD ₅₀ (oral, mouse) g./kg.	1.861 ± .07*	1.394 ± .041	2.328 ± .056
Electroshock protection at 250 mg./kg.	4+	4+	4+

*Toxicity of the DL form is the mean of the D and the L.

TABLE 3 — DATA ON THE EFFICACY OF OPTICAL ANTIPODES OF NIRVANOL AND MESANTOIN

	Toxic dose-50 mg./kg.	ED ₅₀ (mg./kg.) to prevent convulsion due to	
		Electro-shock	Metrazol
DL-Nirvanol	119	10	62
D-Nirvanol	312	32	228
L-Nirvanol	156 } 234	8.5 } 20	145 } 186
DL-Mesantoin	143	10	105
D-Mesantoin	172 } 128	10.2 } 12	167 } 129
L-Mesantoin	83	14.3	91

spectra, but the spectrum of the mull of the racemate is significantly different from that of D- or L-atrolactamide. Pharmacologically, the three have equal anticonvulsant action but the D-isomer is significantly more toxic while the L form is significantly less toxic than the racemate. Both the D and L forms were found to be equally effective as internuncial neuronal depressants.

Swinyard *et al.*²³ have presented some interesting data on the efficacy of the optical antipodes of nirvanol and mesantoin in mice administered orally (Table 3).

The mean of the doses of the two antipodes should theoretically approximate the dose for the racemate. This holds for the LD₅₀'s of atrolactamide and for Swinyard's data on mesantoin. With nirvanol, however, the DL compound apparently acts in the body like a compound of greater molecular weight and biological activity. This leads to an interesting question: Could the hydrogen bonding persist in biological fluids?

The peculiar contrast in toxic effects of optically isomeric drugs cannot be adequately demonstrated by the intravenous route of administration — probably because of non-specific intoxication by the high concentration which floods the vital centres.

Theoretically, the LD₅₀ of the DL salts should be the mean of the D and the L or approximately 199 μmole/kg. The fact that DL-lactoylcholine iodide is a compound with physical characteristics (melting point, infrared absorption in solid state) different from those of the D(—)- and L(+)-enantiomers was already reported²⁴. An equimolar physical mixture of the D(—) and L(+)-isomers will be transformed into an entity possessing the physical properties of DL-lactoylcholine upon heating above 90°C. The fact that the physical mixture of the enantiomers (50:50) was more toxic than the mean of 199 probably indicates slow DL salt formation beginning at room temperature. The comparatively low isomeric ratio (1:3) of the optical isomers in these experiments may be due to the rapid exposure of the organism to large quantities of compounds by intravenous injection. The esters may have acted primarily upon less specific vital centres instead of affecting the more specific ones which are selective in regard to the configuration of a given isomer¹⁶.

Yet another example of DL salt formation which may be sufficiently stable to act as a double molecule

TABLE 4 — ACUTE TOXICITY OF LACTOYLCHOLINES AND RELATED COMPOUNDS IN MICE (INTRAVENOUS)

Compound	m.p. °C.	LD ₅₀ ± S.E. μmole/kg.	Slope	Estimated relative molar activity
Acetylcholine bromide		80.72 ± 2.03	5.4	1000
Propionylcholine iodide		125.4 ± 3.90	5.98	644
D(—)-Lactoylcholine iodide	90°	171.5 ± 3.69	5.98	337
L(+)-Lactoylcholine iodide	90°	225.9 ± 7.92	5.98	357
DL-Lactoylcholine iodide	121°			
(i)		75.0 ± 2.57	5.67	1076
(ii)		74.2 ± 2.57	5.4	1088
Mixture of equal quantities of D(—)-lactoylcholine iodide and L(+)-lactoylcholine iodide		168.2 ± 1.88	5.4	480

TABLE 5 — TOXIC EFFECTS OF OPTICALLY ISOMERIC DRUGS

Drug	LD ₅₀ , mg./kg.			Ratio
	DL	L	D	
Methadone i.v. in mouse ¹⁶	20.9 ± 1.6	28.7 ± 4.5 (mean 29)	30.6 ± 1.0	D/L 1.0
Methadone i.p. in mouse ¹⁶	29.0 ± 2	11.2 ± 2 (mean 37)	63.1 ± 3	D/L 5.6
Ephedrine ²⁷ i.v.	60.0	60.0	80.0	—
Pseudoephedrine ²⁷ i.p.	70.0	80.0	75.0	—
Desoxyephedrine ²⁸ i.p.	—	82.0 ± 2.8	15.0 ± 1.7	L/D 5.5
Desoxyephedrine ²⁸ i.v.	—	35.0 ± 3.6	9.4 ± 0.9	L/D 3.7
Pipradrol* (mouse, subcutaneous)	147.0 ± 8.0	223.0 ± 14.0 (mean 162)	102.0 ± 8.6	L/D 2.2
Pipradrol (rabbit, i.v.)	15.0 ± 1.8	13.0 ± 0.8 (mean 12)	9.7 ± 1.2	L/D 1.3
Amphetamine† (mouse, i.v.)	42.0	79.2 ± 8.5 (mean 42)	5.0 ± 1.3	L/D 16

*Personal communication (Brown and Werner).

†Personal communication (V. Vernier).

in the body is that of the lactoylcholines. The toxicities given by Rama Sastry *et al.*^{24,25} are presented in Table 4.

Chen²⁶ gave the LD₅₀ values for intravenous methadone in the mouse and Pfeiffer¹⁶ for intraperitoneal injection (Table 5). Chen *et al.*²⁷ found the toxicities for ephedrine on intravenous injection. By other tests the difference in pharmacological activity is 3 for ephedrine. Roth *et al.*²⁸ determined the toxicity of desoxyephedrine by both the intravenous and

intraperitoneal routes in mice. The values are given in Table 5. Brown and Werner (personal communication) have similar data (Table 5) on pipradrol (meratran), but in different species. Vernier (personal communication) studied the toxicity of the isomers of amphetamine administered in the mouse by very slow intravenous injection (1.0 ml./2 min.) and found the LD₅₀ values which are given in Table 5.

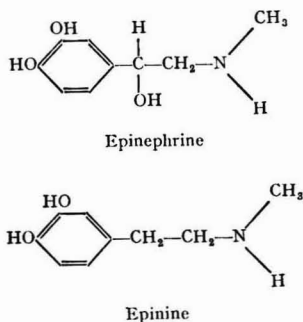
Optical Isomerism versus Functional Groups on Drug Molecules

Cushny²⁹ studied the action of hyoscyamines, DL-(atropine), L- and D-hyoscyamine. As an inhibitor of pilocarpine-induced salivary secretion, 2 molecules of atropine (2DL) were equal to one of L alone in pharmacological action. Both the D- and L-isomers were found to act in the same way, but the L was about 12-20 times as strong as the D, except in the central nervous system in which case the D caused distinctly greater and longer excitation than the L form. Similar observations were made on the hyoscines and on the optically active homatropines (Table 6).

In order to determine if the asymmetric carbon atom or other parts of the drug molecule were responsible for the marked differences in potency of the compounds, Cushny studied tropines with no asymmetric carbon atom and hydratropyltropine which has an asymmetric carbon atom and differs from atropine only in that the CH₂OH group is replaced by a CH₃ group. He found that the pharmacological activity of hydratropyltropine, possessing

an asymmetrical carbon but no OH, was of the same order as that of such bases as benzoyletropine and phenacetyltropine which possess neither asymmetric carbon nor OH in the side chain, while atropine, which possesses both, is about two hundred times as powerful. It is obvious that the activating factor in the atropine molecule is not the asymmetric carbon but the OH. This is important as the third functional group of the atropine molecule and must be alcoholic rather than phenolic for optimal activity. The asymmetric carbon and the optical activity with which it endows the molecule does not lend any specific action.

A similar analogy can be found between epinephrine and epinine where the latter compound is identical to epinephrine in structure, except for the beta hydroxy group.



This hydroxy group provides the asymmetric centre for the optical isomerism of epinephrine. (-)-Epinephrine is 20 times more active than (+)-epinephrine in pressor action, while epinine and (+)-epinephrine are about equivalent. This can be interpreted as evidence that the hydroxy group in (+)-epinephrine is neutral in pharmacological effect³⁰.

Specific Instances of Optical Activity in Molecules without an Asymmetric Centre

In the synthesis of certain acids, such as citric acid, within the body the combination with the template of an enzyme may originate asymmetry by hydrogen bonding or other forces. Steric hindrance in biphenyls may produce asymmetry and optical activity^{31,32}.

Geometrical isomerism—The biological effect of geometrical isomerism has not been studied to any great extent except in the isomers of the substituted cyclohexane insecticide called gammexane, in vitamin A, and of course in various steroid molecules³³. Fancher and Lim³⁴ have presented an interesting example of two geometrical isomers of 2-ethylcrotonylurea (IV and V), one of which is sedative and the other convulsant.

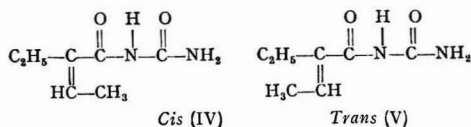


TABLE 6 — PHARMACOLOGICAL ACTIVITY OF TROPINES

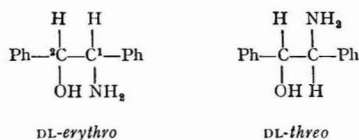
Tropine	Functional group	Pharmacological activity
L-Hyoscyamine	$\text{C}_6\text{H}_5 - \text{C}(\text{CH}_2\text{OH})(\text{H}) - \text{COOT}$	600 Mean 307
DL-Atropine	$\text{C}_6\text{H}_5 - \text{C}(\text{H}) - \text{COOT}$	300 Ratio L/D 40
D-Hyoscyamine	$\text{C}_6\text{H}_5 - \text{C}(\text{OH})(\text{H}) - \text{COOT}$	15
L-Homatropine	$\text{C}_6\text{H}_5 - \text{C}(\text{OH})(\text{H}) - \text{COOT}$	14 Mean 10.4
DL-Homatropine	$\text{C}_6\text{H}_5 - \text{C}(\text{H}) - \text{COOT}$	10 Ratio L/D 2
D-Homatropine	$\text{C}_6\text{H}_5 - \text{C}(\text{H}) - \text{COOT}$	7
Phenylacetyltropine	$\text{C}_6\text{H}_5 - \text{C}(\text{H}) - \text{COOT}$	1
Benzoyletropine	$\text{C}_6\text{H}_5 - \text{COOT}$	1
Oxybenzoyletropines	$\text{C}_6\text{H}_4(\text{OH})\text{COOT}$	1/2-1
Hydratropyltropine	$\text{C}_6\text{H}_5 - \text{C}(\text{CH}_3)(\text{H}) - \text{COOT}$	1-1.5

TABLE 7 — TOXICITY AND PHARMACOLOGICAL EFFECTS OF GEOMETRICAL ISOMER OF 1,2-DIPHENYL-1-AMINOETHANOL-2 DERIVATIVES

Form	Substitutions on C-1	Substitutions on C-2	LD-(i.v., mouse)	Pharmacol. effect	LD ₅₀ (i.p., mouse) (mg./kg.)	Slope
<i>Erythro</i>	N(CH ₃) ₂	OH	37.5	Convulsions	101 ± 4	38
<i>Erythro</i>	NCH(CH ₃) ₂	OH	50.0	Sedation	150 ± 15	16
<i>Threo</i>	N(CH ₃) ₂	OCOCH ₃	50.0	Convulsions	206 ± 15	22
<i>Erythro</i>	N(CH ₃) ₂	OCOCH ₃	50.0	do	133 ± 11	19
<i>Threo</i>	N(CH ₃) ₃	OCOCH ₃	15.0	Curare-like	64 ± 13	9
<i>Erythro</i>	N(CH ₃) ₃	OCOCH ₃	30.0	do	121 ± 2	55

	<i>Cis</i> (IV)	<i>Trans</i> (V)
Sedation, mg./kg.	3 ± 0.6	7 ± 3
Death, mg./kg. rat	2500 (oral)	1130 (oral)
Pharmacological activity	Hypnotic	Clonic convulsions

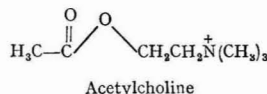
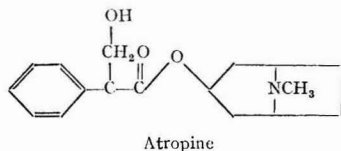
Another example of a difference in the toxicity and pharmacological effects of geometrical isomers is illustrated by some 1,2-diphenyl-1-aminoethanol-2 derivatives (Table 7).



Again, it may be noted that with the exception of the last two compounds, the intravenous route of administration provides less differentiation between the toxicity of the compounds than the intra-peritoneal route. The tertiary compounds produce death by excitation or depression of the CNS, whereas the quaternary nitrogen compounds produce death by respiratory paralysis and the mice may be revived by artificial respiration.

Ten Broek³⁵ studied the antigenicity of racemized proteins and found that racemized egg albumin (prepared by Dakin) was without antigenic effect. Dakin and Dalc³⁶ found that the crystalline albumins from the eggs of chickens and ducks behave as distinct antigens for the anaphylactic reaction and that this difference corresponds with the difference in the structure of the protein molecule. When the proteins were racemized, the amino acids escaping racemization were not identical in the case of the two proteins.

Finally, the action of the optical isomers of drugs may provide clues to the search for more perfect neurohumoral agents. If we return to atropine and compare it to acetylcholine, several differences and similarities in structure are noted.



The third oxygen atom is extremely important in atropine but is not present in acetylcholine. More important — the α -carbon atom of the acetyl group in acetylcholine does not contain an asymmetric carbon. Perhaps one of the hydrogens is activated in an asymmetric fashion, but perhaps also the acid group may be different and contain an asymmetric carbon atom. Such acids might be phosphoric, seryl, lactic or glyceric, each of which would have the additional oxygen functional group and an asymmetric α -carbon.

Summary

Natural asymmetry in plants and animals, and geophysical asymmetry as well as asymmetry at molecular level have been discussed. The pharmacological activity of optically isomeric drugs and the relation between optical isomerism and functional groups on drug molecules have been considered and specific instances of optical activity in molecules without an asymmetric centre are given.

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Mechanism of Infection of Legume Roots by *Rhizobium*

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THE occurrence of nodules on the roots of legumes is the result of symbiosis between bacteria of the genus *Rhizobium* and plants of Leguminosae, one of the largest families of angiosperms. The nodules are the sites of biological nitrogen fixation where both the bacteria and the legume interact to convert atmospheric nitrogen into amino acids. Therefore, an understanding of the mechanism of entry of *Rhizobium* (lower symbiont) into the roots of legumes (higher symbiont) is of vital importance. The primary portals of entry (infection) of *Rhizobium* into the roots of legumes are the root hairs. The first visible evidence of successful infection of a root hair is the appearance of a tube-like structure within it known as 'infection thread'. The bacteria grow and multiply in the infection thread and occur as a line of small rod-shaped bodies with dense granular contents. The infection thread makes its way through the root by intracellular growth. One of the cells of the root cortex becomes meristematic and nodule growth begins by the activity of a nodule meristem constituted sooner or later. However, the establishment of the initial infection thread in the root hair involves a series of biological events that take place in the root environment, some of which are hypothetical. Nevertheless, in the recent decade attempts are being made to provide experimental evidence in support of them.

Indole Acetic Acid

The earliest observation was that of West¹ who found that root secretions stimulate the rapid multiplication of the lower symbiont in the root region. Thus, the initial prerequisite for infection is an accumulation of *Rhizobium* in the rhizosphere. Rhizobial secretions contained in culture filtrates are known to influence the deformation and curling of root hairs of legumes². These filtrates were shown to contain indole acetic acid³. The exudation of tryptophan by roots of legumes has now been demonstrated^{4,5}. Tryptophan is one of the precursors of indole acetic acid and the auxin may be synthesized

by the following mechanism⁶. The amino acid may be deaminated to indole pyruvic acid and then decarboxylated to indole acetaldehyde; alternatively, the amino acid may be first decarboxylated to tryptamine and then deaminated to indole acetaldehyde. The aldehyde thus formed is the immediate precursor of indole acetic acid by either route of formation. Therefore, it would be logical to assume the possible production of indole acetic acid by conversion of tryptophan in the root environment of legumes. However, many interesting questions arise as corollaries to this hypothesis. Is tryptophan exuded by roots of all nodulating legumes and not by non-nodulating legumes and non-leguminous plants? Have the enzymes essential for conversion of tryptophan to indole acetic acid been actually detected in the root environment of legumes? These questions are relevant, but answering them appears inconsequential since it has been shown that deformation and curling of legume root hairs is an unspecific phenomenon; strains of rhizobia non-specific to a given legume induce the deformation and curling effect and also produce indole acetic acid on synthetic media⁷. Besides, the auxin is produced by microorganisms unrelated to rhizobia. Beyond its ability to induce curling of root hairs, the precise role of indole acetic acid in the mechanism of infection of legume root hairs by rhizobia is not known. Therefore, it would be appropriate to say that the auxin plays a likely role in activating the production of pectic enzymes by virtue of its importance in increasing the plasticity of cell walls⁸.

Invagination Theory

In the absence of any explanation for the precise mode of entry of *Rhizobium* into the legume root hair, Nutman⁹ has proposed a theory called the 'invagination theory'. According to this theory, the root hair surface becomes invaginated into the lumen of the root hair to form the infection thread possibly associated with prior incorporation of bacteria into the primary host wall material. The

primary wall of the root hair tip consists chiefly of pectic substances which form a continuous phase within which a discontinuous phase of cellulose fibrils is deposited. During the growth of the hair, the network of cellulose becomes loosened and expanded so that new fibrils may be interwoven to maintain its structure. This process is known as 'intussusception'¹⁰ which is aided by indole acetic acid by virtue of its ability to loosen the cellulose cross-linkages⁸. The merit of the invagination hypothesis lies in that it explains the ingestion of bacteria into the root hair cell without disrupting the structure of the primary cell wall.

It is visualized that rhizobia take part in the root hair growth at the time of intussusception and thus build themselves into the primary wall material of the actively growing tip of the root hair. The 'built-in' *Rhizobium* induces a reorientation of the growth of the root hair so as to produce an invagination of the wall into the lumen of the root hair cell. Thus, invasion of *Rhizobium* into the root hair represents an incorporation of the bacterium into the pectic layer of the root hair tip. Pores have not been observed on the root hair tip and, therefore, it is likely that pectic substances harden after the invasion of *Rhizobium*. The invagination hypothesis underlines not only a close chemical and physical affinity between the bacterial surface and the primary wall of the root hair at the molecular level but also a mechanism by which the growth of the root hair preceding bacterial invasion is directed inwards into the lumen of the hair cell by a process of invagination. The primary infection thread passes through the cells of the cortex. However, the cellulose wall of the infection thread is not continuous from cell to cell of the cortex and is interrupted at the middle lamella. It is known that the cellulose layer in secondary walls is interrupted at pits which may be compound or simple depending on the age of the tissue. It is envisaged that at each cell wall pit, a naked mass of rhizobial cells enclosed by a slime layer initially enters a cell through a pit. After this process, a cellulose sheath is formed afresh around the bacterial mass in each cell. Therefore, it could be visualized that the growth of a fresh section of the thread may start *de novo* at each cell wall pit encountered by it, in the same manner as it initially started at the apex of the root hair cell wall.

Pectic Enzymes

It is hard to account for the entry of *Rhizobium* into the root hairs since no breakage of cell walls near the infection foci has been observed. The invasion of *Rhizobium* is unlike the case of several fungi which are known to gain entry into the tissues of higher plants by sending in outgrowths from propagules such as spores or mycelial fragments. Since the beginning of this century, many fungal infections of plants have been ascribed to the ability of fungi to produce pectinolytic enzymes¹¹. However, a search for pectin degrading enzymes in pure cultures of rhizobia proved negative^{12,13}. Therefore, no serious attention was paid to enzymatic degradation of pectin in cell wall of root hairs by rhizobia until Fahraeus and Ljunggren^{14,15} reported

that polygalacturonase was produced *in situ*, in the root environment, when a specific *Rhizobium* interacted with the root system of a legume belonging to an appropriate cross-inoculation group. This finding need not necessarily alter the suggested invagination theory of Nutman⁹. On the other hand, it would help in explaining the invagination process more clearly as a specific biochemical event originating on the cell wall of root hairs by the activity of both the symbionts. Ljunggren and Fahraeus¹⁵ conducted experiments in which non-infective and infective strains of *Rhizobium* differing in virulence (isolated from the same cross-inoculation group) were allowed to interact with plant species of differing susceptibilities under aseptic conditions. Rigid sterility control was maintained so as to avoid foreign contaminants viciating the study. The results indicated that infection of seedlings was strongly correlated with the production of polygalacturonase. The enzyme produced *in situ* may weaken the cell wall of the root hair resulting in the partial depolymerization of cell wall pectin which would facilitate bacterial invasion as envisaged by the invagination theory of Nutman.

According to Ljunggren and Fahraeus¹⁵, the bacteria secrete highly specific water-soluble substances, mainly polysaccharide in nature and probably containing deoxyribonucleic acid, which pass through the cell walls of root hairs and reach the protoplasm. This substance is believed to react with some specific cell component. The reaction results in the formation of an 'organizer' which governs the production of polygalacturonase when the roots of a legume are in association with a homologous species of *Rhizobium*. Cell-free preparations of *Rhizobium* induced the production of polygalacturonase in the vicinity of roots in the same way as cell suspensions suggesting that the enzyme is produced by the higher symbiont. The presence of nitrate in the root environment, a factor inimical to nodulation, also inhibited polygalacturonase production. Incidentally, this offers a possible explanation for the inhibition of nodulation in the presence of excess of nitrates. These facts indicate that polygalacturonase produced *in situ* in root environment under the influence of a specific *Rhizobium* could be considered as one of the biological links favouring successful infection.

Fine Structure Studies

It has often been suggested that the nucleus of the root hair takes active part in the infection mechanism. Fahraeus¹⁶ clearly showed that the nucleus of root hair cell leads the path of infection thread in the hair and that the thread ceases to grow when the nucleus moves away. The infection of the root hair may be simple, multiple, terminal or lateral on the hair and sometimes found on its side branch. Fahraeus and Ljunggren¹⁴ suspect that the principle secreted by *Rhizobium* which is active in inducing polygalacturonase production may contain deoxyribonucleic acid in addition to a polysaccharide. Since the root hair nucleus is always near the tip of the infection thread, it is very likely that the bacterial principle may in some way react with the host nucleus.

Electron microscopic studies have provided evidence in support of Nutman's root hair invagination theory. Bergersen and Briggs¹⁷ observed that bacteroids (a stage in the life cycle of *Rhizobium* in the nodular tissue when active nitrogen fixation is accomplished) were surrounded by membrane systems in soyabean root nodules. These membranes were thought to be continuous with the host cell membrane, although direct evidence in support of the hypothesis was not provided. However, the studies of Sahlman and Fahracus¹⁸ with infected root hairs of *Trifolium parviflorum* have provided direct evidence in support of the invagination theory wherein electron micrographs have been presented to illustrate that the wall of the infection thread is continuous with the wall of the root hair cell. In a detailed study of changes in fine structure during development of root nodules of *Trifolium subterraneum* and *Medicago tribuloides*, Dart and Mercer¹⁹ have shown from electron microscopic studies that infection threads develop from narrow tube-like projections of the cell wall of host cells. These projections were seen to extend into the cytoplasm of the host cell. They did not observe any difference in the appearance of the wall of the host cell and that of the infection thread. These workers also observed that the infection thread grows towards and comes to lie in close proximity to the nucleus of the host cell; later, a degeneration of the nuclear membrane sets in resulting in the coalescence of the karyoplasm and the ground cytoplasm.

The Legume Factor

A factor or a principle of host origin has been implicated in explaining the origin of atypical morphogenetic structures on plant parts which are formed as a result of intimate association (beneficial or otherwise) of a microorganism with a higher plant. It is now known that a factor called 'M' factor operates in the establishment of ectotrophic mycorrhizas of forest trees, especially pine. In a similar manner, a tumour inducing principle (TIP) is known to govern the origin of plant tumours like 'crown gall' caused by *Agrobacterium tumefaciens*. In the field of legume *Rhizobium* symbiosis, attention is being currently focused on finding out the existence of a nodule inducing principle as causative agent in nodule initiation and development. In this connection, Raggio and Raggio²⁰ used decapitated soyabean plants inoculated with *Rhizobium japonicum* and demonstrated that cotyledons were necessary for nodulation. In a later work²¹, using special technique for growing isolated roots of legumes, it was shown that excised roots of *Glycine max* and *Phaseolus vulgaris* inoculated with their homologous rhizobia developed nodules. However, the involvement of a host factor need not necessarily be precluded since the roots were excised from the tops several days after germination. Valera and Alexander²² found that nodulation of excised roots of *Medicago sativa* was enhanced by an extract of alfalfa seed. Coconut water exerted a similar effect on the formation of nodules on excised roots of *Glycine max* and *Phaseolus vulgaris*. Coconut water is the liquid endosperm of coconut seed and

hence physiologically similar to the cotyledons of Leguminosae. Since excised roots nodulated even in the absence of these stimulatory factors, a further probe into the existence of a specific nodulation factor was made by conducting nodulation studies with three types of plant material²²—excised roots from seedlings obtained through normal seed germination, seedlings from intact embryos (radicle, hypocotyl and plumule) devoid of cotyledons, and explants derived from hypocotyls of embryos. Inoculations with homologous strains of rhizobia revealed that roots derived from hypocotyls of ungerminated soyabean and kidney bean seeds grew normally and did not produce nodules. The results indicated that separation of hypocotyl from the rest of the dormant embryo of ungerminated seed by excision prevents the entry of nodulation factor into the hypocotyl from the rest of the embryo. On the other hand, roots derived from intact kidney bean embryos without cotyledons not only grew well but also produced discernible nodules. Therefore, a substance evidently found or produced in the upper portion of the plant is necessary for nodule genesis. Further experiments also revealed that the legume factor, when examined by a soyabean hypocotyl bioassay procedure, could be replaced by a water-soluble, thermostable and dialysable fraction of coconut water but not by meso-inositol, scyllitol, yeast extract and a number of amino acids. It is interesting to note that meso-inositol could not replace the nodulation factor since it is known that this vitamin, up to a concentration of 1 g. per litre increases nodulation²³⁻²⁵. Therefore, it is quite obvious that meso-inositol is a stimulatory agent for nodulation of legumes and not a factor governing nodule initiation. The work of Hely *et al.*²⁶ reveals that nodulation occurs on *Trifolium ambiguum* roots when grafted to tops of *Trifolium repens* which also suggested the existence of a nodulation factor in the upper portion of the plant. Therefore, the prerequisites for nodulation of a given legume is not only the presence of a specific strain of *Rhizobium* in its rhizosphere but also of a substance produced by the shoot system which is translocated to the root system by an actively growing plant.

In conclusion, a step-wise scheme (Chart 1) may be visualized to summarize the several events that are known to take place in the root environment of legumes leading to successful infection of root hairs by *Rhizobium*.

Summary

While angiosperms in general are able to utilize nitrates from soil, plants belonging to Leguminosae in particular are able to utilize not only combined nitrate from soil but also fix atmospheric nitrogen in nodules occurring on their roots. The nodules are the sites of symbiosis between bacteria of the genus *Rhizobium* and the plants of Leguminosae. *Rhizobium* occurring freely in soil cannot fix atmospheric nitrogen unless it gains entry into the root system of legumes via the root hairs. The *modus operandi* involves a series of events in the root environment and on the cell wall of the root hair (Chart 1). Unlike phytopathogenic fungi, the entry of microorganism into the host is not by penetration

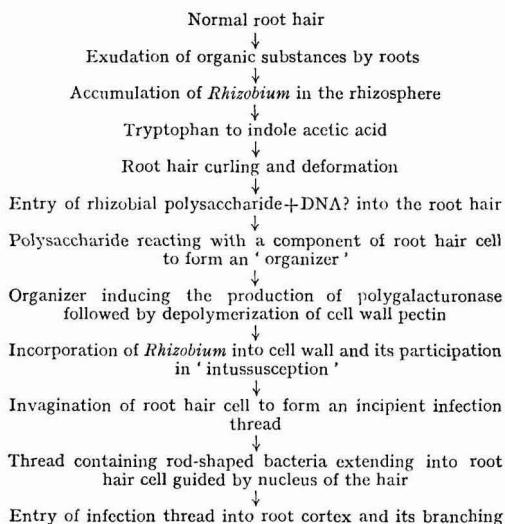


Chart 1— Events taking place in the root environment of legumes leading to successful infection of root hairs by *Rhizobium*

through wounds or pre-existing pores on the plant. Rhizobia do not produce pectinolytic enzymes *in vitro* and, therefore, direct penetration through enzymatic degradation of cell walls cannot be envisaged. While fungal spores produce germ-tubes which gain entry into plant tissue, rhizobia do not produce any propagules. However, recently it has been demonstrated that pectic enzymes are produced when a specific strain of *Rhizobium* is in association with a member of its cross-inoculation group of legumes. Besides strain specificity, even virulence, efficiency and nitrate inhibition of nodulation have been ascribed to the ability of rhizobia to produce different amounts of polygalacturonase. Rhizobia secrete abundant polysaccharides or gums. It has been hypothesized that a water-soluble polysaccharide of rhizobial origin enters the root hair and reacts with a specific component of the root hair protoplasm, which in turn produces an 'organizer'. The organizer is believed to excite the production of polygalacturonase in the root environment. Indole acetic acid is produced by rhizobia and the auxin may accelerate the production of polygalacturonase besides its well-known and unspecific action of inducing curling and deformation of root hairs, a phenomenon generally noticed by many investigators. According to the 'invagination theory' proposed by Nutman, the cell wall of the root hair participates in the infection mechanism. Pectic enzymes produced *in situ* may soften

the pectin substrate of the primary cell wall of the root hair; in this process, the bacterial cells get incorporated into the primary cell wall and then participate in the further growth of the cell wall and the root hair by a process of 'intussusception'. This is followed by a reorientation of the growth of the root hair inwards through invagination of the cell wall to form a tube-like structure within the hair called the 'infection thread'. The infection thread carries rod-shaped rhizobia in it and its growth is guided by the nucleus of the root hair cell. More than one infection thread may occur in a root hair depending on the number of infection foci. The thread enters and traverses the cortex of the root intracellularly at the region of pits. Recent studies on the fine structure of infected root hairs lend support to the invagination theory of the origin of infection thread. Thus, in effect, the infection thread is of host origin and serves to carry the lower symbiont into the root system of legumes.

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REVIEWS

MICROWAVE SPECTROSCOPY OF GASES by T. M. Sugden & C. N. Kenney (D. Van Nostrand Co. Inc., New York), 1965. Pp. ix+322. Price 35s.

The book deals satisfactorily with the quantum mechanical properties of molecular rotation. The topics of inversion and restricted rotation are well treated. The description of the experimental methods of microwave spectroscopy is good. Bibliography regarding microwave spectra of different molecules is well given. The book is useful for physicists and chemists working in microwave spectroscopy and allied areas.

PUTCHA VENKATESWARLU

INTRODUCTION TO ELECTRICAL CIRCUIT ANALYSIS by Robert C. Carter (Holt, Rinehart & Winston Inc., New York; *Distributors in India*: India Book House, Bombay), 1966. Pp. xii+500. Price \$ 9.95

During recent years several books have been written by various authors on circuits and networks, which are primarily meant for the advanced students in electrical engineering. Very few attempts have, however, been made in writing a book suitable for the engineering technicians, whose services are essentially needed by the engineers working in their respective fields. This book is, therefore, a timely contribution for the requirements of technical personnel who do not have the opportunity to go through the elaborate course for the degree in electrical engineering. It may be mentioned, however, that the book has been written in an excellent and systematic manner which will not only be indispensable for the technicians but will also serve as a valuable companion for the undergraduate students of electrical engineering.

The intention of the author to familiarize his readers with the basic concepts of electrical and electronic circuits is amply manifested in the first introductory chapter of the book, which is followed by several other chapters on simple circuits and networks. The achievement in this direction has been specially marked in connection with Chapter 4 on complex algebra and Chapter 8 on graphical technique for the design and analysis of phase-shift networks. It may, however, be mentioned that a brief reference to the oscillatory circuit has inadvertently escaped the notice of the author in Chapter 7 on resonance, although such a circuit is the precondition for the occurrence of resonance.

Chapters 6 and 9 are on single and multi-source networks respectively, which have been clearly defined with several applications. Various methods of analysis have been included with multi-terminal networks, bridges and ladders which are widely used in practice. It may, however, be mentioned that the definitions of poles and zeros of networks have been omitted, presumably due to want of space. Chapter 10 on transformers along with the last two chapters on balanced and unbalanced polyphase

systems are full of information for the practising engineer and the technicians. The large number of examples and problems at the end of each chapter of the book will be of immense help to those who desire to have a working knowledge of the subject.

S. S. BANERJEE

HANDBOOK OF DIFFERENTIAL THERMAL ANALYSIS by W. J. Smothers & Yao Chiang (Chemical Publishing Co., New York), 1966. Pp. ix+633. Price \$ 17.50

The technique of differential thermal analysis (DTA) has attained considerable popularity and importance in recent years. The technique has found application in diverse fields, such as criminology, geology, metallurgy, radio, sugar, polymer, soil, catalytic, analytical and inorganic chemistry and also in wood, coal, ceramic, glass and cement technology. In fact, the application of DTA in the field of cements itself has reached such a stage that the Chemical Publishing Co. has undertaken the publication of a book on cement chemistry by the reviewer.

The publication under review is a very much improved version of the earlier work published by the authors in 1958. The outstanding features of this edition are: A more comprehensive treatment of the application of DTA; inclusion of author, subject and mineral indices; and an exhaustive list of publications on DTA up to 1963 and some publications of years 1964 and 1965. The listed references total 4248. Inclusion of secondary sources, chemical and ceramic abstracts makes this book particularly useful to those who do not have access to many journals published in languages other than English. A worker dealing in a narrow field of study would also find this book very useful, as it comprises references to papers in which DTA has been applied as one of the diagnostic tools.

There are some minor typographical errors pertaining to references 441, 1196, 1343, 2788, 3048, 3071, 3131, 3421, 3429, 3656, 4083, 4092 and 4094. The author and subject indices for the years 1964 and 1965 are not included and this fact ought to have been mentioned in the book. Acknowledgement made to the figure numbers in the preface is not consistent with the numbering in the text.

The value of the book as a 'handbook' would be enhanced further by incorporation of (i) reference number and year as given in the bibliography whenever references to authors are made in the review, (ii) a separate list of books and review articles on DTA, (iii) DTA curves, or at least the temperatures and intensities of endo- and exothermal effects of the materials listed in the appendix, (iv) an index to instrumental and phenomenal aspects of DTA, and (v) e.m.f. charts of thermocouples normally used in DTA.

This book, incorporating as it does many features that are not available in other books, is highly

recommended to beginners as well as research workers interested in thermal investigations.

V. S. RAMACHANDRAN

ENERGETICS IN METALLURGICAL PHENOMENA: Vol. II, edited by William M. Mueller (Gordon & Breach Science Publishers Inc., New York), 1965.

Pp. ix+203. Price \$ 11.00 (cloth); \$ 5.50 (paper) This attractive volume contains the contributions made at the second annual seminar on energetics in metallurgical phenomena held in the summer of 1963 at the University of Denver. The contents of the volume are: (1) The kinetics and thermodynamic properties of surfaces by J. P. Hirth; (2) Solid solution formation by B. L. Averbach; (3) The statistical mechanics of nucleation and crystal growth by G. M. Pound; and (4) Point defects in metals by C. J. Dienes.

The contributions are comprehensive, well documented as well as up to date and should be of great assistance to all those concerned with the intelligent and effective transfer of fundamental scientific discoveries into the fields of engineering and technology.

T. R. ANANTHARAMAN

MASS SPECTROMETRY: A NATO ADVANCED STUDY INSTITUTE ON THEORY, DESIGN AND APPLICATIONS HELD IN GLASGOW, August 1964, edited by R. I. Reed (Academic Press Inc., New York), 1965. Pp. x+463. Price 105s.

This book is a collection of twenty-three articles representing the lectures delivered at the NATO Advanced Study Institute on mass spectrometry held at Glasgow University in 1964. The authors are all specialists in their respective fields and the topics discussed cover a broad range, including description and design of various types of mass spectrometers, the theory of mass spectra and typical applications in physical, inorganic and organic chemical problems. Mass spectrometry is a rapidly advancing field of endeavour and up-to-date reviews of the various developments will be of considerable interest to users of the technique. The editor and the publishers are to be congratulated for bringing out this useful volume.

The arrangement of the articles does not follow any rigid order. Instrumental aspects are covered in the first four chapters, three of which are written by scientists from manufacturing firms. These are followed by a chapter on field-ion mass spectrometry by the originator of the technique. Two chapters are devoted to the theory of mass spectra and one to the special features of the spectra of compounds like phthalocyanine which contain large aromatic rings. Physico-chemical themes like ionization by photon and electron impact, measurement of ionization and appearance potentials, high temperature mass spectrometry and its applications, and mass spectrometric studies of ions in flames, ion-molecule reactions and heterogeneous kinetics are all dealt with in separate chapters.

Two articles of interest to inorganic chemists are on the quantitative determination of impurities using the spark source and the mass spectrometry of volatile inorganic compounds. Several aspects of

the mass spectrometry of organic compounds are also reviewed, but only briefly, and these accounts are meant to be supplementary to the more comprehensive treatments available in several recent books on the subject.

This book is a welcome addition to the growing literature on mass spectrometry.

P. MADHAVAN NAIR

INTRODUCTION TO MOLECULAR ORBITAL THEORY by Arno Liberles (Holt, Rinehart & Winston Inc., New York; *Distributors in India*: India Book House, Bombay), 1966. Pp. ix+198. Price \$ 5.95

After the publication of the excellent book by A. Streitwieser and the notes by J. D. Roberts, any author writing a book on molecular orbital theory for organic chemists, or what is otherwise termed as an 'introduction' faces a formidable challenge. Judged in this background the present book by Dr Liberles is a fine presentation of a very useful and interesting subject at an introductory level. The knowledge of mathematics needed for understanding this book is not high and the details of the various steps in the m.o. calculations are clearly presented. Anyone wishing to start a study of this subject by himself will be greatly benefited by this book. Although the book is by no means adequate for a survey of the subject, it will undoubtedly serve as a good supplement to the existing books on this topic.

P. T. NARASIMHAN

KURZES LEHRBUCH DER PHYSIKALISCHEN CHEMIE by Hermann Ulich & Wilhelm Jost (Dr Dietrich Steinkopff Verlag, Darmstadt), 16th revised edition, 1966. Pp. xviii+482. Price DM 29,40

This is a well-known physical chemistry text-book in German. Originally written by the late Prof. Ulich and published in several editions, and revised from the sixth and seventh editions by Prof. Jost of Göttingen University, it now appears as the freshly revised sixteenth edition. It was and is still meant to be an introductory physical chemistry course for chemists, physicists, metallurgists, engineers and biologists.

The book is divided into six parts. Part I (Properties of states of matter) contains 10 sections dealing with ideal gases, laws of thermodynamics, solids, diffusion and rotation in solids, non-ideal gases and liquids, thermodynamic functions, solutions, boundaries between surfaces and colloids, etc.

Part II (Chemical thermodynamics and equilibria) deals with chemical equilibrium, thermodynamics of chemical reactions, homogeneous and heterogeneous equilibria, phase rule and equilibria in solutions. Part III (Electrochemistry) is concerned with electrolyte equilibria, electromotive force, electrolysis, conductance and ionic mobility, and electrode processes. Part IV (Chemical kinetics) deals with reactions in homogeneous and heterogeneous systems, and photochemistry. Part V (Chemical force and structure of matter) describes the electronic structure of atoms, ionic and covalent bonding, molecular properties and covalent bond, metallic bond and intermolecular forces. Part VI deals with quantum theory and laws of radiation and the new quantum

mechanics rather briefly. Each section of the main part is completed with some exercises and further reading material. There are some useful appendices containing standard enthalpy, entropy and free energy data for a number of substances, brief biographical notes on eminent chemists and the meaning and translation of special expressions used in chemistry, besides author and subject indices.

The general approach is basically experimental; the book starts with experimental results presented in 72 tables and 161 individual graphs and diagrams, and then deduces theoretical considerations and generalizations. This sort of treatment can easily get lost sometimes in minute details and mathematical derivations. The book is strong in thermodynamics and chemical kinetics in the classical sense, and does not elaborate sufficiently the quantum mechanical theory of valency. Teachers of physical chemistry will find the book very useful and informative.

L. M. YEDDANAPALLI

ORGANIC CHEMISTRY by L. O. Smith (Jr) & S. J. Cristol (Reinhold Publishing Corp., New York), 1966. Pp. xv+966. Price \$ 12.50

This text is designed for a complete course on organic chemistry. This branch is no longer considered as factual and descriptive chemistry testing the memorizing power of the student. In the words of the consulting editors of the text, "Organic chemistry has emerged as a vigorous and relatively exact science based on well-developed theory". Everyone will agree with this view. The need for stressing the principles and the theory underlying the facts to the beginning students of organic chemistry cannot be overemphasized. However, organizing the material with a proper balance between factual and descriptive chemistry on the one hand and the theory and reaction mechanisms on the other is a real problem to teachers of organic chemistry. This will be felt particularly in our Bachelor's degree course. The authors of the text have done a good job in presenting "a sound body of experimental facts in a systematic organization, correlated and explained by the best and the most recent basic theories possible".

The text is organized in five major units. The first unit begins with an introduction to the nature and study of organic chemistry and includes a short review on chemical bonding. The second unit deals with the fundamental principles of organic structure and classification of organic compounds. The chapter on nomenclature is to be welcomed and should prove useful. The third unit is the largest and presents organic reactions on the basis of theory and mechanism. This mode of presentation rather than the one based on functional groups should make the student have a good grasp on the general principles underlying the organic reactions. There are twenty chapters in this unit. Each chapter deals with one type of reactions, such as nucleophilic displacements, elimination reactions, electrophilic additions, electrophilic aromatic substitution, molecular rearrangements, etc. Optical activity, optical rotatory dispersion, molecular spectra and other constitutive physical properties are included in the

fourth section. The last one deals with topics of special interest and more complex organic compounds like carbohydrates, proteins, terpenes, steroids and heterocyclics.

All the chapters have been written in an excellent style and are up to date. The structures and illustrations are profuse and are well drawn. Stimulating questions and problems are given at the end of each chapter. Useful appendices added give list of reference works, glossary and index of named reactions, general nomenclature tables, and answers to selected questions and problems. On the whole, this is an excellent text on organic chemistry which should give a comprehensive understanding of the subject to the student and should be able to stimulate his interest in the subject.

M. BALASUBRAMANIAN

FATTY ACIDS — INDUSTRY, TECHNOLOGY AND RESEARCH IN INDIA (Regional Research Laboratory, Hyderabad), 1966. Pp. viii+89. Price Rs 5.00

Fatty acids manufacture is a developing industry in this country. A seminar where all interests meet for fruitful discussion contributes substantially to this development. Such a seminar was held at the Regional Research Laboratory, Hyderabad, from 10 to 12 February 1965, and the publication under review gives the proceedings of this seminar.

Section A of the publication gives statistical data on licensed capacity, installed capacity and present production of fatty acids, nature of raw materials available, specifications for fatty acids as laid down by the manufacturers, consumers, the ISI and other countries. The information shows how production is impeded by shortage of suitable raw materials. To a certain extent, inability to utilize the available large quantities of cottonseed oil soapstock and to exploit the potential fish oils and non-edible oils has retarded the expansion of our fatty acid industry.

Part I of Section B gives the present position about the Indian plants for splitting and fatty acid distillation and fractionation. Much of the equipment has yet to be imported, although it appears that some Indian firms have proposals to start fabrication of such equipment on their own or in collaboration with foreign firms. In Part II of the same section, the type of equipment available from eight foreign firms has been described and flow sheets of the processes have been provided. Performance and utilities requirement data for the various processes add to the importance of this section.

A summary of the research work currently being carried out and/or recently accomplished at different Indian research institutes has been given in Section C, while detailed abstracts of papers presented at the seminar have been given in Section D. Both these sections are valuable as they indicate the lines in which research in this field is being directed and the status attained by it.

The research papers of both academic and applied nature number 43. One-third of them relate to chemistry and properties of fatty acids. About 25 papers deal with processing and preparation of

derivatives. Paper Nos. 23, 28-30, 36, 37 and 42 are out of place. The abstract of paper No. 27 seems to be incomplete.

The publication is an important addition to the technical literature on fatty acids.

J. G. KANE

PROGRESS IN BIOPHYSICS AND MOLECULAR BIOLOGY:

Vol. 16, edited by J. A. V. Butler & H. E. Huxley (Pergamon Press Ltd, Oxford), 1966. Pp. 276. Price 84s.

Like the preceding volumes of this series, the number under review highlights some of the achievements in molecular biology resulting from the application of biophysical techniques to biological problems. Included in the present volume are eight lucidly presented reviews on recognition of antigens by cells, DNA-dependent RNA synthesis or the transcription phenomenon, cybernetic reactions in epigenetics, the organization and function of the sarcoplasmic reticulum, the molecular organization of cell membranes, high resolution autoradiography, molecular genetics of bacteria and bacteriophages and electrophoretic behaviour of cells followed by subject index and titles of previous volumes of the series.

The range of subjects discussed in this volume indicates how precise techniques of investigation can reveal hitherto unavailable information on the electrical nature of the cell surface, or the molecular organization of cell membranes. The functional role of phospholipids in such structural organization is a lively field currently being investigated and the knowledge emanating from this front is as fascinating as the role of nucleic acid replication in molecular genetics. Some of the reviews not only present in a nutshell the current status of knowledge on the subject discussed but also stress the areas where further investigations are urgently needed. The editors deserve all our grateful congratulations on presenting the rich fare contained in the volume.

C. R. KRISHNA MURTI

ESSENTIALS OF PALYNOLOGY by P. K. K. Nair (Asia Publishing House, Bombay), 1966. Pp. vii+96. Price Rs 9.00

This book is meant for graduate and postgraduate students of palynology and should be very welcome to them. Its emphasis is on structure and distribution and not on functions of pollen. The first chapter gives the general background of the subject dealing with dispersal of pollen and spores, their chemical composition and scope of the palynological science. The second chapter deals with preparation of pollen for morphological studies, their characters and terminology, their distribution and the facilities which fossil pollen offer for study of evolution. The next chapter deals with the principle of aeropalynology, analysis of pollen and allergy of pollen and spores. The fourth chapter is concerned with collection of pollen by bees, analysis of honey pollen and its chemical composition and uses. The final chapter deals with fossil palynology. There is a selected bibliography and index of authors and genera and families of plants.

In the preface the author states that "the whole science of palynology had its origin in fossil studies and a major part of the literature on palynology is (*sic*) on fossil spores and pollen", though he mentions that "palynological studies are important also in the field of forestry, agriculture and medicine" (p. 1). This book, however, does not seem to be concerned with the physiology of pollen. In a recent article in the *Annual review of plant physiology* (1964), H. F. Linskens, University of Nijmegen, The Netherlands, noted that "during the past 50 years, at a rough estimate, more than 8000 articles on pollen physiology have been published".

Perhaps the author is not familiar with the literature on pollen physiology. This may explain his uninformed regret that in India "at the graduate and postgraduate level, almost no information about the science is provided, accounting for the almost total neglect of it in the field of research". From an article by B. M. Johri and I. K. Vasil, 'Physiology of pollen', in the *Botanical review* (1961), the author will be able to get an idea of the research work on palynology now being carried out in the Indian universities and laboratories.

There are a few printing mistakes. One that catches the eye is on page vii, '139' should be read '93'.

B.S.

INSECT SEX ATTRACTANTS by Martin Jacobson, with a foreword by Stanley A. Hall (John Wiley & Sons Inc., New York), 1965. Pp. xi+154. Price \$ 7.75

The public consciousness of the adverse side effects of broad spectrum insecticides, commonly used for plant protection work, on human health has made it mandatory to search for less hazardous yet effective methods of insect control. Since insect sex attractants (sex pheromones) have a tremendous potential in this direction, it has led to a proliferation of literature on this subject. Moreover, the recent developments in analytical techniques that enable the isolation and chemical characterization of very minute quantities of chemicals have also helped the research on insect sex attractants, which are nothing but chemicals released by one sex of an insect to lure or sexually excite the opposite sex for mating purposes. Because of this increased emphasis on insect sex attractants the need for a reference book bringing together all the relevant information up to date at one place has been very acutely felt. Indeed the present book by Dr Jacobson who has done commendable research on the subject fills this very need admirably. The author, within 154 pages (twelve chapters), has done a good job of presenting all the available information in a concentrated form. The book will prove extremely useful to research workers in this field as the author has given an extensive list of insect species in which the occurrence of sex pheromones has been demonstrated (Tables 1 and 2). Moreover, the list of references given is by far the most extensive compilation of the available literature on sex pheromones. The book also gives a good indication as to the type of researches that have been done and that which is currently in progress.

Unfortunately, the author has not tried to generalize from the information available to him. This has minimized the value of the book to a general reader not familiar with the latest developments in the field. Because of this the book appears at places redundant and non-critical. The chapter on mechanism of attractant perception (Chapter 6) especially suffers from this drawback.

The book is free from printer's mistakes and has been nicely printed. In spite of the drawbacks mentioned above the book is an excellent reference source and is strongly recommended to the workers actively engaged in research on various aspects of sex attractants in insects. The author deserves congratulations for producing the first reference book on the subject.

K. N. MEHROTRA

ENERGY — ITS PRODUCTION, CONVERSION AND USE IN THE SERVICE OF MAN by Philip Sporn (Pergamon Press Ltd, Oxford), 1966. Pp. xiv + 69. Price 7s. 6d.

In this age of rising expectations in standards of living, consciousness of energy resources and their availability is widespread all over the world. Particularly noticeable since the Second World War is the common exercise in many countries and regions of the world of attempts at energy survey and planning. As a result of the great interest in such studies, a widely held notion is the paramount importance of energy resources and their availability in adequate measure as a major deciding factor for economic development and human welfare.

The book under review is a collection of three lectures delivered by Mr Philip Sporn, a recognized US authority in electrical engineering. It attempts at focusing the correct perspective regarding energy and its impact on society. Mr Sporn convincingly challenges and advances arguments to prove the fallacy of current concepts on the role of energy, fear of its world shortage and the premature optimism about the early replacement of fossil fuels by nuclear power. To Mr Sporn, energy is only one of the factors limiting world economic development. The machinery and methods of production and productivity are of greater significance for economic advancement.

In his first lecture Mr Sporn traces the history of energy use and development with some emphasis on the art and technology of conversion of primary sources into electrical energy. The second lecture — perhaps the most interesting — deals with a close examination of the true nature of energy and the mechanism of its contribution to the service of man. In the third and concluding lecture, Mr Sporn evaluates critically the rate of growth of energy and its likely availability in adequate supply in future, with particular reference to conditions in USA. It is a pity that the examination is not on a wider canvass. It may be difficult for some to agree with the author's optimism regarding the continued availability of fossil energy in adequate quantities in many parts of the world.

The book is highly interesting and is recommended for a critical study by economists, planners and others interested in human welfare.

S. RANGA RAJA RAO

PUBLICATIONS RECEIVED

- SOME PROBLEMS IN THE THEORY OF CREEP IN CONCRETE STRUCTURES:** International Series of Monographs in Civil Engineering, Vol. 1, by N. Kh. Arutyunyan (Pergamon Press Ltd, Oxford), 1966. Pp. xi+290. Price 80s.
- ZONE MELTING** by Hermann Schlicknecht (Verlag Chemie, Weinheim, and Academic Press Inc., New York), 1966. Pp. xii+222
- MINERAL PROCESSINGS PATENTS ISSUED DURING 1965** by Oliver North (Noyes Development Corp., New Jersey), 1966. Pp. 139. Price \$ 15 00
- INTRODUCTION TO MASS SPECTROMETRY** by H. C. Hill (Heyden & Son Ltd, London), 1966. Pp. xi +135. Price £ 1 10s.; \$ 4.50
- PROGRESS IN LAND REFORM, FOURTH REPORT** (United Nations, New York), 1966. Pp. 178. Price \$ 2.00
- A MANUAL FOR PROGRAMME AND PERFORMANCE BUDGETTING** (United Nations, New York), 1965. Pp. ix+103. Price \$ 2.00
- ECONOMIC SURVEY OF ASIA AND THE FAR EAST, 1965** (United Nations, New York), 1966. Pp. ix +320. Price \$ 3.50
- REPORT OF THE INTERREGIONAL SYMPOSIUM ON INDUSTRIAL PROJECT EVALUATION, PRAGUE** (United Nations, New York), 1966. Pp. iv+92. Price \$ 2.00
- ELECTRONICS IN INDIA, Report of the Electronics Committee, February 1966** (Secretary, Electronics Committee, Government of India, Bombay), 1966. Pp. 396.
- HORTULUS** by Valahfrid Strabo, translated by Raef Payne, commentary by Wilfrid Blunt (The Hunt Botanical Library, Pittsburgh, Pennsylvania), 1966. Pp. xi + 131. Price \$ 12.00
- SCIENTIFIC RESEARCH IN BRITISH UNIVERSITIES AND COLLEGES, 1965-66** (Her Majesty's Stationery Office, London), 1966. Vol. 1 — PHYSICAL SCIENCES. Pp. xix+413. Price £ 1 17s. 6d.; Vol. 2 — LIFE SCIENCES. Pp. xxi+426. Price £ 2
- SCIENCE AND THE NATION DURING THE THIRD PLAN:** Vol. 1, edited by Y. R. Chadha (Thorne's Private Ltd, Calcutta), 1966. Pp. xviii+369. Price Rs 30.00
- AN INTRODUCTION TO COMPUTER PROGRAMMING** by Henry Mullish (Gordon & Breach Science Publishers, New York), 1966. Pp. xi+244. Price \$ 5.00 (paper); \$ 14.50 (cloth)
- RESEARCH IN ELECTRIC POWER** by Philip Sporn (Pergamon Press Ltd, Oxford), 1966. Pp. xvi +64. Price 7s. 6d.
- INSTRUMENTS OF COMMUNICATION, AN ESSAY ON SCIENTIFIC WRITING** by Patrick Meredith (Pergamon Press Ltd, Oxford), 1966. Pp. xx +645. Price £ 7
- INDUSTRIAL CHEMISTRY: Part 1 — METALLURGY** by R. K. Das (Asia Publishing House, Bombay), 1966. Pp. 147. Price Rs 8.00
- APPLIED OPTICS AND OPTICAL ENGINEERING: Vol. 3,** edited by R. Kingslake (Academic Press Inc., New York), 1966. Pp. xiv+374. Price \$ 15.00

A team of Westinghouse physicists has succeeded in creating a plasma with a laser beam and in confining the plasma by means of a specially shaped magnetic field called a 'magnetic bottle'. This marks a step towards achieving controlled thermonuclear power generation. In the present experiment, aluminium metal has been used in place of a thermonuclear fuel.

A powerful ruby laser, designed to give 20 nanosec. long bursts of light, creates the aluminium metal plasma inside a vacuum chamber. In the absence of the magnetic field a broad glowing plasma lasting about a millionth of a second is obtained. The electrically charged particles forming the plasma are mainly aluminium ions, which expand outward from their origin at a speed of about 10 million cm./sec. When the magnetic field is turned on, it spins the aluminium ions in circular orbits forming a pulsating ball of ionized gas that lasts 50 times longer than without the magnetic field. The average energy of motion of the ions is about 300 eV. The laser beam delivers 100 MW power to the aluminium target, which is a disc about the size of a pinhead. The target absorbs about 80 per cent of the power delivered to it; in less than 10 nanosec., it is changed from a solid into an electrically charged gas.

The aluminium target is located at the centre of a glass vacuum vessel with three pairs of arms at right angles to each other. The laser beam passes through one pair of arms, the second pair supports the two coils of the electromagnet which creates the plasma-confining magnetic field and the third provides connections for a vacuum pump and measuring apparatus [*Int. Electron.*, 12 (2) (1966), 9].

Two-dimensional electron gas on semiconductor surface

A 'gas' of electrons exhibiting two-dimensional behaviour has been observed on semiconductor surfaces in two experiments conducted at the research laboratories of the IBM Watson Research

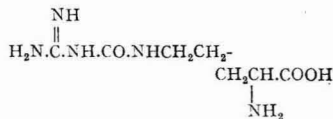
Centre, New York, USA. The experiments were carried out using a field-effect transistor, the current controlling portion of the transistor consisting of a sandwich of metal, insulation and silicon. The two-dimensional condition was created by applying a field of over 1 million V./cm. across the sandwich. The extremely high voltage field, made possible by the development of high purity insulation material, quantized the electrons in the direction perpendicular to the surface. Because of the large field at the silicon-insulation interface, the electric potential in the silicon drops sharply near the surface creating a potential well. Electrons exist only as standing waves in this well. At low temperatures (4.2°K.) electrons have the same wave shape and energy with respect to the perpendicular direction. In the plane of the surface, electrons can be thought as running waves implying that they can have motion and different values of energy. Thus, electron motion and interaction in the plane of the surface can be approximated by a two-dimensional model, i.e. electrons can move and collide with each other in the same way as molecules in a gas. As the temperature of the gas is increased slightly by adding energy to the system, the vivacity of electron motion perpendicular to the plane is increased, but the electrons still retain the same discrete, stationary quantum state with respect to the perpendicular direction.

In the first experiment, using a silicon surface prepared on the (100) face, it was found that the field-effect mobility and surface conductivity decrease with increasing charge density at high voltages. If the gas were three-dimensional, both high and low mobility states would have been occupied simultaneously and the conductivity would not have been expected to decrease as observed. In the second experiment, Shubinikov-de Haas like oscillations were observed. The oscillations

could be observed as the carrier concentration and hence the Fermi energy in the surface layer was changed by changing the electric field perpendicular to the surface. The constant period of these oscillations as a function of the carrier concentration confines the two-dimensional behaviour of electrons in the surface [*Int. Electron.*, 12 (2) (1966), 6].

Gigartinine: A new amino acid

The isolation of a new amino acid, gigartinine, from red algae, *Gymnogongrus flabelliformis*, has been reported [*Nature, Lond.*, 211 (1966), 417]. Analytical studies involving elementary analysis, reaction with Sakaguchi's reagent and ninhydrin, identification of the degradation products and spectral analysis have shown the amino acid to be L- α -amino- γ -(guanlyureido) valeric acid with the structure



Among fifteen species of red algae tested with the help of paper chromatography, the presence of gigartinine has been demonstrated only in the following seven species: *Gelidium amansii*, *Grateloupia livida*, *Polyopes polydeoides*, *Carpopeltis flabellata*, *Hypnea Japonica*, *Gracilaria textorii* and *Gymnogongrus flabelliformis*. Brown or green algae do not contain gigartinine.

The filtrate from aqueous ethanolic extracts of the algae, after removing gongrine as precipitate, is passed through a column of Dowex 50X-8. The basic amino acids are eluted from the cation exchange resin with ammonium hydroxide and gigartinine is purified by repeated crystallization of its nitrate from 50 per cent aqueous ethanol. Gigartinine nitrate is obtained as thin plates, melting at 197°C.

Fruit storage at subatmospheric pressures

Studies at the School of Medicine, University of Miami, Florida, USA, aimed at understanding the biological mechanism underlying the prolonging of storage life of fruits at subatmospheric pressures have indicated the removal of the fruit-refining hormone ethylene rather than reduced availability of oxygen to be the probable factor responsible for this. The storage life of bananas was found to be approximately doubled when they were flushed with air at 0.5 atm. pressure and doubled again when the pressure was reduced to 0.3 atm. That this was not due to the reduced availability of oxygen was indicated in an experiment where ripening in air at 760 mm. and in pure oxygen at 180 mm. Hg was compared. Though the partial pressure of oxygen was almost same in both cases, ripening was decidedly slower at 180 mm. pressure. Green bananas contain 0.1-0.2 p.p.m. of ethylene which is enough to trigger ripening, but when the ambient pressure is reduced by 75 per cent, the ethylene content declines by the same amount and the life of the fruit is increased correspondingly. It has also been observed that more ethylene is required to ripen fruits when the concentration of oxygen is lowered [Science, 153 (1966), 314].

Microbial production of food from coal

Paraffin-rich, higher molecular weight fractions derived from low temperature coal tar and from Fischer-Tropsch synthetic liquid fuel have been found to compare favourably with petroleum-derived fractions as substrates for microbial production of food [Nature, Lond., 211 (1966), 736].

The yeasts *Candida lipolytica* (strains 409, 409A, 409B), *C. tropicalis* 410 and six unidentified species of soil bacteria were used as test organisms. The basal growth medium (pH 7.0) contained 5.0 g. ammonium nitrate, 2.5 g. potassium monohydrogen phosphate, 1.0 g. MgSO₄·7H₂O and 0.1 g. yeast extract per litre of tap water and to this was added, as the major source of carbon and energy, a weighed quantity

of different substrates. Triplicate cultures (50 ml. of medium in 300 ml. flasks) were incubated at 30°C. for 4-6 days (bacteria) or 6 days (yeasts) on a rotary shaker. The resultant growths were collected on tared solvent resistant membrane filters washed free of residual substrates with acetone and *n*-hexane, and then dried and weighed.

The substrates tested included four different Fischer-Tropsch synthetic liquid fuel fractions, viz. FTL (boiling range, 0-204°C.), FTD (boiling range, 204-316°C.), FTW (boiling, >316°C.) and SASOL (C₁₁-C₂₀; mainly C₁₂-C₁₈; 64 per cent paraffins; 33 per cent olefines; both mostly normal); low temperature tar fractions, HSF and HSD (both 'hexane solubles' with 7-12 per cent phenol); paraffin-rich fraction CTP and paraffin, olefin fraction CTPO; and petroleum-derived fraction PET (C₉-C₁₄ *n*-paraffins). Other substrates used for comparison were three coal acid mixtures and an artificial mixture of polynuclear aromatic hydrocarbons found in high temperature coal tar.

The highest yields of growth with yeasts were obtained with *n*-hexadecane as substrate. Normal paraffin fraction PET gave yields ranging from 244 mg. dry wt (*C. tropicalis* 410) to 430 mg. (*C. lipolytica* 409B). Fischer-Tropsch fractions FTD (*C. lipolytica* 409B), FTW and SASOL (*Candida* strains) as well as low temperature tar fraction CTPO (*C. lipolytica* 409, 409A, 409B) gave yields within the broad range obtained on fraction PET. The crude protein content of *C. lipolytica* 409B grown on SASOL was 41.1 per cent, which compares favourably with those on petroleum hydrocarbons. The results suggest that, in general, higher yields are obtained on substrates (carbon and energy source) which (i) are higher boiling and thus of higher molecular weight, (ii) contain a high ratio of normal paraffins to olefins, and (iii) are low in phenols or other toxic compounds.

A method for extracting mucoproteins from epithelial tissues

A simpler method has been reported for extraction of poly-

saccharide protein complexes from the epithelial tissue of the stomach wall by I. Hakkinen and his coworker at the Department of Pathology, University of Turku, Finland. The mucoproteins are preserved unaltered as far as possible and only mechanical breakdown of the tissue structure is effected, while allowing the macromolecules to be isolated from the extract.

The method consists in homogenizing the tissue in distilled water followed by washing with 0.15M or more concentrated sodium chloride solution. The extracted polysaccharides in the sodium chloride solution are precipitated with a threefold volume of ethanol and redissolved in water, whereupon the greater part of the coprecipitated tissue proteins remains undissolved. The clear supernatant is quantitatively transferred to a column packed with cellulose treated with 1 per cent solution of cetylpyridinium chloride in 0.05M sodium chloride. The polysaccharides are eluted successively with 0.3, 0.6, 1.0, 1.2, 1.6 and 2.4M sodium chloride solution. Each of these solutions eluates a fraction that is precipitated by cetylpyridinium chloride after dilution of the effluent with distilled water. Portions of the four precipitates are subjected to hydrolysis with papain and re-fractionated on cellulose column pretreated with cetylpyridinium chloride as described earlier [Acta chem. scand., 19 (1965), 800] and polysaccharide contents estimated [Nature, Lond., 210 (1966), 1263].

A cold box for biochemical work

A laboratory cold box (Fig. 1) wherein temperatures down to -15°C. could be reached and maintained for biochemical, enzymological and other works has been designed at the Boris Kidrič Institute of Nuclear Sciences, Beograd, Yugoslavia. The box permits the worker to work in a sitting position and is free from the disadvantages of working with a cold bath or in a cold room. It permits work at reduced temperature while the experimenter himself works at room temperature. The box (working space 100×66×44-66 cm.) uses 'Freon

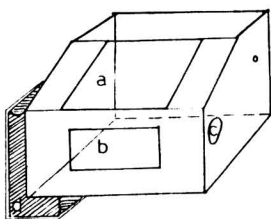


Fig. 1 — Schematic view of the cold box [(a) window (60 × 40 cm.); (b) door (50 × 22 cm.); (c) side opening (14 cm. diam.); and (d) heat insulation]

12' mixture for refrigeration. The tubes are insulated with a 10 cm. thick layer of 'stiroper'. The door and window are made of double phlexiglass, and a wind-screen wiper is attached to clear off the moisture collecting on the outside [*Bull. Boris Kidrič Inst. nucl. Sci.*, 17 (No. 2) (1966), 155].

Studies on communication and dissemination of scientific information

Among the various projects sponsored recently by the National Science Foundation, USA, towards effective communication and dissemination of scientific information are: (1) a study of the abstracting and indexing of scientific literature in USA to be carried out over a 7-month period by the System Development Corporation, Santa Monica, California, under a \$ 185,622 contract; and (2) the establishment of a joint committee on scientific and technical communication by the National Academy of Sciences and the National Academy of Engineering. The purpose of the first study is to determine the current status and effectiveness of abstracting and indexing services and to explore ways of making the entire complex of services more effective. The results of the study will be used primarily to aid the formulation of federal agency policies concerning abstracting and indexing services. The committee set up under the second study will provide a forum for participation by scientific societies in the planning for a national network of science information systems. The committee will give special attention to information activities in the private sector, both

at home and abroad. Of particular concern will be: (1) methods for promoting more effective relationships between information systems and information producers and users; and (2) techniques for improving information transfer, especially those that provide greater selectivity and consolidation of information. The committee will make recommendations both to private organizations and to federal agencies on actions required to maintain effective communication in science and technology.

World scientific information system

At its Eleventh General Assembly held in Bombay during 6-10 January 1966, the International Council of Scientific Unions decided to set up a Committee for a World Scientific Information System. Main among the initial terms of reference of the committee are: (1) ascertaining the factual data concerning existing and contemplated programmes, both international and national; (2) identifying the actual requirements of the system as indicated by the various needs of research scientists, research management, and governments and intergovernmental organizations; (3) outlining a feasible world scientific system giving due consideration to how both national and international services can contribute to, and receive from, the world system; and (4) identifying the gaps to be filled to enable the system to function and the means by which the initial world system can develop in order to keep pace with the developing needs of science.

Automatic Documentation and Mathematical Linguistics

Commencing from January 1967, the Faraday Press Inc., 84 Fifth Avenue, New York, will publish this new quarterly, an English translation of the Russian journal, *Nauchno-Tekhnicheskaya Informatsiya*. Of outstanding interest to mathematicians, engineers and documentalists, the translation will include articles on automatic documentation, mechanical translation, information retrieval and mathematical linguistics. Subscription

rate is \$ 145 per year. Sample contents can be had from the publishers.

Concrete

This monthly, the organ of the Concrete Society, London, starts publication from January 1967. The new journal incorporates *Structural Concrete* and *Concrete and Constructional Engineering*, both of which cease publication. Besides reporting the activities of the Society and events to be organized by it and publishing papers and reports, *Concrete* will include contributed articles and news features on all aspects of concrete design and construction. The annual subscription for the journal is £ 2 10s. or \$ 9.00.

Comments on Nuclear and Particle Physics

This journal, the first of a series of journals to be devoted to critical commentaries on significant current developments appearing in the scientific literature, announced by Gordon & Breach Science Publishers, New York, will start appearing from January 1967. The journal will publish comments on outstanding developments in the fields of particle physics, nuclear physics and astrophysics contributed by eminent physicists. To be issued bimonthly, the journal has a special annual subscription rate of \$ 10.00 for individuals and \$ 20.00 for libraries.

British Scientific Instrument Research Association

The annual report of the Association for the year 1965-66 records significant achievements in its three major areas of activity, viz. (1) Precision processes and optical systems; (2) Industrial measurement and control; and (3) Instrument performance. During the year there were accessions to the Association from diverse user industries such as baking, confectionary, rubber, steel and special chemicals and from manufacturers of plant and machinery for these industries, demonstrating a growing awareness in the industry of the importance of measurement and control.

A technique involving the use of a close-fitting metallized plastic skin has been developed to release the green body from its forming electrode during electrophoretic forming of alumina redoxes; the work is being extended to larger objects. It has shown to be possible to control the thickness of alumina films up to an accuracy of ± 1 per cent by automatic methods. The object of a project in hand is to investigate the possibility of fabricating computer stores by electrophoretically depositing ferrites on to prefabricated wire networks. It has been demonstrated that (1) uniform layers of ferrite can be deposited and will encapsulate the intersection of two or more wires; (2) these coatings can be processed to give suitable magnetic properties; and (3) the intersections can exhibit memory properties.

During studies on surface treatments for reducing the surface resistivity of transparent materials it has been found that low surface resistivities can be obtained, by introducing ionizable groupings into methacrylate polymers such as perspex, at the expense of a slight degree of surface damage. The introduction of polar groups, like sulphones on plastic surfaces, has been found to increase the metallic film adhesion. In the case of sulphonated polystyrene the bond strength of silver films was found to increase by five times. From studies on precise control of the deposition process in the preparation of thin film electronic devices, such as resistors, electron bombardment evaporation has been found to be the best solution for the control problem. For the fabrication of passive elements of micro circuits from previously evaporated thin film multilayers, selective chemical etching processes have been successfully employed.

A theoretical design of a process for depositing and controlling improved anti-reflection films has been worked out. Metal films of the order of few micrometres have been deposited, to the correct contour for aspherizing spherical mirrors, by measuring the optical

FORTHCOMING INTERNATIONAL SCIENTIFIC CONFERENCES, 1967

<i>Date</i>	<i>Conference</i>	<i>Place</i>
14-19 February	Symposium on the Triple State	Beirut
15-17 February	International Solid State Circuits Conference	Philadelphia
1-3 March	Second International Particle Accelerator Conference	Washington, DC
Spring 1967	International Symposium on the Biochemistry of Ribosomes and <i>m</i> -RNA	Germany (Dem. Rep.)
March 1967	Second International Congress for Stereology	Gainesville, Fla.
2-8 April	Seventh World Petroleum Congress	Mexico
3-5 April	Third International Conference on Fluid Sealing	Cambridge, England
5-7 April	International Conference on Nonlinear Magnetics	Washington, DC
9-15 April	International Cryogenic Engineering Conference	Kyoto, Japan
10-14 April	International Symposium on Physical Separation Methods in Chemical Analysis	Amsterdam
18-28 April	Ninth International Hydrographic Conference	Monte Carlo
1-5 May	Second Asian and Oceanian Congress of Neurology	Melbourne
2-4 May	International Conference on Research Reactor Utilization and Reactor Mathematics	Mexico, DF
7-14 May	Fifth International Conference of Engineers	Athens
15-18 May	International Rubber Conference	London
21-26 May	Fifth International Conference on Non-destructive Testing	Montreal
May	Fourth International Heating and Air-conditioning Congress	Paris

performance of the mirror during the deposition with a device utilizing position sensitive photo cells.

During the studies for the control of the structure of thin film, iron has been found to be the most effective underlayer metal governing the structure and electrical properties of the overlying gold film. The nucleation density of the gold films deposited in both normal and ultra-high vacuum has been studied as a function of film thickness and the nucleation energies calculated.

As a first step towards the evolution of an automatic lens-mounting machine, a laboratory model instrument has been fabricated to correct the centring errors of an optical system. In this, a narrow beam of light falling on the lens is transmitted through the lens and as the latter is rotated, the centring errors cause the transmitted beam to rotate. The reflected beam is also deflected if the lens is not accurately centred. This deflection is detected by photo cells,

the signals from the photo cells are processed electronically and transmitted to force transducers which will correct the position of the lens.

For on the line automatic inspection of moving highly specularly reflecting materials, an illumination system has been devised which gives a high contrast image of the surface. To observe the defects from surfaces of poorer specular reflecting properties, a test rig has been built to observe the intensity of the scattered light.

For the measurement of humidity in air at high temperatures, with particular reference to baking ovens, two methods using water-cooled condensers are being investigated. One involves the measurement of flow or drip rate of water from the condenser at known input gas flow rate, and the other is based on the measurement of pressure prior to and after the condenser. This pressure difference after compensation for the temperature drop can be correlated to humidity.

FIRST GET-TOGETHER OF RESEARCH AND INDUSTRY PUBLICATIONS

The Council of Scientific & Industrial Research (CSIR) organized a two-day First Get-Together of Research and Industry in New Delhi during 20-21 December 1965.

The gist of speeches delivered and the key papers prepared are presented in one volume entitled *Addresses and Key Papers*. Out of the papers received, some selected papers having a direct bearing on the objectives of the Get-Together are being published separately under the 15 Working Groups embracing related groups of industries.

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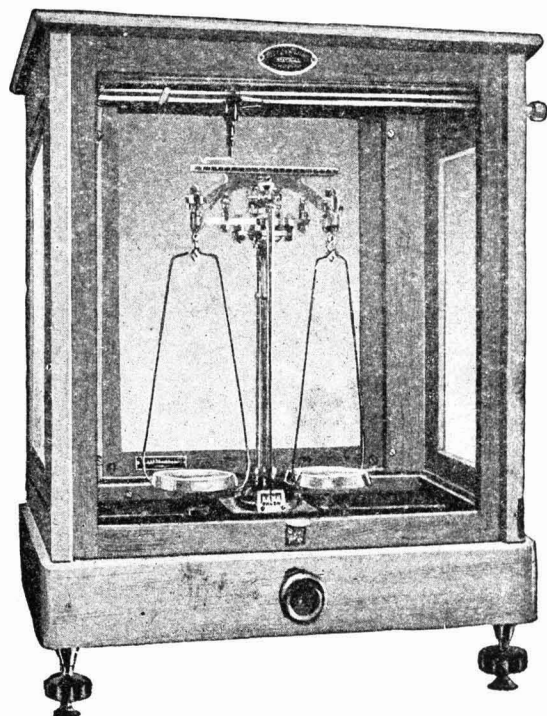
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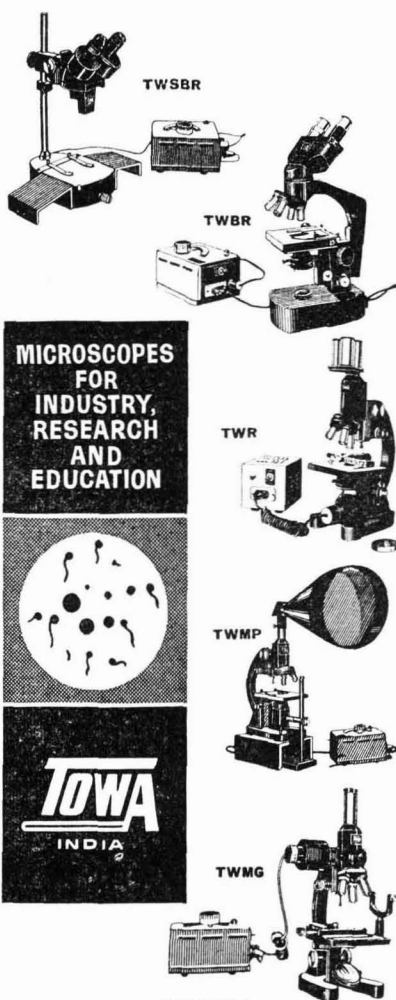
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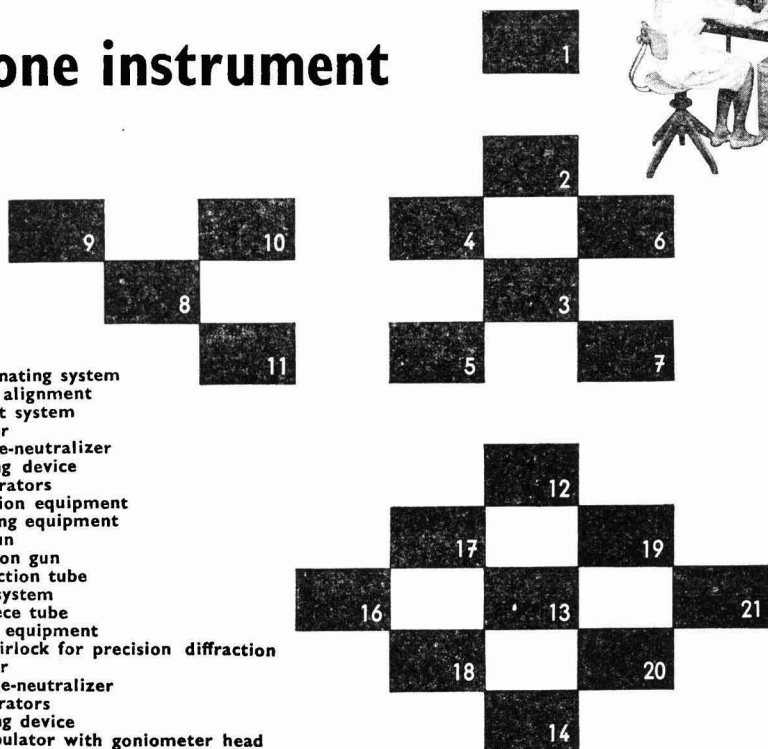
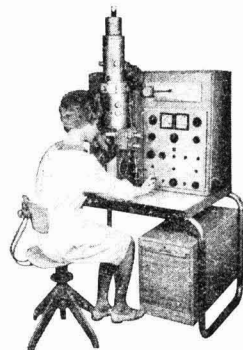
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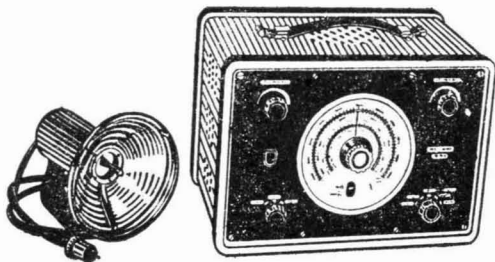
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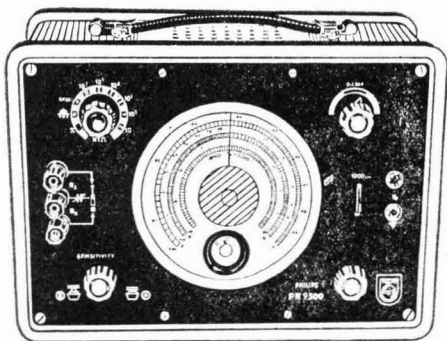
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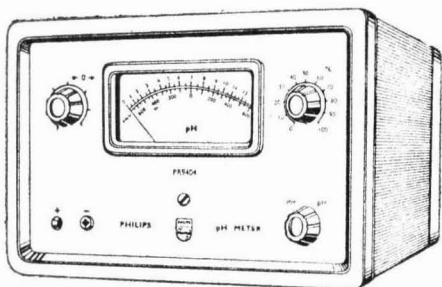
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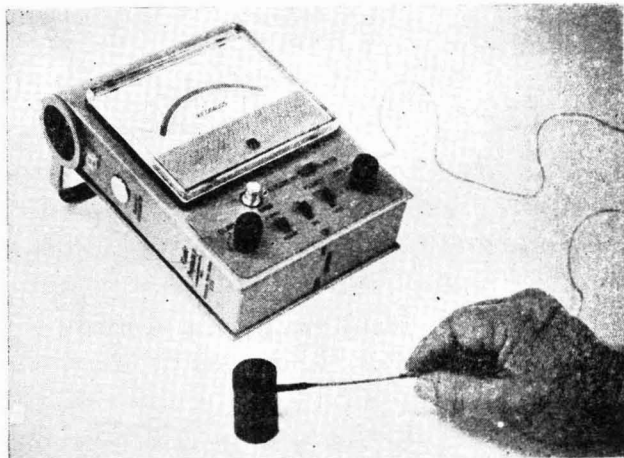
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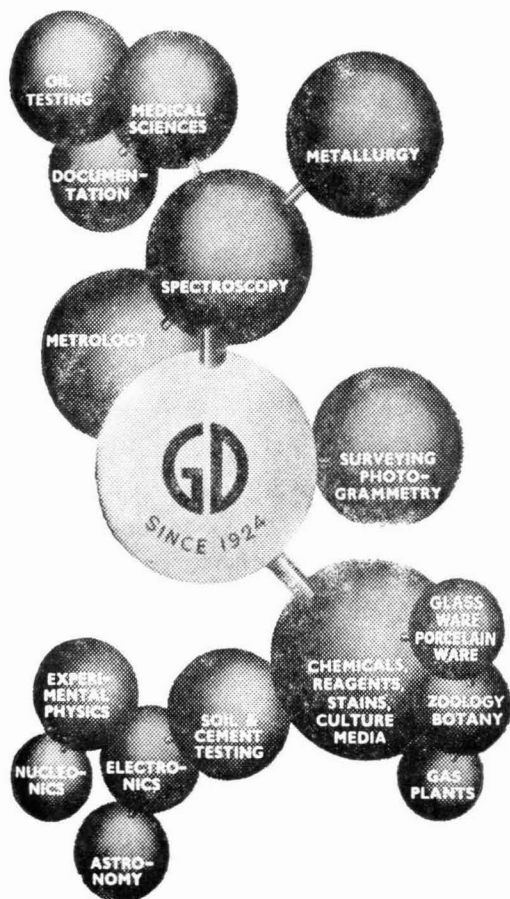
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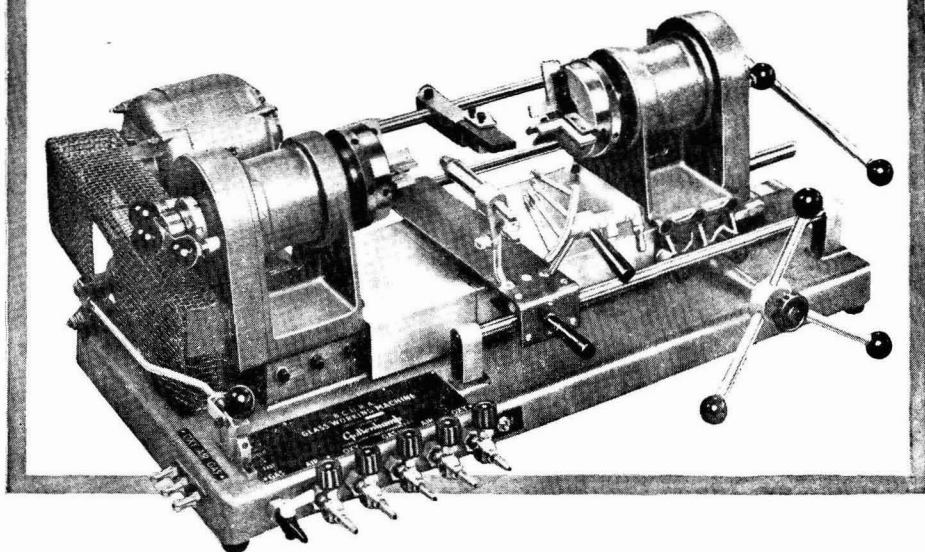
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