

Journal of Scientific & Industrial Research



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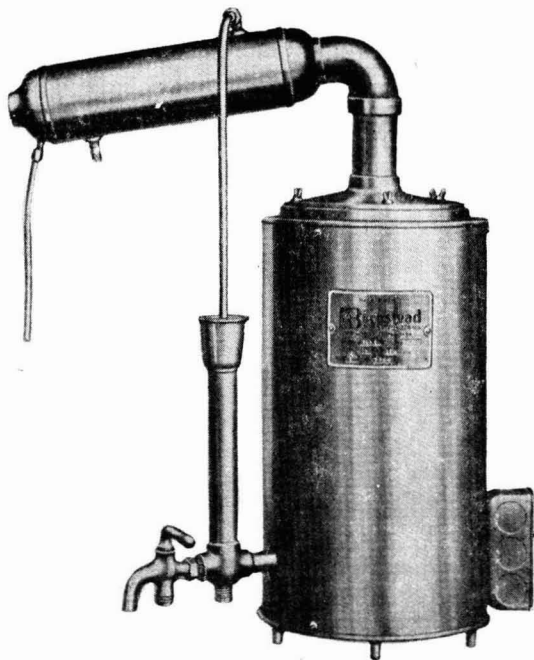
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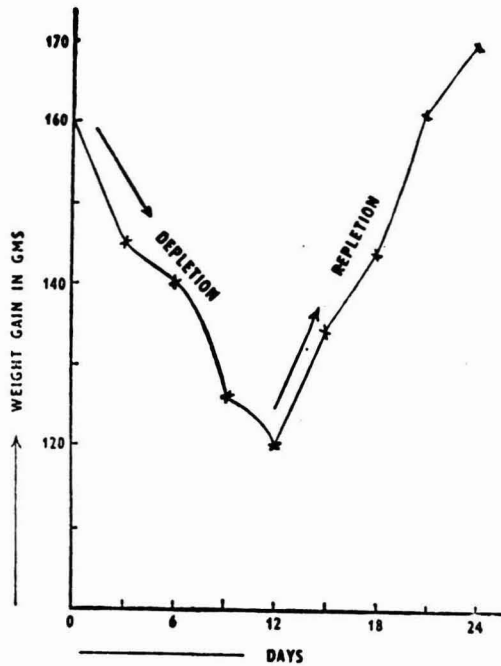
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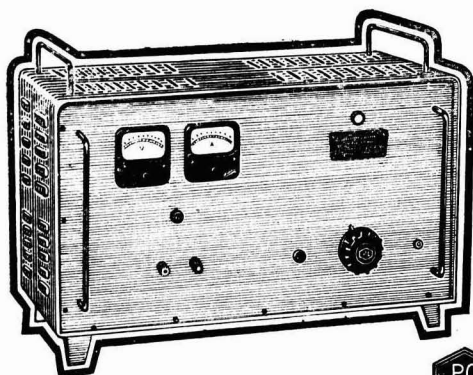
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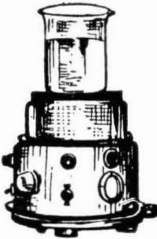
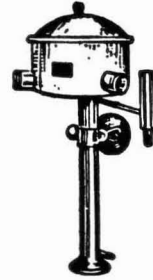
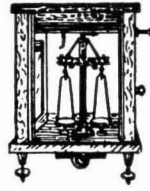
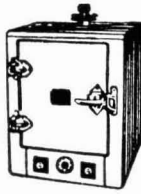
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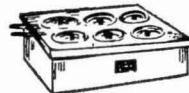
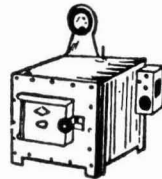
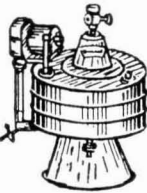
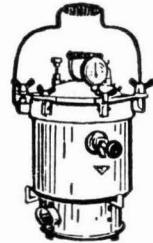
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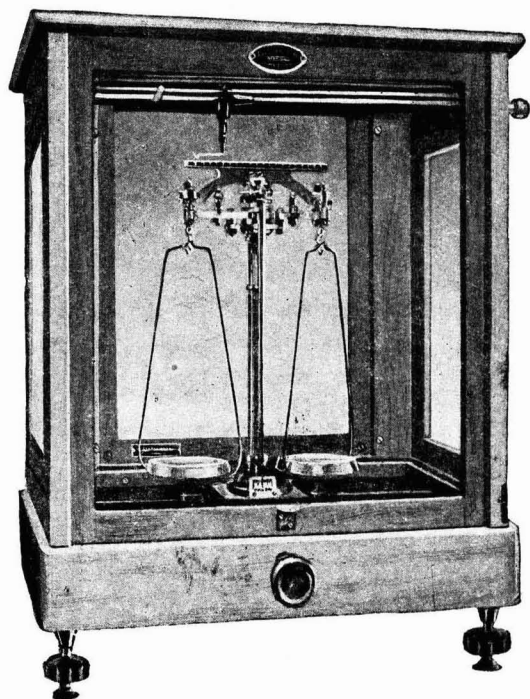
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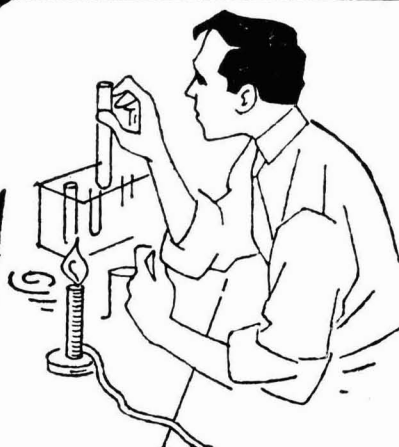
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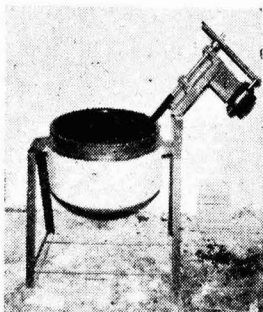
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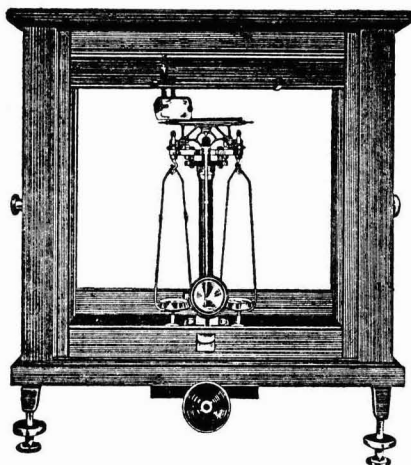


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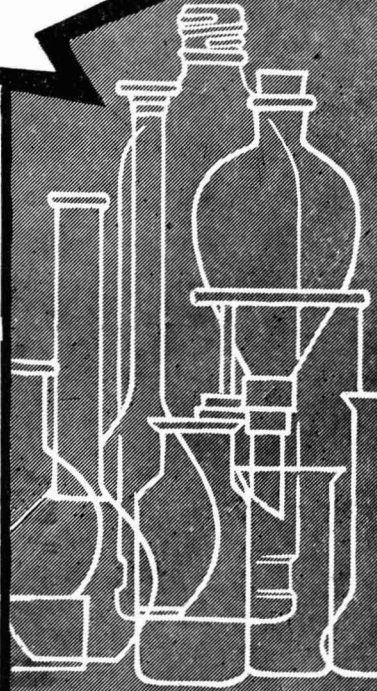
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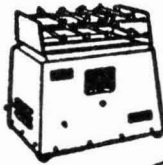
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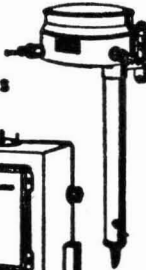
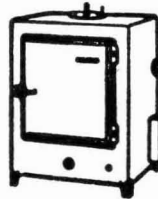
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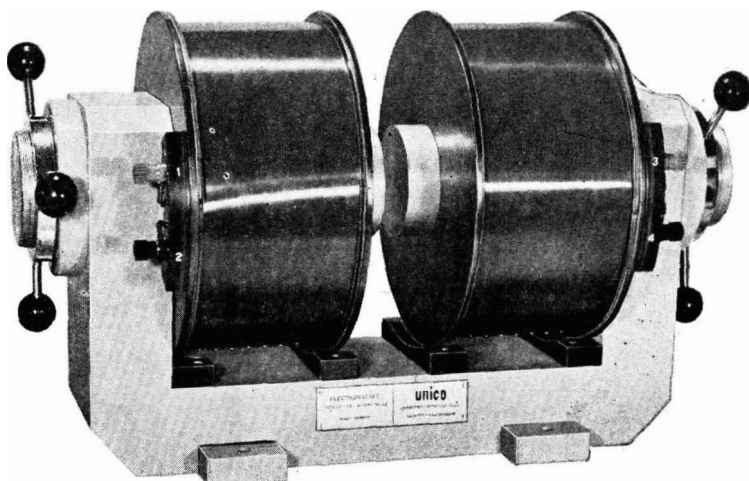
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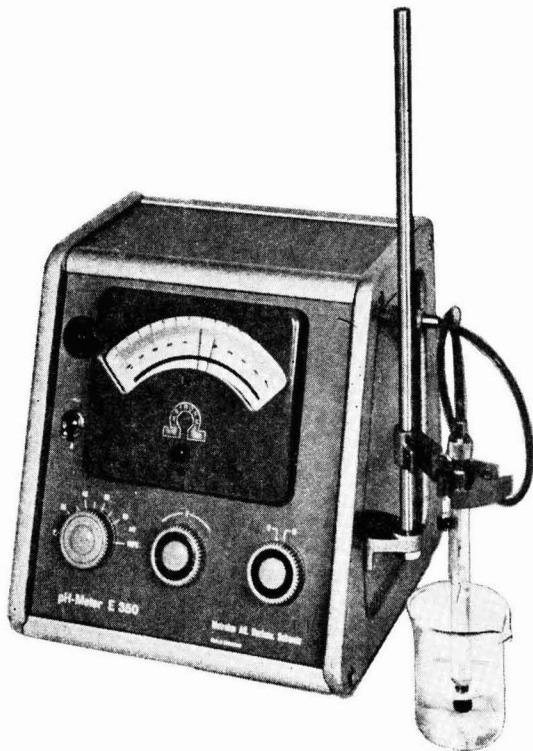
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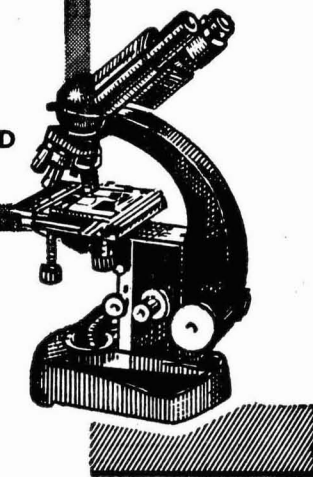
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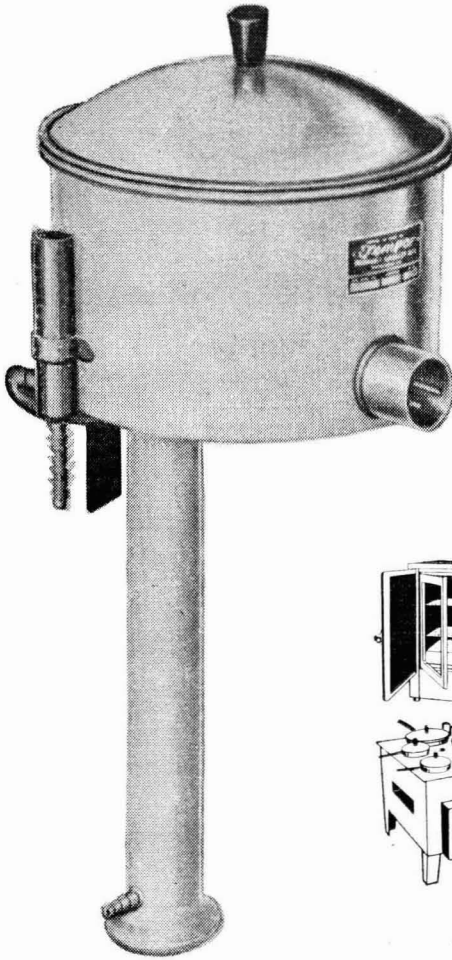


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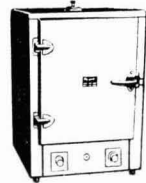
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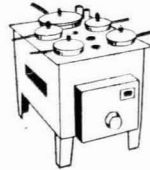
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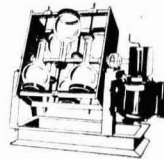


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Current Topics

Indian Agricultural Research Institute: Diamond Jubilee

THE Diamond Jubilee Celebrations (29 March to 3 April 1965), marking the 60th anniversary of the establishment of the Indian Agricultural Research Institute (IARI), New Delhi, is an occasion not only for recapitulating the many useful contributions of the Institute during the past six decades but also for stocktaking of the progress and development of Indian agriculture towards self-sufficiency in food. It should also be an occasion to ponder over further ways and means of efficiently gearing agricultural research to bridge the rapidly widening gulf between food production and demand in the country.

Established in 1905 at Pusa in North Bihar, IARI is the premier agricultural research institution in the country and an important centre for post-graduate training and research in agriculture in South East Asia. The Institute has maintained high traditions of research in both fundamental and applied aspects of agriculture. Since Independence, the Institute has greatly expanded and possesses many unique research facilities. It comprises at present 12 major divisions manned by 700 research workers. In addition to its own research projects, the Institute carries out much collaborative research along with other national, foreign and international agencies. IARI has one of the finest agricultural libraries in the country. It has a large collection of 22,000 insect specimens, known as the National Pusa Institute Collection. The Mycological Herbarium of the Institute is a unique repository of 27,000 specimens of fungi representing 5000 species. The Indian National Type Culture Collection at the Institute is the largest collection of its kind in the country. Many modern research facilities have been added to the Institute in recent years which include a Radiotracer Laboratory and a Gamma Garden with a 200 Curie source of radioactive cobalt.

An idea of the contributions made by the Institute to various branches of agriculture can be had from about 3500 research papers published by the Institute. The Institute has now the status of a university in regard to post-graduate research and training. Since 1958, the Institute has produced 395 M.Sc.s and 132 Ph.D.s in different branches of agriculture.

The Institute has done pioneering work in the field of plant viruses and it has also carried out extensive work in the field of phytopathology, with special reference to fungal and insect diseases of crop plants. Studies on physical characteristics of soil have led to the development of a method for measuring soil structure and indices for various types of soils have been worked out in relation to their fertility. Many of the additive laws which hold good in respect of chemical reactions in soils have been

invalidated as a result of strong interactions noticed in mixtures of soil clays; this finding is of great significance in evaluating the mineralogical composition of soils. A technique for assessing the maximum yield potential of wheat varieties developed at the Institute has been worked out. In entomology, a new biotic theory which would help in controlling locust epidemics has been put forward. This theory explains, for the first time, that both periodicity of locust cycles as well as distribution of locust breeding grounds are determined by fluctuations in the population of non-winged cold-blooded predators which in turn are brought about by variations in sunspot density, and vegetation density. Electro-physiological methods for detecting the toxicity and specificity of insecticides have been worked out. Many important advances in cytology and cytogenetics have emerged from the Institute. Techniques for artificially culturing young embryos of intergenic and interspecific crosses have been developed. Studies on the meiosis of the hybrid wheat, *Triticum zhukovskyi*, have shown that it is the first hexaploid species to have a genomic composition other than ABD. A topological analysis of brown *rust* has revealed that a marked reduction in fitness is not an inevitable consequence of inbreeding in a self-compatible organism as *brassica*. Studies on the effect of drought and light on grain development in wheat have shown that grain weight increases in the dark at the expense of stem sugar and that the ultimate number of grains per ear is due to starch synthesizing enzyme factors residing in the grain itself. Genome analysis in wheat in relation to species adaptability has shown that diploid species are more sensitive to temperature than hexaploid species. These studies have shown that while the B-genome contributes substantially to the adaptability of the wheat plant, the part played by D-genome is negligible. The finding that the mutation rate in *Drosophila* cultures raised from flies fed on irradiated media increases 3- to 5-fold provides a method for assessing the indirect effects of ionizing radiations and their possible harmful effects on the wholesomeness of irradiated food. Among other useful contributions mention may be made of the investigations on the biogenesis of fats and oils.

On the practical side, the Institute has many notable contributions to its credit. The development of the famous *Co.* varieties of sugarcane revolutionized the Indian sugar industry. The Institute has developed the first wheat variety (*N.P.* 809) resistant to all the three rusts of wheat. Other significant achievements relate to evolution of superior varieties of food, vegetable and commercial crops capable of giving high yields and possessing tolerance or resistance to drought, pests and diseases. These include barley, wheat, oats, paddy, maize, *jowar*

(*Sorghum vulgare*), *bajra* (*Pennisetum typhoides*), gram, oilseeds, tobacco, cotton, fodder plants, etc. The Institute has developed the first hybrid *bajra* which yields 100 per cent more grain and 30 per cent more fodder than the strains under cultivation; the highly productive Pusa Giant Napier Grass developed gives 2 lakh pounds of green fodder per year per acre; and the Pusa Giant Berseem (with 32 chromosomes as against the usual 16) gives 20-30 per cent more fodder than other varieties. For grain storage, an inexpensive structure has been fabricated in which a film of polyethylene is incorporated in the body of the mud wall. Inexpensive and efficient methods for controlling a number of plant diseases and pests have been worked out. Improved techniques of crop production, soil management, mixed farming and improved agricultural implements are among other important contributions of the Institute. To the Institute also goes the credit of preparing the first soil map of India.

The record of achievements of the Institute both in fundamental and applied aspects of agriculture is indeed impressive, and with modern research facilities being provided one can look forward to many more important contributions from the Institute. But, as Dr Zakir Hussain, Vice-President of India, observed in his inaugural address, the application of agricultural knowledge in raising agricultural production in the country has lagged far behind its acquisition. Agricultural production made some headway during the first two plan periods (1951-61); the index of agricultural production rose by about 40 per cent by the end of this 10-year period, largely due to more land being brought under the plough, more irrigation facilities, greater use of fertilizers, adoption of better agricultural practices, etc. However, the pace of progress has not been kept up during the Third Plan (1961-66) period. Whereas the target for production of food grains for the Third Plan has been set at 100-105 million tons, the production figure for 1963-64 is only 79 million tons. The gap between demand and production of food grains is continuously widening with the increase in population. This situation is a pointer to more intensive efforts to raise agricultural production.

Dr Zakir Hussain has also observed that the development and progress of agriculture in the country is beset with many intricate problems of a socio-economic nature. Agriculture is India's major industry and its contribution to national income is more than 50 per cent. Therefore, unless agricultural production is stepped up, all other planning activities will not show any tangible results in improving the country's economy. The Institute should devote

more serious attention to the study of problems coming in the way of speedy and efficient translation of the results of research to the field and for adaptation by the farmer. This aspect of agricultural research should receive as much, if not greater, attention and emphasis as other programmes of the Institute and should form an integral part of its activities.

It has been observed that agricultural education, research and extension work are not properly co-ordinated and geared to the solution of the practical problems of the farmer. Measures to bring about such a coordination should form the subject of study and research by IARI, and should receive high priority. Some basic deficiencies in the manner the national agricultural extension service functions have been pointed out as the principal contributory cause for agricultural research not making an impact on agricultural production. If this is a serious bottleneck, it is necessary to inquire closely into what the deficiencies are. This is an important prerequisite for the national agricultural research and development programme which is to be initiated this year and for which an outlay of Rs 2400 crores out of Rs 22,000 crores has been earmarked during the Fourth Plan period.

Advances in agriculture brought about by research can be effectively and with benefit put to use by the professional farmer only. IARI should, therefore, take up, as one of its activities, the evolution of the professional farmer in the country. A lacuna in the Institute's programme of work appears to be the study of and research into socio-economic aspects of agriculture. The Institute should establish a full-fledged division to initiate long- and short-term research projects in this area. It should concern itself with the training of prospective farmers and farm workers. The farmer should not only be educated in marketing practices, but advice should also be provided as regards standards of quality and guidance as to how and where to sell. This is important, as without such advice all measures to improve the country's agricultural structure will not produce the expected socio-economic results. An organization on the lines of the Committee for Agriculture set up by the Organization for Economic Cooperation and Development in Europe, comprising various agricultural interests, may be considered to go into these problems. This body should stimulate inquiry into the practical measures necessary for the adaptation of agricultural research for the benefit of the farmer and to the needs of the general economic growth of the country. In short, much more has to be done in the field of agricultural extension work and the Institute should give a lead in this area as in other areas of agriculture.

Prof. P. Maheshwari, F.R.S.

PROF. P. MAHESHWARI, Professor and Head of the Department of Botany, University of Delhi, has been elected *Fellow of the Royal Society, London*, for his significant contributions in the field of botany, chiefly in the morphology and embryology of seed plants. Born on 9 November 1904, Prof. Maheshwari had his academic education at the University of Allahabad, where he later worked as a research scholar under the late Dr Winfield Dudgeon. He obtained the D.Sc. degree of the University of Allahabad in 1931. He began his teaching career as Lecturer in Botany at the Ewing Christian College, Allahabad (1928-30), Agra College (1930-37) and the University of Allahabad (1937-39). In 1939 he was appointed Reader in Botany and Head of the newly started Biology Department at the University of Dacca, and was later promoted to a Professorship. In March 1949 he was invited by Sir Maurice Gwyer, then Vice-Chancellor of the University of Delhi, to join the University of Delhi as Professor and Head of the Department of Botany. From 1954 to 1956 he also served the university as Dean of the Faculty of Science.

Prof. Maheshwari's chief interest lies in the morphology and embryology of seed plants, on which some 250 papers have been published by him and his collaborators. Some of his important contributions include studies on cryptogams, gymnosperms and angiosperms with special reference to taxonomy, anatomy, embryology and metabolism. As early as 1935, he published an account of the gametophytes of *Ephedra foliata* and cleared the confusion on the terminology for designating the constituents of the male gametophyte and also demonstrated that the nucellus is of dual origin: from the derivatives of the epidermis and from parietal cell. His work also threw light on the life history of certain angiosperms and the taxonomic assignment of several disputed genera and families. He has also successfully grown the reproductive organs of several plants on synthetic nutrient media in aseptic cultures. He is the author of a book entitled *Embryology of angiosperms*, published in 1950 by the McGraw-Hill Book Co., and of a monograph entitled *Gnetum* (jointly with his pupil Vimla Vasil), published by the Council of Scientific & Industrial Research. He has edited a book entitled *Recent advances in embryology*, published in 1963 by the International Society of Plant Morphologists and has in preparation a fourth on the *Morphology of gymnosperms*.

Prof. Maheshwari was elected Fellow of the Indian Academy of Sciences in 1934, Fellow of the National Institute of Sciences in 1935, a Corresponding Member of the American Botanical Society and Honorary Foreign Fellow of the American Academy of Arts and Sciences in 1947, President of the Botany Section of the Indian Science Congress in 1949, and President of the Indian Botanical Society in 1951. In 1950

he was elected one of the Vice-Presidents of the International Botanical Congress held at Stockholm, in 1954 President of the Embryology Section of the International Botanical Congress held at Paris, in 1959 Vice-President of the Morphology Section of the International Botanical Congress held at Montreal, in 1964 one of the Vice-Presidents of the International Botanical Congress at Edinburgh, and in 1965 President of the International Society of Plant Morphologists. From 1956 to 1960 he was Biological Secretary of the National Institute of Sciences of India and now Editor of Publications. In 1959 he was the recipient of the Birbal Sahni Memorial Medal of the Indian Botanical Society and in 1964 of the Sunderlal Hora Memorial Medal awarded by the National Institute of Sciences of India. In 1959 he was elected Foreign Member of the Kaiserlich Deutsche Akademie der Naturforscher, and later he received the honorary doctorate of the McGill University, Montreal. In 1961 he was elected Corresponding Member of the Deutsche Botanischer Gesellschaft and in 1963 of the Royal Dutch Botanical Society.

Prof. Maheshwari has travelled widely in Europe and USA. At the invitation of Unesco he visited Indonesia in 1952 and Egypt in 1954, and in 1958 he paid a short visit to the USSR as a member of a scientific delegation sponsored by the Government of India. In the summer of 1959 he was Visiting Professor at the University of Illinois. In 1961 he visited Germany and England, and in 1964 he gave lectures at many universities in the USA.

Prof. Maheshwari has repeatedly drawn attention to the importance of embryological studies in botanical research and teaching, both at national and international meetings. He is an excellent teacher, technician, research worker and organizer. He aims at nothing but the very best and does not tolerate poor standards. He is held in high esteem by his fellow botanists all over the world.

Prof. Maheshwari has been associated with the Council of Scientific & Industrial Research as a Member of the Governing Body, Chairman of the Biological Research Committee, and Member of the Executive Councils of the Regional Research Laboratory, Jammu, and the Indian National Scientific Documentation Centre, New Delhi. He has been a Member of the Editorial Board of the *Journal of Scientific & Industrial Research* and the *Indian Journal of Experimental Biology*.



Prof. P. Maheshwari, F.R.S.

First International Symposium on Isothiocyanates

P. L. NARASIMHA RAO

Department of Biochemistry, Indian Institute of Science, Bangalore 12

THE first International Symposium on Isothiocyanates was held during 15-17 October 1964 at the picturesque Smolenice Castle (the 'Home of Scientists' of Slovak Academy of Sciences) about 80 km. from Bratislava, Czechoslovakia. The symposium was organized jointly by the Chemical Society of Czechoslovakian Academy of Sciences, the Chemical Faculty of the Slovak Technical University, and the Biological Institute of Czechoslovak Academy of Sciences, Bratislava.

In all 41 papers were presented and discussed in the following two sections: (i) Preparation, structure and reactions of isothiocyanates (20); and (ii) Biological activity of isothiocyanates and their practical applications (21). Majority of the papers were contributed by the scientists from the Slovak Polytechnical University, Bratislava. The proceedings of the symposium were devoted to the following aspects: (i) Synthesis, reactions and derivatives of isothiocyanates (ITC); (ii) Isolation of ITC and heteroglucosides from plants and their biogenesis; (iii) Physico-chemical properties and structure of ITC; (iv) Antimicrobial activity and further biological effects of ITC; (v) Mechanism of action of ITC, structure activity relationship; and (vi) Applications of ITC in chemistry, biochemistry, medicine and agriculture. A novel feature of the symposium was the presentation of the results in the form of lectures with up-to-date information in the respective fields serving as an introduction. These lectures were supplemented by short communications and personal discussions. The striking advance in recent years as to the wide theoretical and practical potentialities of ITC was vividly focused by the proceedings of the symposium.

Earlier work on the isolation of ITC from the seeds of plants belonging to the N.O. Cruciferae was reviewed by C. M. Suther in 'Medicinal chemistry' (1951). They exist in plants in the combined form as glycosides. Several plants containing ITC have antibiotic properties and have been used in household remedies for a number of ailments. In 1939, M. J. Foter found allyl-ITC occurring in horse-radish to have antibacterial properties.

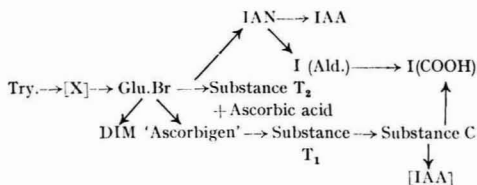
During the years 1948-57 our knowledge on the antimicrobial potentiality of ITC progressed with the demonstration of the high activity of pterygospermin, the antibiotic principle isolated from the roots of *Moringa pterygosperma*, as well as of its decomposition product, benzyl-ITC (P. L. Narasimha Rao and coworkers, Department of Biochemistry, Indian Institute of Science, Bangalore). The latter is the most potent among the unsubstituted homologues and better than the aliphatic members. The isomeric thiocyanates as also the naturally occurring thioglucosides before hydrolysis are not active, while the derived thioureas and other

compounds evoke relatively feeble antimicrobial responses. Benzyl-ITC could be used in seed treatment as it does not inhibit seed germination at antimicrobial concentrations. The wide range of antimicrobial response of the benzyl ester (including antiviral activity against vaccinia virus) was thus brought out and, further, it was postulated that only compounds that conceivably produce it *in vivo* evince significant antimicrobial activity — 'the isothiocyanate theory' — an observation subsequently invoked by several workers to account for the activity of synthetically prepared compounds. ITC inhibit transaminases and the inhibition qualitatively parallels their antistaphylococcal action which is significantly influenced by exogenous pyridoxine, thiamine and glutamic acid. The demonstration of the anti-influenza activity of European cress extracts containing benzyl-ITC in 1957 by Prof. A. G. Winter in Germany reinforced the importance of these studies. The compound itself is now widely used in Czechoslovakia under the name Urogran for treating genitourinary tract infections. Since 1957, hundreds of ITC containing diverse groups as well as the related thioureas, thiosemicarbazides, thiosemicarbazones, rhodanides, etc., have been screened for antimicrobial properties. While extensive search and characterization of thioglycosides, particularly from members of Cruciferae and Capparidaceae, has been in progress in Prof. A. Kjaer's laboratory at Copenhagen and the general biochemical interest in Edman degradation of polypeptides and proteins gathering momentum, comprehensive studies on the preparation, physico-chemical and biological properties of ITC (effect on bacteriophages, bacteria, yeasts and other fungi, algae, protozoa, animal cells in tissue culture and multicellular organisms) were instituted at Bratislava by Prof. P. Nemeč and his associates (published in Czechoslovakian journals).

Distribution and Biogenesis

Prof. Kjaer (Royal Veterinary and Agricultural College, Copenhagen, Denmark) described ITC of natural derivation and their botanical distribution pointing to 'the chemical character of a uniform and highly characteristic group of constituents from higher plants'. He expressed the belief of discovering yet new members of the series. Glucobrassicine has been shown by the workers from the Institute of Experimental Botany, Czechoslovak Academy of Sciences, Prague, to occur in all species of Brassica and Rhabanus. It has also been detected in other genera, viz. Nasturtium, Cochlearia, Sisymbrium and Barbarea while its presence, besides other glucosides, has been demonstrated in Sinapus by H. Schraudolf and in Cardaria by N. Michajlovsky (Institute of Endocrinology, Czechoslovak Academy of Sciences, Bratislava). The following scheme has been suggested as representing the results so far

obtained with labelled compounds in regard to the glucoside metabolism:



The discussion included reports on the effect of γ -radiation, zinc (N. Mašev *et al.*, Institute of Experimental Botany, Czechoslovak Academy of Sciences, Prague) and sulphate (J. Sedlák, Institute of Endocrinology, Czechoslovak Academy of Sciences, Bratislava) on the glucoside levels in the Brassica plants as well as its biosynthesis in cauliflower plant using $^{35}\text{SO}_2$ (M. Kutáček *et al.*, Institute of Experimental Botany, Czechoslovak Academy of Sciences, Prague).

Preparation

A convenient method of labelling ITC is the isotopic exchange with ^{35}S in the absence of solvent at 180° for 4 hr. Purification of the product involves treatment with freshly precipitated copper powder and passage through Brockman's alumina. Gradient elution technique for the chromatographic separation of thioglucosides was communicated by Dr D. L. Carreras Matas (Instituto J. C. Mutis, Madrid, Espanole) but not discussed at the symposium. Among the new ITC derivatives reported by East German workers is an azofluorescein-ITC considered to give better performance in staining procedures.

Physico-chemical Studies

The physico-chemical data included (i) dipole moments, (ii) infrared and Raman spectra, (iii) reaction kinetics and Hammett constant for the reactivity of isothiocyanate group in substituted ITC (and discussed in relation to antimicrobial action), (iv) polarography, and (v) physico-chemical and electro-chemical investigations of electro-conductive systems formed by ITC and amines.

The dipole moments of a series of *m*- and *p*-substituted phenyl-ITC as well as *m*- and *p*-phenylene-di-ITC (K. Antoš *et al.*, Slovak Polytechnical University, Bratislava) are interpreted as indicative of a linear arrangement of NCS radical with the aromatic nucleus. Electron density at individual atoms of phenyl-ITC has been calculated by the methods of Ri and Eyring from the dipole moments of phenyl-ITC and *tert*-butyl-ITC. The theoretical yields of products in nitration of phenyl-ITC have been computed. The NCS group has been considered as similar to halogens in respect of the course of nitration and consonant with the expectations on the basis of the Hammett constant for NCS group. Measurements of reaction velocities of addition of OH^- to ITC have indicated the higher activity of ITC with deactivating substituent groups in the aromatic nucleus. The kinetics of the nucleophilic addition of *m*- and *p*-substituted benzyl-ITC and

glycine at 25° under conditions favouring a first order reaction has demonstrated the effect of the electron accepting substituent in raising the reaction rate.

Dr A. Fava (Department of Chemistry, University of Perugia, Italy) reviewed the available data on the mechanisms of transformation of thiocyanates to isothiocyanates and mentioned that generally two pathways are available, viz. (i) ionization and (ii) nucleophilic attack by the N^- end of isothiocyanate ion. A third possibility applicable to allylic thiocyanate involves an intramolecular non-ionic cyclic mechanism. Tracer and stereochemical evidence now reported indicates that the ionization mechanism is not a simple dissociation ($\text{R-SCN} \rightarrow \text{R}^+ + \text{SCN}^- \rightarrow \text{RNCS}$) but seems to involve an internal ion pair.

The infrared spectra of a series of *m*- and *p*-substituted phenyl-ITC (Š. Kováč *et al.*, Slovak Polytechnical University, Bratislava) have been measured in solvents of varying polarity (cyclohexane, carbon tetrachloride, chloroform, acetonitrile, nitromethane, trichloroacetonitrile and dimethylformamide) and the vibration frequencies correlated with Hammett's constants — *m*- or normal or σ_n constants. A linear plot is obtained in the case of $\sigma_{m\text{-NCS}}$ and $\sigma_{p\text{-NCS}}$ in all solvents and the integrated intensities of absorption bands at 2100 cm^{-1} vary linearly with σ . On the basis of differences in vibration frequencies and integrated intensities of NCS group, various mesomeric structures have been discussed. A broad band around 2100 cm^{-1} , a strong band in the region of 1250 cm^{-1} and a weak one at 927 cm^{-1} (also at 650 cm^{-1} ?) have been reported in the Raman spectra of eleven *p*-substituted phenyl-ITC, the precise values depending upon the nature of the substituent (M. Pisarcik, Slovak Polytechnical University, Bratislava). The variation of the prominent band around 2100 cm^{-1} with Hammett σ_p constants indicates a linear relationship with the negative tangent. A value 0.34 ± 0.09 for σ_p for NCS group has been obtained by extrapolation.

The results of investigations on the polarographic reduction of eighteen *m*-substituted phenyl-ITC (A. M. Kardoš *et al.*, Institute of Polarography, Czechoslovak Academy of Sciences and Slovak Polytechnical University, Bratislava) show that the electrode process is strongly irreversible. The charge-transfer coefficient $\alpha = 0.25 \pm 0.02$ in the alkaline medium, for derivatives for which the shift of half-wave potentials occurs because of substitution, has been quantitatively evaluated. The slope $\beta_{1,1R} = 0.22$ has been calculated for linear correlation of half-wave potentials and the polar constants of the substituent σ_{m-x} . The kinetics of the nucleophilic addition of OH^- ion to the ITC in water-methanol-dioxane systems, studied polarographically, indicate Hammett's correlation of $\log K$ values with polar constants of the substituents σ_{m-x} with slope $\rho = 1.57$.

A dual reactivity of allyl-ITC has been detected in twenty electro-conductive systems formed with various primary, secondary and tertiary amines where it could form thioureas (non-electrolyte) or ammonium thiocyanates (electrolytes) (S. Miskidžjan

et al., Medical Institute, Lvov, USSR). Adequate interpretations have been sought in the light of current as well as Nesmejanov's theories. It is shown that the more basic the amine, the longer is the course of the reaction.

The data on the electrolytic dissociation constants in various solvents obtained by the workers of the University of Lvov have indicated the acid-base character of the reaction of the ITC with amines.

Physical Characteristics and Antimicrobial Activity

In a series of 45 *m*- and *p*-substituted phenyl-ITC, the biological activity, expressed in terms of log ED₅₀ (inhibition of growth, reproduction, spore germination or motility) or/and log ID₅₀ (inhibition of respiration or glycolysis), has been found to be related to the chemical reactivity of the NCS group (log *K*, Hammett constant). Alternatively, it is related to logarithm of molecular solubility or distribution coefficient implying the entire molecular characteristics. Prof. Nemeč and his associates (Slovak Polytechnical University, Bratislava) find it possible to express the observed antibacterial effects of the ITC along with any determinable chemical rate constant by means of a modified Hammett equation. On the other hand, the inhibitory activity against yeasts and EAC-cells is best explained by the alternate relationship. Explanations are put forth for this preference on the basis of localization of ITC on bacterial cell membranes by interaction with protein component (enzyme), and in the second case, as due to adsorption, distribution and transport into the cells favoured by lipophilic nature. While these observations and deductions undoubtedly increase our understanding of the physical and functional aspects of ITC in relation to microbial inhibition, it should not be forgotten that systemic action of ITC involves, besides cells of the parasite, diverse host cells conceivably exposing number of reaction sites for their attack. Apparently factors such as time of contact, specificity, primary interaction and degree of reactivity, nature of binding, reversible or irreversible, with the host cells are equally significant for the choice of suitable ITC and interpretation of their therapeutic action.

Potential Mustard Oil Producers

Some compounds (apart from the substituted thiomethane sulphonates referred to by the author) that conceivably give rise to ITC *in vivo* were described by Prof. A. Rieche (German Academy of Sciences, Berlin) and others. These include esters of dithiocarbamic acids prepared by treatment of salts of the acids with alkyl halides or olefines and by the action of diazonium salts. They display high bacteriostatic and fungistatic activities *in vitro*. The *N*-substituted thiocarbamic acid-*O*-aryl derivatives obtained on acylation, particularly *N*-phenylethyl and *N*-benzyl derivatives, are strongly fungistatic. Further, these compounds exert virostatic effect on influenza virus and thus show marked prophylactic action. 3,5-Substituted tetrahydro-1,3,5-thiadiazine-2-thione, formed by cyclization of sodium dithiocarbamate with formalin, is highly fungistatic and, in young animals, allows

better growth than aureomycin when given in feed. The ready breakdown of the thiadiazines into ITC in aqueous solutions has been observed by Dr D. Martin (Berlin Academy of Sciences, Berlin). The influence of substituents on this fission has been demonstrated by quantitative separation and identification of the phenol and ITC, formed from the *O*-aryl derivatives, by thin-layer chromatography. While this fission is little dependent upon temperature, it is strongly affected by the polarity of the solvent and by the electron attracting substituents in the *O*-aryl residue. The breakdown into benzyl-ITC *in vivo* has been further demonstrated using S³⁵ labelled substances. The compounds referred to inhibit protein and RNA synthesis in bacteria and markedly lower respiration.

Other types of compounds conceivably coming under this group are substituted rhodanines, thiazolidindiones, 2,4-pseudothiohydantoins as also the six-membered analogues which could be advantageously prepared from the appropriate ITC as described by Dr N. M. Turkjevič and Dr E. V. Vladimirska (Medical Institute, Lvov). 3-Substituted rhodanines are obtained by treating potassium or sodio derivative of methyl α -thiocyanacetate with ITC. 2' and 2',3'-Substituted pseudothiohydantoins are formed by condensation of substituted thioureas with α -chlorocarbonic ester. Use of autoclaves or sealed tubes may be obviated if ITC is boiled in alcoholic solution for long periods for obtaining thiourethanes which yield 3-substituted thiazolidindione-2,4 and thiazodione-2,4 by condensation with monochloroacetic or chloropropionic acids.

Biological Effects

By far the largest number of communications dealt with antimicrobial effects of ITC and formed the central theme of interest in the symposium. The data presented were not only distinguished by the number and diversity of ITC and related compounds examined, but also the wide range of microorganisms and other parasites on which tests had been conducted.

Antimicrobial activity — Apart from the structural contributions of ITC, Prof. T. Zsolnai (Medical University, Debrecan, Hungary) focused the significance of genetical factors and further suggested the intracellular inactivation of sulphhydryl microbial constituents (cf. transaminases) as a probable mode of action of ITC and potential ITC compounds — an observation rather pertinent in view of the unpublished results of Dr S. Mahadevan (Department of Biochemistry, Indian Institute of Science, Bangalore) on inhibition of certain other thiol enzymes (e.g. mold nitrilase) by phenyl thiocyanate, phenyl- and benzyl-ITC. The antagonism between the potent benzyl ester and thiamine has been referred to, which, in the light of recent studies, has led to the postulate that their isosterism is a basic factor in the expression of antimicrobial action of benzyl-ITC. Antimicrobial activity of numerous mono- and polynuclear aromatic and heterocyclic compounds has also been presented by Prof. Nemeč and Prof. Rieche groups. The antimicrobial, including antiviral activity of nuclear and side-chain substituted benzyl isothiocyanates as well as of salts of

substituted benzylthiomethane sulphonates, was discussed by the author proposing the extension of the earlier isothiocyanate theory to embrace isosterism with thiamine to explain their action.

Antifungal activity — Dr M. Zemanova (Comenius University, Bratislava) brought out the industrial importance of ITC in the protection of textiles, leather, paper, optical construction materials, etc., against fungal attack by practical trials after having determined the potentialities of hundreds of isothiocyanates in tests on spp. of *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium*, *Tricoderma*, *Alternaria*, *Monillia*, etc., and other organisms, e.g. yeasts, algae and protozoa. The successful clinical trials with *p*-bromophenyl-ITC in *Tricophyton* infections at Dermatology Clinic, Komensky University, Bratislava, have been summarized by Dr L. Chmel (Comenius University, Bratislava). Cases of skin and beard infections as well as infiltrative suppuration of capillaries respond favourably within 24-48 hr and become negative within 6-9 days. The veterinary applications have been described by Dr M. Torda (Agricultural Polytechnical University, Nitra, Czechoslovakia) who recommends 10 per cent triethyleneglycol solution in the form of spray (or in an oil base) for effective control of *Tricophyton* infections in heifers and horses which are quite common in Czechoslovakia.

Antihelminthic activity — Screening for antihelminthic activity of about 100 natural and synthetic mono- and polynuclear aromatic and other ITC has been reported by Dr D. Bačíková (Slovak Polytechnical University, Bratislava) using *Anguillula aceti* as the test organism, but the findings of M. Breza *et al.* (Institute of Helminthology, Czechoslovak Academy of Sciences, Kosice) are of more practical interest in veterinary medicine, in that they describe an effective means of dehelminthization in cattle by *p*-bromophenyl-ITC. *Müllerius capillaris*, *Heamonchus contortus*, *Nematodirus*, *Trichostrongylus* and *Chabertia ovina* infections have been successfully controlled at oral dosage levels of 0.1-0.2 g./kg. in lambs (*F. hepatica* and *D. dentriticum* do not respond favourably to this treatment). Thus an effective means of controlling strongylates of ruminants is now readily available. Using the above compound as well as *p*-ethoxyphenyl-ITC the helminthic infections in fowls have been effectively controlled as reported by J. Pačenovský (Agricultural Polytechnical University, Nitra, ČSSR).

Antiviral activity — The effectiveness of the potential mustard oil producers in experimental mouse influenza infections has already been referred to elsewhere in the report. It has been demonstrated in this laboratory that the highly bacteriostatic *o*- and *p*-substituted as well as the unsubstituted benzyl-ITC inhibit both vaccinia and PR₈ strain influenza

viruses. A few derivatives of aminothiotriazole which hold clinical promise are, however, exceptions ('Amine-X' which holds the best therapeutic promise among drugs till now known) to this rule, in that they exert specific antivaccinia activity without inhibiting bacteria or fungi. *o*- and *p*-Methoxybenzyl-ITC, however, retain the anti-influenza activity. Thus the specificity of antiviral action among this group of compounds has been focused. *p*-Methylbenzyl-ITC is more toxic to eggs and animals than the unsubstituted or *o*-methyl compounds. These results were discussed by the author in relation to their isosterism with vitamin B₁ and in the light of biochemical and clinical observations on cocarboxylase and vitamin B₁ deficiencies in vaccinia infections.

Neoplastic processes — Prof. M. Lopez (University of Pisa, Italy) first observed in 1951 that α -naphthyl-ITC (ANIT) causes proliferation of the bile ductules in rats. He has now added further information on this phenomenon on its action as well as on those of 1- α -naphthylthiourea and di- α -naphthylthiourea, benzylsulphocyanic acid and phenyl-ITC. However, the changes caused by ANIT were discussed in great detail by Prof. H. Ungar (The Hebrew University, Jerusalem, Israel). The biochemical changes include rise in serum bilirubin, total cholesterol and alkaline phosphatase; while the glutamic oxaloacetic transaminase levels increase in serum, they decrease in liver recalling in this context our earlier findings on the inhibition of transaminases by benzyl-ITC and other ITC. The main morphological change at the acute stage is a severe necrotizing cholangitis and cholangiolitis. Bile ductule proliferation and fibrosis in the walls of the intrahepatic bile duct dominate the picture in the chronic stage. Acute poisoning produces transient jaundice of the obstructive type and stimulation of mitotic activity in the epithelium of liver and bile ductules, protractive poisoning resulting in biliary cirrhosis accompanied by bile ductule proliferation. The cholangioplasmic effect can, however, be reversed if the administration of ANIT is stopped. The liver cells are capable of acquiring resistance to the agent. The striking effect noticeable, however, is the ability of ANIT to inhibit haepatoma formation induced by azo dyes (e.g. butter yellow), but not by certain other haepatomogen, which is reflected accordingly in the antigenicity of the liver cells. Immunological and histological (including historadiographic) evidence has been adduced in support of these observations.

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Symposium on Puzzolanas—Their Survey, Manufacture & Utilization

A SYMPOSIUM on Puzzolanas — Their Survey, Manufacture and Utilization was organized by the Central Road Research Institute, New Delhi, from 18 to 20 December 1964. The problem is of great importance to civil engineers interested in the use of cement-saving materials and admixtures imparting beneficial properties to mortars and concrete. It is also of interest to engineering geologists engaged in the survey of puzzolanic materials as well as to industry. Thirty-seven papers including about a dozen by delegates from abroad were presented at the symposium. The following main fields were covered: (1) Occurrence of puzzolanic materials and their processing; (2) Properties of different types of puzzolanas; (3) Fundamental studies on puzzolanic reactions; (4) Manufacturing methods; and (5) Use of natural and artificial puzzolanas.

Occurrence, Properties and Processing of Puzzolanic Materials

The natural puzzolanas like volcanic tuffs and trass available in Italy and Germany and their general properties were discussed by Giovanni Malquori (Italy), Henz Kremser (Germany), U. Ludwig and H. E. Schwiete (West Germany), A. Steopoe (Rumania) and Erich Johann Zirkel (Austria). The properties of Austrian fly ashes were discussed by O. W. Blumel (Austria). H. P. Oza (Engineering Research Institute, Baroda) surveyed the puzzolanic material resources of Gujarat State. In a paper from the Central Road Research Institute, New Delhi, the results of a country-wide survey on the puzzolanic clay deposits of different regions conducted during 1960-64 were presented. A list of the major deposits (70) was appended. The survey has, in general, indicated the Indo-Gangetic plains to be mainly composed of alluvial, silty soils and deficient in good deposits of puzzolanic clay. Black cotton soils, despite their high clay mineral content, have generally been found to contain medium reactive puzzolanas. Lateritic clays of the West Coast composed of South Maharashtra, Mysore and Kerala contain highly reactive puzzolana deposits. Highly reactive clays have also been found to be associated with kaolin deposits. Besides white kaolins many impure kaolins in Kerala, Mysore, Gujarat, Bihar, Orissa, West Bengal and Madhya Pradesh lying as waste heaps in mining areas as well as some of the colliery shales in Bihar, Madhya Pradesh and Rajasthan have been found to be quite suitable for the manufacture of good puzzolanas.

Chemical and mineralogical composition, physical characteristics and puzzolanic activities of fly ashes from the major existing thermal power stations in India were reported in a paper presented by S. K. Chopra, S. S. Rehsi and S. K. Garg (Central Building Research Institute, Roorkee). Compared to fly ashes produced elsewhere, the Indian fly ashes have been found to contain a higher content of unburnt fuel and to be coarser. Their main mineralogical

constituents are quartz, haematite, magnetite and mullite. In general, the Indian fly ashes have been found to fulfil the specified requirements of lime reactivity test and Fratini test. The finer fly ashes have been found to have higher activity; no definite relationship has been established between the puzzolanic activity and any other physical characteristic of a fly ash or its chemical or mineralogical composition. Puzzolanic cements containing 20 per cent of fly ash have been found to fulfil all the chemical and physical requirements as well as to possess requisite strength for portland-puzzolana cement laid down in the Indian standard specification (IS: 1489-1962). The authors also reported a method of proportionating fly ash-concrete mixes for attaining 28 days' strength equal to that of the corresponding portland cement concrete.

During the discussions following the presentation of papers at this session, the occurrence of natural puzzolanic deposit near Ranavav cement factory in Gujarat and the possibilities of its use for puzzolanic cement manufacture were mentioned. Interesting information on the alunite deposits in Trinidad and its possible use as a puzzolana was given by the delegate from Trinidad. Similar information has been obtained from Central Road Research Institute's survey of a few clays of the Rann of Kutch with high aluminium sulphate content which showed high puzzolanic activity after calcination. Interesting discussions ensued on the quality of Indian fly ashes compared to those of Austria and Japan. While recognizing that Indian fly ashes available at present needed improvements through better methods of processing to attain those standards, these were felt to be good enough for use under conditions of good quality control. The effects of high calcium and carbon content of some Indian fly ashes were discussed.

Fundamental Studies on Puzzolanic Reactions

U. Ludwig and H. E. Schwiete (Germany) reported the observations on the nature of products formed during trass-lime and puzzolana-lime reactions. The hydrates formed in the trass-lime-gypsum-water system have been found to be same as in the portland cement-water system. Giovanni Malquori (University of Naples) also discussed the lime-puzzolana reactions with reference to their influence on the strength of the resulting products. Tests were described for determining the puzzolanic activity of various materials. A. Steopoe (Rumania) reported a modified method for the determination of active components of trasses based on the treatment of hardened lime-trass or cement-trass paste with hydrochloric acid ($d, 1-12$) as a result of which only the bound SiO_2 passes into solution and can be determined analytically. Studies on different varieties of trasses have revealed that a trass rich in active silica gives binding materials which require more mixing water than pure cement. Therefore, trass-based hardened pastes have lower bulk density and show greater shrinkage. The existence of a

close correlation between the puzzolanic activity and lime absorption, hydration characteristics and silica and mixed oxides contents of a variety of puzzolanic materials, based on studies on lime-puzzolana-sand mortars was reported by M. K. Chatterjee and D. Lahiri (Department of Applied Chemistry, Calcutta University). The following equation has been derived on the basis of statistical analysis, correlating soluble silica plus alumina content (S) and packing density (P) with compressive strength (Y):

$$Y = 364.9S^{0.737}P^{1.549}$$

From studies on coprecipitated silica-alumina gels, N. R. Srinivasan (Central Road Research Institute, New Delhi) has been able to draw a parallelism between the structural requirements of silica-alumina catalysts used for petroleum cracking and silica-alumina synthetic puzzolanas. Thus, in both there is tetrahedral coordination of alumina and the electrostatic balancing of the structures is through protons. The mechanism of puzzolanic reactions has also been shown to involve a quick adsorption reaction followed by a slower diffusion process, besides certain quick reactions approaching the rate of ionic reactions.

From studies on alkali aggregate reaction (the reaction between reactive form of silica in the aggregate and alkali in the cement), S. P. Brahma and P. Ganguli (Calcutta) have attempted to explain the mechanism of the reaction and the effectiveness of puzzolanas in inhibiting it on the basis of the difference in the diffusivity rates of Ca and Na ions. The provision of a large surface by the puzzolanas has been shown to reduce the alkali concentration at the reaction sites. Puzzolanic replacement of cement also reduces the amount of alkali released from the cement. H. L. Uppal and Mohinder Singh (Land Reclamation, Irrigation and Power Research Institute, Amritsar) discussed some aspects of the development of strength in cement-puzzolana and lime-puzzolana mixes during hardening. It has been observed that the original physical characteristics of the puzzolanic material, such as its hardness and porosity, greatly influence the performance characteristics of the puzzolanas.

Discussions took place on the following topics: Malquori's definition of pseudo puzzolanas; the possibilities of formation of Gehlanite hydrate under conditions of high lime concentration; the identification of the hexagonal C_3AH_{12} phase; the efficacy of acid extraction method to evaluate trasses; the 'potential strength' of puzzolanas and their Ca absorption capacity; the 'pessimism ratio concept' in alkali-aggregate reaction; and the parallelisms between silica-alumina catalysts and the puzzolanas.

Manufacturing Methods

Production techniques and the uses of a special puzzolana-metallurgical cement developed in France were outlined by La Loïse (France). The cement is prepared by grinding together in specific proportions portland cement clinker, granulated blast furnace slag and fly ash obtained from thermal generators using pulverized coal, together with a

small addition of gypsum. For obtaining a satisfactory product, the fly ash used should have a low lime content ($CaO < 4$ per cent), low unburnt carbon content (< 6 per cent) and $SiO_2 + Al_2O_3 + Fe_2O_3$ content of at least 85 per cent. Concrete obtained from the resulting cement attains strength comparable to that obtained using portland cement even at early ages and surpasses the strength of ordinary cements at later ages. A survey of the developments in Japan in the direction of utilizing fly ash as a constituent of special cements was presented by Masatano Kokubu (University of Tokyo). Besides discussing problems like handling and transport of fly ashes, the influence of such factors as carbon content, uniformity of composition, etc., on the quality of the processed fly ashes was dealt with. Ardaman Singh (Beas-Sutlej Link Project, Nangal) described India's first modern plant set up for the production of high quality burnt shale puzzolana used in the construction of Bhakra Dam. The various quality control measures adopted and the economics of production were detailed. Two papers contributed from the Rana Pratap Sagar field laboratory, Chambal Project; Kota, dealt with comparatively small-scale production of *surkhi* by calcination of clay in a small rotary kiln and the use of *surkhi* in the construction of the dam. In a paper resulting from studies jointly conducted by the Central Road Research Institute, New Delhi, and the Central Water & Power Commission, New Delhi, the economics of different modes of grinding *surkhi*, viz. (i) pan grinding, (ii) ball mill grinding, and (iii) ball mill grinding followed by grinding in a vertical flour mill, were given. The use of ball mill has been found to be the most economical, the cost of grinding being Rs 10.25 per ton. Two other papers, one by H. L. Uppal and Grusharan Singh (Land Reclamation, Irrigation and Power Research Institute, Amritsar) and the other by S. S. Ahmad (PWD Research Institute, Lucknow) gave results of studies on the puzzolanic properties of *surkhi* prepared from waste bricks prepared from alluvial soils in Punjab and UP respectively. The results of the latter studies have shown that (i) *surkhi* prepared from semi-burnt bricks has superior puzzolanic properties than that prepared from well burnt bricks, (ii) the puzzolanic properties improve with fineness, and (iii) fat lime-*surkhi* (1:2) mortar has the same performance as cement-sand (1:6) mortar. M. L. Bhatia, S. C. Banerjee, Bahadur Singh and K. S. Bhalla (Central Road Research Institute, New Delhi) presented results of studies on the production of reactive *surkhi* using down draft and rotary kilns. The estimated cost of production in these kilns varies from Rs 42 to 50 per ton, depending upon fuel costs. The various technological and economic factors related to the efficient utilization of different types of puzzolanas—both those derived from natural clays and those obtained by processing of industrial wastes, like fly ashes—were discussed in a paper presented by R. K. Ghosh and N. R. Srinivasan (Central Road Research Institute, New Delhi). Reactivity limits for puzzolanas intended for different applications were given. Specific uses for puzzolanic materials of different degrees of reactivity were recommended. Fly ashes with a

reactivity of about 800 lb./sq. in. have been considered useful for the manufacture of puzzolanic cements, those with medium reactivity (600-800 lb./sq. in.), in combination with lime, for use in foundation concrete and base course under pavement and those with very high reactivity (1300 lb./sq. in.) for preparing lime-puzzolana mortars.

Interesting discussions followed on the relative economics of production of different types of puzzolanas coupled with their reactivity properties. The very high reactivity of certain burnt clay puzzolanas developed at the Central Road Research Institute was recognized, but the need to bring down the cost of production was stressed. Much interest was shown in the French paper on the manufacture of metallurgical puzzolana cement and on the Japanese account of the manufacture of portland-fly ash cements. The upgrading of the medium reactive fly ash available in India through suitable processing methods was also discussed. The difficulties of the portland cement manufacturers in India towards the manufacture of puzzolana cements in their factories were outlined. The production of medium reactive *surkhi* from brick-bats wherever these satisfied the specification requirements was also discussed. It was generally felt that there was need for and scope to develop production of different types of locally available puzzolanic materials, keeping in view the relative economy and the structural requirement in the case of each work. The need to develop lime industry on modern lines was also stressed and some measures towards this end were discussed.

Utilization of Puzzolanas

The use of puzzolanas in irrigation projects featured in four papers. Pais Guddou, N. C. Rawal and M. B. Roy (Central Water & Power Commission) presented the results of studies on the use of *surkhi* in various river valley projects, with particular reference to calcination temperature, fineness, compressive and flexural strength, modulus of elasticity and shrinkage. Laboratory tests have indicated that the puzzolanic properties of *surkhi* are closely related to calcination temperature and fineness of grinding. The strength of mortar decreases with increase in cement replacement by *surkhi*. The drying shrinkage of mortar increases progressively with increase in water content in the mix. The finer the *surkhi*, the greater the shrinkage of the *surkhi*-cement mortar. Similar results have been obtained from the use of puzzolana in the Chambal Project where two types of *surkhi*, obtained by calcination of soil and shale, have been tried. The experience gained from the use of puzzolanas in the construction of Bhakra Dam formed the subject of a paper by B. R. Palta and P. S. Rao.

The advantages accruing from the use of fly ash as partial substitute for cement in the construction of dams, roads, tunnels and foundations were enumerated in a review article by T. Mizukoshi and H. Hasegawa (Tokyo Electric Power Co.). Notable among these are: (1) after 6 months' curing, the compressive strength of fly ash concrete is about 110 per cent compared to that of ordinary concrete; (2) additions of fly ash improves the tensile strength,

particularly at later ages; and (3) the workability of concrete is improved and, therefore, the unit water content of concrete can be decreased with consequent reduction of bleeding water.

About half a dozen papers dealt with the use of puzzolanas in building and road construction. In a joint paper from the Central Water & Power Commission and the Central Road Research Institute, New Delhi, the results of detailed studies on cement-reactive *surkhi*-sand mortars with and without lime were presented. The incorporation of reactive *surkhi* alone and lime-reactive *surkhi* has been found to result in a higher flexural strength/compressive strength ratio, lower modulus of elasticity and higher strain-taking capacity compared to pure cement mortars. Cement-reactive *surkhi* mortars show higher percentage shrinkage compared to the corresponding plain cement mortars. M. G. Padhye, M. R. Vinayaka and S. V. Hasabnis (Maharashtra Engineering Research Institute, Nasik) presented a new approach to lime mortar mix design based on absolute volume basis, keeping the lime content constant. The new approach is claimed to be a marked improvement over the conventional fixed proportion system, since the optimum ratio of lime to *surkhi* relative to the sand content varies with the lime content and the optimum cannot be exactly arrived at in the fixed proportion, since all the factors — lime, *surkhi* and sand contents — and consequently the yield change simultaneously with change in proportion.

The results of actual large-scale trials with reactive *surkhi* in mortars for masonry and plastering and in lime pointing were presented by O. P. Kapur (Punjab PWD, Rohtak). By replacing the usual cement-sand (1:6) mortar by lime-reactive *surkhi*-sand (2:3:8) mortar, a saving of 2.2 per cent for masonry work and 14 per cent for plastering work has been shown possible. R. K. Ghosh, N. R. Srinivasan, M. L. Bhatia and S. G. Banerjee (Central Road Research Institute, New Delhi) reported the finding that for similar compressive strength, lime-*surkhi*-sand mortars possess higher flexural strength compared to leaner (1:5 or 1:6) cement mortars usually used in masonry works. Lime-*surkhi* mortars have lower values of modulus of elasticity and greater extensibility, which improves the resistance to cracking due to load stresses.

A mix design procedure for fly ash concretes developed at the University of Glasgow was described by P. D. Arthur, Visiting Professor at the P.S.G. College of Technology, Coimbatore. The procedure involves the determination of the ratios (by wt) of ash to cement (F), water to cement (W), and total aggregate to cement (A). Knowing these, the mix proportions are given by

$$\text{water : fly ash : cement : aggregate} = W : F : 1 : A$$

The reliability of the procedure has been established for a wide range of British fly ashes by an exhaustive series of tests.

Two papers from the Central Road Research Institute, New Delhi, dealt with the use of reactive *surkhi* in road construction. The first paper by R. K. Ghosh, N. R. Srinivasan and Ram Lal

discussed the performance of a test concrete road, in which 20 per cent cement was replaced by *surkhi*. It has been concluded that for satisfactory performance, the puzzolana used in road construction should have a lime reactivity value of at least 1000 lb./sq. in. The second paper by R. K. Ghosh, M. P. Dhir, N. R. Srinivasan and M. L. Bhatia presented a new approach in road construction using lime-reactive *surkhi* concrete as an underlay with a thin plain concrete bonded overlay. The design of such a slab, the efficiency of bonding and the behaviour of the composite slab under loading conditions were discussed. A few other possibilities of sandwiched construction with lime-reactive *surkhi* as the sandwiched layer were indicated. Considerable saving in cement and overall costs has been claimed for the new technique.

The problem of corrosion of reinforced steel embedded in fly ash concrete due to sulphur and free carbon in fly ash was dealt with in a paper from the Bihar Institute of Hydraulic and Allied Research, Patna. It has been observed that if the fly ash content in concrete is below 50 per cent, there is negligible effect of both sulphur and carbon on steel. The last paper by S. Raymond (Royal College of Advanced Technology, Salford, UK) dealt

with the shear strength and other characteristics of compacted fly ash in the context of its use as an embankment material. The compaction characteristics of fly ashes, with and without binder additions, were described. Binderless fly ash embankments were specially recommended for regions where the sub-soil is poor. Being lighter compared to those made of denser soil, these embankments stress the ground on which they stand to a lesser extent.

During the discussion, the experiences of using lime-reactive *surkhi* mortars for building works around Delhi, their merits and scope for improvement were given in some detail by engineers who used these. The requirements of puzzolanic mortars and concretes for different types of works were discussed. The economy both in cement and in costs resulting from the use of puzzolanas were stressed and the need to use such alternate materials to cement wherever possible was emphasized.

At the conclusion of the symposium visits were arranged to the building projects at the Indian Institute of Technology, New Delhi, where reactive *surkhi* produced at the Central Road Research Institute is being used and to the puzzolana concrete pavement laid down near Faridabad.

Kalinga Prize to be Awarded to Dr Warren Weaver

Dr Warren Weaver of the United States, who has had an illustrious triple career as a professor, administrator and popularizer of science, has been selected as the thirteenth winner of the international Kalinga Prize of £1000 for the popularization of science.

Born in 1894, Dr Weaver began his career as a professor of mathematics at the California Institute of Technology and the University of Wisconsin.

He is now Vice-President and executive director of the Alfred P. Sloan Foundation. In 1957, he was President of the American Association for the Advancement of Science.

Dr Weaver is the author of numerous articles and books. Among them are: *Lady luck—The theory of probability*; *The mathematical theory of communication*; *Science and complexity*; and *People, energy and food*.

Internal Friction Due to Defects in Solids*

M. A. QUADER

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IT is well known that deformation in a solid consists of two parts even at a stress level far below the elastic limit. The first part is virtually instantaneous and reversible as it disappears on removing the stress and this part represents the usual elastic deformation of a solid.

The second part of the deformation is time dependent, grows slowly by the operation of some mechanisms within it and becomes constant after a certain time. This part of the deformation is also reversible provided sufficient time is allowed. This shows that in the true sense no solid is virtually elastic and Hooke's law does not properly explain the static stress-strain curve. This property of the solid, by virtue of which a solid does not obey Hooke's law even under a very small stress, is termed by Zener¹ as anelasticity. The usual manifestation of the anelasticity of a solid is the damping or internal friction which is often defined as the capacity of a vibrating solid to damp out its vibrational energy by the operation of some specific mechanism within it. There are three main sources of anelasticity, viz. thermoelastic effect, magneto-elastic effect, and defects within the lattice. Experience over the last twenty-five years has shown that a larger number of internal friction effects are due to defects in the lattice and that internal friction measurements are extremely sensitive indicators of events involving these defects. The method has been applied very successfully, particularly in the field of physical metallurgy in the study of diffusion, recovery, precipitation and other related phenomena and has led to their deeper understanding on an atomic scale. Nowadays it is being used as a powerful tool in the experimental solid state physics studies and this paper reviews the recent advancements made in understanding the role played by defects in the manifestation of various types of internal friction effects.

The defects considered in this review are: (1) Interstitial solute atom, (2) Substitutional solute atom, (3) Grain boundary, (4) Dislocation, (5) Vacancies, and (6) Precipitation. The centre of relaxation in a vibrating solid can be either single defects or a number of defects interacting with each other depending on their densities and interaction energy. As regards the condition under which the presence of defects in a crystal can be expected to give rise to internal friction, it may be stated in general terms that internal friction can be expected whenever introduction of defects produces distortion, thus resulting in a lower symmetry for the doped crystal lattice than that of the original lattice. The application of a uniaxial stress will cause a separation of the energy states

of the equivalent points surrounding the asymmetric defect, and a redistribution of the defect population amongst the various states will ensue. This phenomenon of the stress induced ordering of the defect populations endows the crystal with anelastic properties¹ in general and internal friction in particular. This mechanism of stress induced ordering of the defect populations in interstitial and substitutional alloys gives rise, in a number of cases, to internal friction peaks when measured either at a constant frequency as a function of temperature or at a constant temperature as a function of frequency. According to the formal theory of anelasticity the temperature dependence of internal friction (Q^{-1}) is given by¹

$$Q^{-1} = \frac{\delta}{\pi} = \Delta \frac{\omega\tau}{1 + (\omega\tau)^2} \quad \dots(1)$$

where δ is the logarithmic decrement in amplitude; ω , the angular frequency of vibration; Δ , the relaxation strength; and τ , the relaxation time obeying the Arrhenius type equation given by

$$\tau = \tau_0 e^{H/RT} \quad \dots(2)$$

where H is the activation energy of the relaxation process. The internal friction peaks observed in α -iron containing carbon and in α -brass are the best known examples for the type of peak predicted by Eq. (1).

In the following sections, though detailed analysis has been made of some examples of relaxation behaviours which can be accounted for by different kinds of defects and their interactions, it was necessary in view of the brief nature of the review to exclude certain topics altogether, by merely mentioning them.

Internal Friction due to Interstitial Solute Atoms

The internal friction due to relaxation of isolated elementary point defects caused by interstitial solute atoms is largely accounted for by the Snoek peak² found in body-centred cubic metals containing interstitial solute atoms. This is the first internal friction phenomenon to be understood well enough to be usefully applied as a tool in metallurgical research. Observations of the internal friction peak which results from stress induced ordering of interstitial solute atoms are now used regularly in the evaluation of the concentration and state of dispersion of carbon and nitrogen³⁻⁵ atoms in iron and other transition metals.

In α -iron the interstitial atoms (carbon) are located at the centre of one of the cube edges, i.e. position corresponding to $(\frac{1}{2}, 0, 0)$; they have two close neighbours in the direction of one of the cube axes and thus produce most distortion in this direction. In an unstressed crystal the interstitial atoms will

*Paper presented at the Symposium on Solid State Physics, held at the National Physical Laboratory, New Delhi, in April 1964.

be randomly distributed, but on applying a tensile stress, the energy of an interstitial atom (carbon) will be less in certain sites because of the distortion produced by the applied stress. Hence the stress produces a redistribution of the carbon atoms so that some sites are occupied preferentially. This process of redistribution gives rise to an internal friction peak which was first observed by Snoek² in iron containing carbon or nitrogen atoms as impurities. The theory of the Snoek relaxation, which explains the observed peak in internal friction, was first developed by Polder⁶ and according to him (i) the relaxation time is directly proportional to the mean time of jump of carbon atoms from one position in the crystal lattice to the next neighbouring position; (ii) the activation energy of this relaxation is decided by the rate of diffusion of carbon in α -iron; and (iii) the relaxation strength is directly proportional to the number of carbon atoms in solution. These three predictions of this theory have been verified by Dijkstra⁴ and Wert⁵ who also used the method for studying the rate of precipitation of carbon and nitrogen from their solution in iron.

Similar Snoek relaxation peaks have been observed due to the presence of interstitial oxygen, carbon and nitrogen in tantalum by Ke⁷ and by Marx *et al.*⁸. These were examined in detail by Powers and Doyle⁹, who showed that in addition to the usual peak due to the presence of oxygen or nitrogen there appeared another smaller peak in alloys having high oxygen content (above 0.1 per cent by wt of alloy) on the high temperature side, and these peaks correspond to higher activation energy. This peak was supposed to be due to relaxation of pairs of interstitial atoms which are formed due to the strong interaction force between interstitial oxygen atoms. Snoek peaks were also observed in columbium^{8,10} and molybdenum¹¹. Heller¹² has recently reported an internal friction peak at 29°K. (frequency 1 c/s.) in iron wires charged with hydrogen due to stress induced ordering of interstitial hydrogen atoms.

So far we have considered only the body-centred cubic lattice containing interstitial solute atoms. Now in the face-centred cubic lattice the interstitial atoms may occupy either the centre of the lattice or the centre of the cube edge. In either position the distortion caused by it is symmetrical and hence should not show relaxation. However, if one of the nearest neighbour position of the interstitial atom is occupied by an impurity atom of different atom size, the distortion caused by the interstitial atom will be asymmetrical along that direction. Such an atom pair should show stress induced ordering and hence internal friction¹³. Such peaks have been observed by Ke¹⁴ and his associates in austenitic chrome-nickel steel, Mn-steel and pure nickel containing carbon. The height of the peak in pure nickel¹⁵ containing carbon was found to be proportional to the square of carbon concentration and was thus assumed to be due to pairs of carbon atoms.

Pratt *et al.*¹⁶ have reported an internal friction peak due to oxygen in hexagonal titanium. In Ti the oxygen atoms occupy the octahedral sites which

remain isotropic under external stress and hence should not show relaxation peak. However, Gupta and Weing¹⁷ have definitely shown that the internal friction peak in Ti is due to oxygen atoms in octahedral sites distorted by the substitutional solute atoms.

Another interesting example is the observation by Southgate¹⁸ of an internal friction peak at 1030°C. at 100 kc/s. due to interstitial oxygen atom in silicon with diamond lattice. In this diamond lattice the largest site for an interstitial atom is octahedral in shape for one sublattice and tetrahedral for the other sublattice. An interstitial atom located in this site has symmetrically disposed neighbours and would not be expected to give rise to stress induced ordering. However, Southgate¹⁸ explained this internal friction peak as due to stress induced ordering of oxygen atoms located in asymmetrical position where it can be bound preferentially with just two silicon atoms. This idea of asymmetrical position of oxygen atoms was put forward by Hrostowski and Kaiser¹⁹ from their infrared optical measurements.

Internal Friction due to Substitution Solute Atoms

In any lattice the substitution of one type of atom by another of different size leaves the lattice with the same cubic symmetry. However, a pair of solute atoms in nearest neighbour position will produce asymmetric distortion along the axis joining them, so that an applied stress will induce preferential orientation of the solute atom pairs. Such a process should, therefore, cause internal friction. An internal friction peak due to this mechanism was first observed by Zener²⁰ at 415°C. at a frequency of 620 c/s. in a single crystal of α -brass having a composition of 70 per cent of Cu and 30 per cent of Zn. This is the well-known Zener peak and the process is known as Zener relaxation. Subsequently, the Zener peak was observed in many other alloys such as α -brass²¹, α -AgZn²², α -AgCd²³, β -brass²⁴, MgCd alloys²⁵, etc. In Fig. 1 is shown the variation of the internal friction with temperature in the case of α -AgCd alloys.

Although the original pair orientation hypothesis of Zener could explain the relaxation effects qualitatively, the quantitative agreements between measured and predicted values of relaxation strength were not good. Leclair and Lomar²⁶ have treated the phenomenon of relaxation in terms of a stress induced short-range ordering of the directions of different nearest neighbour atoms and obtained satisfactory agreements between theoretically evaluated values and observed relaxation strengths in several α -brass alloys. However, there is at present no definite method of predicting as to which of the alloys will show the Zener relaxation phenomenon. From a survey of the effects in a number of alloy systems, Nowick and Seraphim²⁷ have shown that for solid solutions of noble metals there is no relation between the relaxation strength Δ and the size factor, but there is a rough correlation between Δ and the magnitude of the deviations from Vagards law. Also, the next nearest neighbour atom pairs have an important role in the relaxation process.

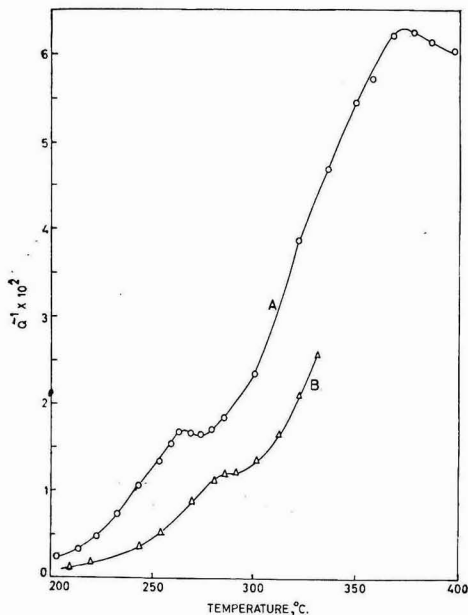


Fig. 1 — Variation of internal friction of α -AgCd alloy as a function of temperature [Curve A, specimen containing 29.3 atomic per cent of Cd, and frequency of measurement 0.84 c/s.; and curve B, specimen containing 24.3 atomic per cent of Cd, and frequency of measurement 1.1 c/s.]

Now the relaxation time τ obtained from the internal friction peaks due to both Snoek and Zener relaxation obeys the Arrhenius type equation, viz. Eq. (2), and the activation energy obtained from Eq. (2) was found to be the same as that of the diffusion energy of the solute atoms in the alloy. This fact led to the assumptions (i) that the relaxation time τ , associated with the stress induced ordering process, must bear a simple relation to the mean frequency of atomic jumps, and (ii) the mechanism of the change of state of order of arrangement of the atoms must be the same as that in the case of volume diffusion. It follows that

$$\frac{1}{\tau} = \alpha \Gamma \quad \dots(3)$$

and from diffusion theory

$$D = \beta a^2 \Gamma \quad \dots(4)$$

where Γ is the jump frequency and α is a dimensionless constant and equal to unity; β is a geometrical factor and equal to 1/12 for face-centred cubic lattice; a , the lattice parameter; and D , the diffusion coefficient.

The above relations show that the internal friction measurement presents a very efficient method of obtaining diffusion data at relatively lower temperatures and in the case of alloys having a relaxation time which is of the same order as that of the mean stay time of an atom in any lattice position. Such low temperature data are necessary to study (i) the effects of cold work; (ii) the excess

vacancies trapped by quenching from a higher temperature, on diffusion; and (iii) the kinetics of atomic movements in precipitating alloys.

Wert²⁸ has shown that in the case of interstitial solution the relaxation time $\tau_r = 2/3\tau$ where τ is the mean time of stay of an atom at a particular lattice site. For the Zener relaxation in substitutional solutions, Leclaire²⁹ obtained $\tau_r = 11/8\tau$ for a stress applied along the (111) direction and $\tau_r = 11/12\tau$ for stress applied along the (001) and (101) directions. However, Nowick²² has suggested that the relaxation process is controlled by the slower moving component in the alloy. He thus obtained

$$\tau_r = \frac{1}{2} \left(\frac{\tau_A}{f_A} + \frac{\tau_B}{f_B} \right) \quad \dots(5)$$

where f_A and f_B are the mole fractions of A and B respectively in the alloy. Nowick²², however, could not verify the above relation in the case of Ag-Zn alloys since the necessary diffusion data were not available. In the case of α -brass, Childs and Leclaire³⁰ have shown that Eq. (5) holds good approximately with the proportionality constant α of Eq. (3) equal to unity.

The author and his coworkers have studied²³ the Zener relaxation in α -AgCd alloys containing 24.3, 29.3 and 32.8 atomic per cent of cadmium, using a Ke type torsional pendulum and the results of this study are shown in Fig. 1. The activation energy H was calculated and found to be 39.36, 38.77 and 38.45 kcal./mole for the three alloys respectively. The diffusion data for the α -AgCd alloys are known from Manning's³¹ data reported for the three temperatures, 900°, 1000° and 1053°K. using the tracer technique. It has been found that the diffusion coefficients at a lower temperature ($\approx 540^\circ\text{K}$.) calculated from internal friction data agree well with those obtained by extrapolating the high temperature values reported by Manning³¹.

The stay time of the atoms in any lattice position site, τ_{Ag} and τ_{Cd} for silver and cadmium, were calculated from the diffusion data of Schoen³² and these were used to evaluate the theoretical value of relaxation time τ_r using Eq. (5). In Fig. 2 is shown plotted these values of τ_r as well as values of τ_{Ag} and τ_{Cd} as a function of temperature. The measured values³³ of τ_r are also marked in the same figure for comparison. It is seen that the variations of τ_r (measured) and τ_{Cd} with temperature agree closely with each other. From this observation it may be inferred that the anelastic relaxation phenomenon, like the diffusion process, is controlled by the rate of movement of the faster atoms in the alloy. Similar results were also obtained by Turner and Williams³⁴ in the case of Cd-Ag alloy having 32 atomic per cent of Cd and in the case of a Ag-Au alloy.

Internal Friction due to Grain Boundary

Relative motion of the grains of polycrystalline material at relatively high temperatures gives rise to a viscous like dissipation of energy at the grain boundaries, and hence an internal friction. The energy dissipated is proportional to the product of the relative displacement of adjacent grains and

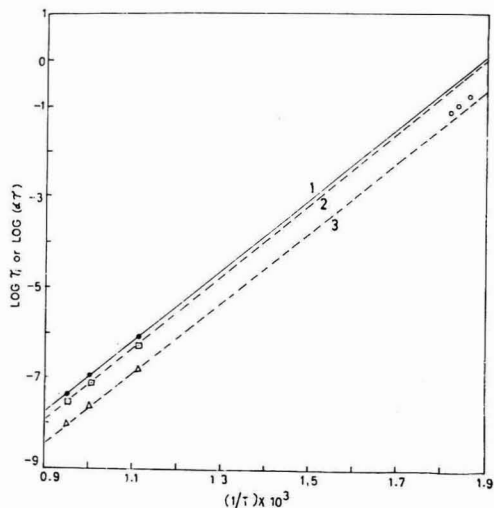


Fig. 2 — Variation of the values of $\alpha\tau$ [calculated from Eq. (5)], τ_{Ag} and τ_{Cd} as a function of temperature for a Ag-Cd alloy containing 29.3 atomic per cent of Cd along with the observed values [Curve 1, variation of $\alpha\tau$; curve 2, variation of τ_{Ag} ; and curve 3, variation of τ_{Cd}]; \circ , observed points for the variation of τ_r ; \triangle stands for τ_r , τ_{Ag} and τ_{Cd}]

the shear stress responsible for this displacement, and is small at low temperatures, where the displacement is small, and also at high temperatures where the shear stress is small. But the energy dissipated becomes appreciable at intermediate temperatures. This behaviour, typical of a relaxation process, has been observed by Ke³⁵ in polycrystalline pure aluminium where a large internal friction peak was found to appear at about 300°C. at a frequency of 1 c/s., whereas the peak was absent in single crystals. Ke³⁶ also measured the internal friction of specimens of aluminium with different grain sizes at frequencies between 0.6 and 2.2 c/s. and showed that the internal friction may be expressed as a function of the parameter

$$[\text{grain size} \times \text{frequency} \times \exp(H/kT)]$$

where $H = 1.5$ eV., k is the Boltzmann's constant, and T , the absolute temperature.

Grain boundary peaks were also observed in pure copper and in copper base alloys, in pure silver and silver base alloys³⁷. It has been observed that in either case the activation energy for grain boundary slip is slightly higher than that for the diffusion in these alloys. The grain boundary peak is also affected by the presence of impurity due to precipitation and oxidation in the alloys.

Internal Friction due to Dislocation Damping

Since one of the principal results of the application of stress to a crystal is to set dislocations in motion, it would be expected that internal friction due to moving dislocations could be observed. This was first suggested by Read³⁸ and further investigations showed that there exist a large

variety of damping effects due to dislocations. The unusually high damping in well-annealed crystals of pure metals and its dependence on temperature and strain amplitude are due to the dislocations. The general type of internal friction both at high and low temperatures could be in some way related to the dislocations. While measuring internal friction at low temperatures, Bordoni³⁹ first observed a peak at lower temperatures in cold-worked copper, aluminium, lead and silver. These peaks are also due to dislocation movements. Nowadays dislocation damping is being used as an experimental technique in experimental solid state research. A very complete and thorough review of these developments has been prepared recently by Niblett and Wilks⁴⁰.

The Bordoni peak in copper was studied in detail by Niblett and Wilks⁴¹, Bordoni *et al.*⁴², Caswell⁴³, etc., over a range of frequencies. Similar studies have also been made in the case of aluminium and lead, all of which being of face-centred cubic type of crystal. The principal results of these investigations are as follows: (1) The Bordoni peak appears in both single crystal and polycrystalline specimens. (2) The peak is not generally observed in fully annealed specimen. (3) The height of the peak is independent of amplitude, and is reduced by the presence of impurity. (4) The temperature at which the peak occurs is dependent on the frequency of measurement.

The results presented above suggest that the Bordoni peak may arise from a relaxation type process. The activation energy has been calculated to be 0.1 eV. in the case of copper, assuming the relaxation type process. Bordoni³⁹ put forward a theory taking into account the sliding of the imperfections in the crystal and was able to explain the internal friction peak observed in crystals. But his theory could not account for the specific properties of the dislocations. Theories based on the motion of the dislocations have been put forward by Mason⁴⁴ and Seeger⁴⁵, to account for the evaluated value of the activation energy. According to Seeger⁴⁵, the Bordoni peak is due to a relaxation process involving dislocations which run parallel to one of the closely packed directions in the crystal. The peak could be expected when the vibration of the dislocation is in resonance with the applied periodic stress.

Koehler⁴⁶ first suggested that the general type of damping is most probably due to forced vibration of free dislocation segments lying between pinning points. Based on this idea a detailed and comprehensive theory of this type of internal friction was given by Granato and Lucke⁴⁷. The Granato-Lucke theory is based on a model shown in Fig. 3. The dislocation segment lying between two immobile node points is further pinned at intermediate points by impurity atoms or vacancies. Under a relatively small stress the dislocation segments between the pinning points will bow out as shown in Fig. 3(b) and under alternating stress this damped motion will result in an internal friction contribution δ_r independent of strain amplitude but dependent on frequency. However, under a sufficiently greater stress the dislocation is torn

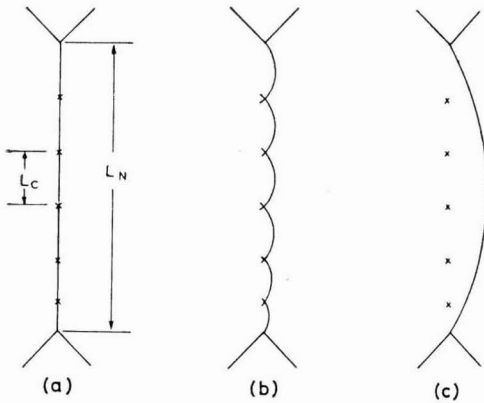


Fig. 3 — Granato-Lucke model of dislocation vibration [(a) Immobile node points and intermediate points due to impurity atoms; (b) mode of vibration at low amplitudes; and (c) mode of vibration at large amplitudes. L_N , dislocation length between immobile node points; L_C , distance between nearest neighbour impurity atoms]

away from their pinning points (break-away phenomena) and the dislocation takes the shape shown in Fig. 3(c). This motion will result in a damping δ_H which will be amplitude dependent. Thus the total damping will have two parts — one amplitude dependent and the other amplitude independent — so that the total damping δ_T can be written as

$$\delta_T = \delta_H + \delta_I$$

In Fig. 4 is shown the variation of the internal friction as a function of strain amplitude obtained by the author in the case of a Ag-Cd alloy containing 29 per cent by weight of Cd⁴⁸. It is obvious that in a quenched alloy up to a certain strain amplitude the damping is amplitude independent and above this the damping increases rapidly. The gradual decrease of this damping on standing the specimen at room temperature is due to increase in pinning due to migration of vacancies to dislocations.

According to the Granato-Lucke theory

$$\delta_H = \frac{C_1}{\epsilon} \exp\left(\frac{-C_2}{\epsilon}\right) \text{ and } C_2 = \frac{K\epsilon'a}{L_c}$$

where ϵ is the strain amplitude and C_1 and C_2 are constants and ϵ' is Cottrell misfit parameter; K is a factor depending on the orientation and anisotropy of the specimen and is equal to 0.02 for polycrystal-line samples; a , the lattice parameter; and L_c the dislocation length between impurity atoms.

Thus the plot of $\log(\epsilon \times \delta_H)$ versus $1/\epsilon$ will be a straight line. This is known as Granato-Lucke plot and is shown in Fig. 5 for Ag-Cd alloys. From the slope of the Granato-Lucke plot, the characteristic impurity pinning dislocation length L_c can be calculated. For the Ag-Cd alloys it is found that $L_c = 2.2 \times 10^{-6}$ cm.

The effect of factors such as impurity, quenching, cold work and recovery, on the dislocation damping (both amplitude dependent and amplitude indepen-

dent) has been studied⁴⁸. The results obtained as a result of these studies could be explained on the basis of Granato-Lucke theory and the Cottrell-Bilby law⁴⁹ of precipitation of point defects at dislocations.

Although the Granato-Lucke theory could explain most of the experimental results, there are, however, a number of observations⁵⁰, particularly where the density of point defects is very high, which are not satisfactorily explained by the Granato-Lucke theory. Very recently, Gelli⁵¹ proposed a qualitative model of dislocation vibration where it is assumed that dislocations freed from the pinning points do not move freely but through an atmosphere of impurities or defects which temporarily damp the dislocation during oscillation. Thus there will be a deviation from the Granato-Lucke theory at higher strain amplitudes. It has been found that while the Granato-Lucke plots in the case of Al-Cu alloy⁵² having 1 per cent by weight of Cu are straight lines in agreement with the theory, the plots in the case of the Cu-Al alloy having 2 per cent by weight of Cu are curved indicating complicated dislocation movement in different environments.

Internal Friction due to Precipitates or Atom Clusters

As stated earlier, Dijkstra⁴ and Wert⁵ have made use of the internal friction studies in evaluating the concentration of the precipitated carbon and nitrogen from supersaturated solid solution of α -iron. In this study quenched specimens were step annealed at a series of temperatures and using the height of the Snoek internal friction peak after each annealing as a direct measure of the concentration of interstitial atoms in solution, the concentration

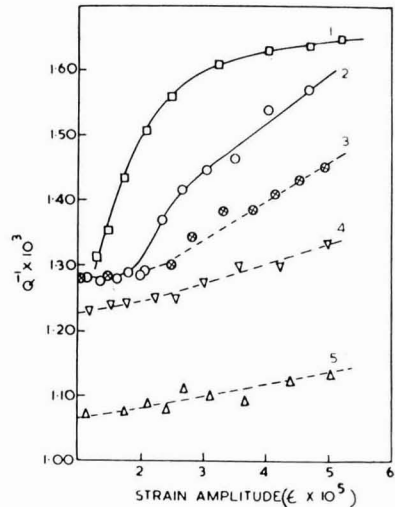


Fig. 4 — Variation of the internal friction of Ag-Cd alloy containing 29.3 atomic per cent of Cd as a function of strain amplitude [Curves 1, 4 and 5 are for furnace cooled specimens and curves 2 and 3 for water quenched specimens. Period of ageing of specimens at room temperature: curves 1 and 2, 15 min.; curve 3, 5 days; curve 4, 3 hr; and curve 5, 52 hr]

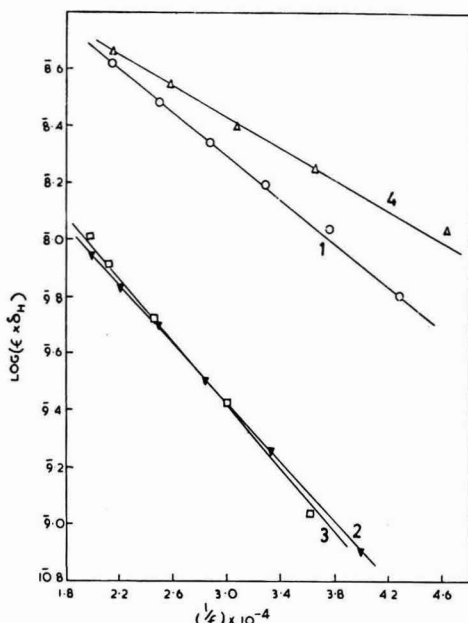


Fig. 5—Granato-Lücke plots for Ag-Cd alloy containing 29.3 atomic per cent of Cd [Curves 1 and 2 show the effect of ageing; ageing period: curve 1, 15 min.; and curve 2, 5 days. Curves 3 and 4 show the effect of temperature at which measurements are made: curve 3, 72°C.; and curve 4, 120°C.]

of the impurity remaining in solution was obtained. Wert analysed his results on the kinetics of precipitation with the semi-empirical equation

$$u = \frac{C - C_{\infty}}{C_0 - C_{\infty}} = \left(\frac{t'}{\lambda}\right)^m$$

where u is the fractional decrease in supersaturation and both λ and m are constants. It may be stated that $t' = \lambda$ when the precipitation is 67 per cent complete. For precipitations in the form of flat disks, cylinders and spheres the values of m are respectively $5/2$, 2 and $3/2$. Wert's analysis gave that $m = 3/2$ for the precipitation of Fe_3C and $5/2$ for precipitation of Fe_4N , thereby indicating Fe_3C and Fe_4N precipitates to be spheres and flat disks respectively.

The depletion of both carbon and nitrogen from cold-worked α -iron has been investigated by Harper⁵⁸ who found that for both the elements the growth exponent m was changed to $2/3$. This fact provided good support for the theory of Cottrell and Bilby⁴⁹ which predicted this value for the exponent assuming that depletion would occur by the formation of atmospheres of interstitial atoms around dislocations.

In the above case internal friction is used to study precipitation. However, it has been found that in a number of cases precipitations give rise to a new type of relaxation processes and hence internal friction peaks. These are discussed in the following paragraphs.

In investigations carried out by Nowick⁵⁴ on Al-Zn alloy containing 39 per cent by weight of Al, no internal friction peak was observed, but the internal friction value was found to increase rapidly with increase in temperature. In this alloy precipitation occurs in a discontinuous manner, and the anelastic behaviour was explained in terms of coupled relaxation across the new interfaces produced by precipitation. In Al-Ag alloy containing 20 per cent by weight of Ag, Damask and Nowick⁵⁵ observed an internal friction peak. As a result of ageing which followed the solution treatment and quenching, the position of the peak was found to shift and also change in height in four successive steps. These stages were attributed successively to (i) annealing of the quenched vacancies, (ii) dispersed silver clusters in the matrix, (iii) reversion of silver clusters, and (iv) formation of intermediate precipitation (γ'). According to Damask and Nowick⁵⁵, the peak originated due to an interaction of the applied stress with the local internal stresses around a precipitate particle.

In commercial duralumin alloy Entwistle⁵⁶ obtained two internal friction peaks after ageing at a low temperature in the case of the solution treated as well as the quenched specimens. However, these peaks were not found in the case of the Al-Cu alloy containing 4 per cent by weight of Cu. These observations were later explained by Berry⁵⁷ as due to complex atom clusters containing several different species of atoms and vacant lattice sites.

In Al-Cu alloy containing 4 per cent by weight of Cu, both Ke⁵⁸ and Maringer *et al.*⁵⁹ observed a peak at about 200°C. Berry and Nowick⁶⁰, in their detailed study of this alloy, observed a peak at 173°C. which was identified as the Zener peak. In partially aged alloy, they observed another peak at about 135°C. associated with the precipitation of θ' phase. A possible mechanism for the second peak observed when a torsional stress is applied may be due to changes in the shape of the coherent precipitate particles.

The author has measured the internal friction in Al-Cu alloys containing 2 and 3 per cent by weight of copper using a torsional pendulum⁶¹. The observed variation of internal friction as a function of temperature is shown in Fig. 6. In solution treated and quenched alloys two internal friction peaks were observed during heating. The first peak occurred at the same temperature range at which grain boundary relaxation occurred in pure aluminium. This is supposed to be the grain boundary peak. This peak disappeared after a few minutes of ageing at the peak temperatures, due to the blocking of grain boundary relaxation on precipitation. On the high temperature side, the internal friction was found to increase to another peak at a temperature few degrees below the solubility temperature. The rate of increase of friction and also the peak height were found to be frequency sensitive although the peak occurred at the same temperature. It is supposed that the increase of the internal friction at the higher temperatures is due to a coupled relaxation arising from viscous slip across the new interfaces produced by

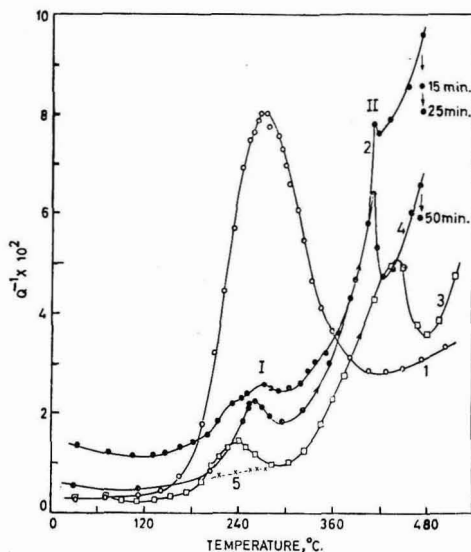


Fig. 6 — Variation of internal friction of different specimens as a function of temperature [Type of specimen and condition of study: curve 1, pure aluminium at $f=1$ c/s.; curve 2, Cu-Al alloy containing 2 per cent by wt of Cu and water quenched from 530°C. at $f=1.3$ c/s.; curve 3, Cu-Al alloy containing 2.95 per cent by wt of Cu and water quenched from 530°C. at $f=1$ c/s.; curve 4, Cu-Al alloy containing 2 per cent by wt of Cu and water quenched 3 times from 530°C. at $f=1.3$ c/s.; and curve 5, specimen same as for curve 3 but aged 10 min. at 180°C. I and II indicate respectively the position of first and second maxima observed. The lowering of internal friction for the alloy containing 2 per cent by wt of Cu on standing for different periods at the corresponding temperature is shown by vertical arrows]

precipitation. The fact that the peaks do not occur exactly at the solubility temperature shows that the coupled relaxation ceased to exist at a critical size of the precipitate. Thus the relaxation process is similar to that observed by Nowick⁵⁴ in Al-Zn alloys.

Internal friction measurements have been made in Cu-Ni alloys containing 25, 50 and 75 per cent by weight of Cu⁶². In these alloys internal friction peaks were observed in specimens quenched from above a certain temperature which is different for different compositions. This aspect is clearly seen in Fig. 7 where the variation of the internal friction of a Cu-Ni alloy containing 75 per cent by weight of Ni is presented as a function of temperature. The internal friction peaks are found to be less sensitive to frequency and to be absent during cooling from 200°C. and on subsequent reheating. It is assumed that the peaks are due to formation of some atom clusters or a metastable precipitate. Although there is no evidence from X-ray or microscopic observations for a phase separation or miscibility gap in the Cu-Ni alloy system, some recent measurements of resistivity, magnetic susceptibility and other thermodynamic properties⁶³ indicate the presence of a miscibility gap or atom clustering at lower temperatures. Internal

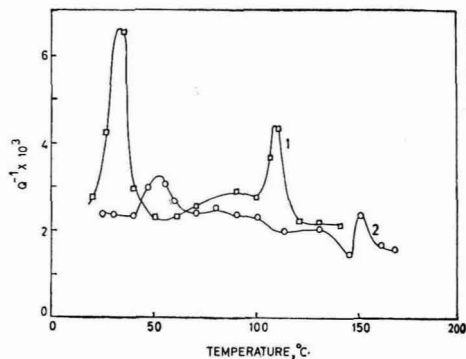


Fig. 7 — Variation of internal friction of Cu-Ni alloy containing 75 per cent by wt of Ni as a function of temperature [Both specimens studied are water quenched and studied at $f=2.2$ c/s.; curve 1, initial temperature 720°C.; and curve 2, initial temperature 600°C.]

friction measurements made by the author's co-worker also indicate a miscibility gap or atom clustering in Cu-Ni alloy systems. Similar internal friction studies have been made by Ang *et al.*⁶⁴ in the case of several Au-Ni alloys and they observed two transient internal friction peaks in quenched alloys. While one of the peaks was due to the Zener relaxation, the other peak which was found to be absent in specimens quenched from just below the immiscibility temperature is due to the formation of atom clusters.

The precipitates (coherent or partially coherent) strongly interact with dislocations whose existence has been observed in electron microscopic studies in case of various aluminium base alloys. Thus, theoretically, the formation of precipitates should decrease the dislocation damping in precipitating alloys. The measurements made by the author and coworkers with Al-Cu and Cu-Ni alloys show that the initial high damping observed after quenching decreases considerably on annealing. Also the amplitude dependent internal friction has been found to decrease on ageing the quenched alloys.

Summary

Defects in the lattice are considered as the source of internal friction in solids. The internal friction is caused by a stress induced ordering mechanism in all cases where the introduction of the defects sets up local asymmetric distortions of the surrounding lattice. Measurements of internal friction provide valuable information about the structure of the solid, movement of atoms and defects in diffusion, and interaction between various types of defects. The energies involved in these movements and interaction could also be estimated from internal friction data. The effects of the following defects on the internal friction of solids have been considered in this review: (1) interstitial solute atoms, (2) substitutional solute atoms, (3) dislocations, (4) grain boundaries, (5) vacancies, and (6) precipitates. Specific examples of internal friction effects, believed to arise from relaxation of

either the single elementary defects or two or more interacting defects, have been considered. The Snoek effect commonly observed in the body-centred cubic crystal type transition elements containing carbon and nitrogen as interstitial solutes has been explained as due to relaxation involving single carbon or nitrogen atom. This relaxation effect is directly proportional to the concentration of the solute in solution and has been used widely to study precipitation of these elements from α -iron. The internal friction effects observed in face-centred cubic austenitic steels containing interstitial carbon and nitrogen atoms in hexagonal titanium are, on the other hand, due to interaction of the interstitial atoms with substitutional impurity atoms like oxygen which occupy octahedral sites.

The type of internal friction observed in substitutional alloys such as α -brass, α -AgZn, α -AgCd, etc., has been explained as due to relaxation of pairs of interacting solute atoms. Although the mechanism of this relaxation, known as Zener relaxation, is not known precisely, recent measurements in AgCd alloys suggest that this relaxation process like the normal diffusion process is most probably controlled by the movement of the faster moving component of the alloys.

The results of the dislocation damping measurements in Al-Cu alloys containing 1 and 2 per cent by weight of copper are interesting as no appreciable deviation from the Granato-Lucke theory of dislocation damping has been observed for these alloys. The internal friction characteristics observed in Cu-Ni alloys show evidence for the clustering of impurity atoms in these alloys at lower temperature. These results are interesting for, in the Cu-Ni alloy, there is no direct evidence for clustering or phase separation or miscibility gap, although a miscibility gap in this system has been predicted from a number of investigations of other physical and thermodynamic properties of the system.

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Organic Photochemistry*

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LIGHT plays an important part in carrying out many organic reactions including those occurring in Nature. Photosynthesis is the oldest biological reaction known to take place in the presence of light. To an organic chemist, until recently, only such light-catalysed reactions as halogenation, phosphorylation and oxidation were more commonly known in which inorganic compounds like halogens undergo photochemical change to give active species which subsequently react with organic compounds. However, those reactions in which light brings about direct chemical transformations of an organic molecule have gained importance only during the last few years. The study of organic photochemistry was made earlier on simple organic compounds at the beginning of this century by chemists like Ciamician, Silber and Paterno in Italy. Also spectroscopists have long been interested in the physical study of the action of light on organic compounds and have contributed substantially to the study of primary photochemical processes. In recent times, those who have inspired the study from the organic chemistry point of view are Barton, Büchi, Schenk, Schönberg and others, and the main reasons for the increased interest are as follows.

Firstly, light is a very good agent for generation of free radicals and brings about many novel reactions, particularly rearrangements which are not met with in ordinary chemical processes. Secondly, it has been possible to synthesize by short routes many compounds which are otherwise difficultly accessible. Thirdly, a greater understanding of the mechanisms of photochemical reactions has become possible as a result of the discovery of new techniques such as flash photolysis. Flash photolysis permits introduction of a large dose of radiant energy over a very short period. Under such circumstances, the concentration of molecules in excited states is sufficiently high to permit spectra to be taken and rates of reactions determined. Hence a study of transient species involved in photoreactions has become possible.

Realizing the vast scope of the subject and a need to infuse interest among younger scientists, an international symposium on 'Organic Photochemistry' was held at Strasbourg during 20-24 July 1964 under the auspices of the International Union of Pure & Applied Chemistry (IUPAC). The main object was to coordinate the earlier knowledge and to reach a common understanding of the subject. It was attended by both organic as well as physical chemists, actively engaged in this field of investigation. Besides, a number of younger scientists who wanted to develop interest in this subject also attended. For the benefit of the latter

group, a course of seven lectures preceded the symposium. Both the course and the symposium were addressed by eminent scientists in the field. The course was conducted by Prof. G. S. Hammond (Director), Dr P. Courtot and Prof. G. Quinkert and the symposium was addressed by Professors W. A. Noyes, E. J. Bowen, Mme. M. Mousseron, H. E. Zimmerman, G. Porter, P. de Mayo, G. Quinkert, K. Gollnick, H. M. Frey, W. G. Dauben, O. Jeger, M. D. Cohen, R. C. Cookson, O. L. Chapman and N. C. Yang. Among other distinguished persons who made discussions lively and useful could be mentioned Professors P. D. Bartlett, J. N. Pitts, L. Horner, D. Schulte-Frohlinde, R. H. Thomson, E. Havinga, G. Ourisson and J. Levisalles. The last two were also the main hosts and they are to be congratulated for the success of the symposium. On the last two days of the symposium, the afternoons were devoted to reading of 14 original research papers. The lectures covered practically all the aspects of organic photochemistry. As the subject is still comparatively new in India, a résumé of the highlights of the subject presented here may stimulate organic chemists in the country to take more interest in this field.

Mechanism of Photochemical Reactions

When an organic molecule is subjected to the action of light, ultraviolet or visible, the primary process is the excitation of one of the electrons in the most readily available bonding orbital of the molecule to a non-bonding orbital. The excited electron in the first instance has always an opposed spin to that left in the vacated orbital; this excitation is called singlet excitation (Chart 1). In some cases, the spin inversion of the excited electron takes place; this spin inversion process is called 'intersystem crossing' and the excited state in which the excited electron has the same spin as that of the electron in the vacated orbital is called triplet state (Chart 1). The unshared pair of electrons,

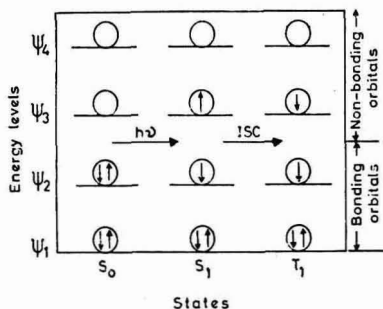


Chart 1 — Electronic configuration of ground and lowest excited states of a molecule [ISC = intersystem crossing]

*Based on the Symposium on Organic Photochemistry, held at Strasbourg during 20-24 July 1964 under the auspices of the International Union of Pure & Applied Chemistry.

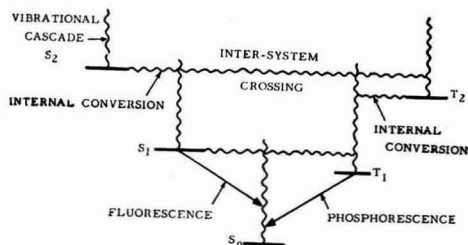


Chart 2 — Electronic transitions of a molecule

i.e. n electrons, present on an atom of the molecule are the most readily excitable, followed by pi (π) electrons; sigma (σ) electrons are very difficult to be excited. Hence two transition states, $n-\pi^*$ and $\pi-\pi^*$, are possible, and the reactions involve unsaturated compounds such as polyenes, carbonyl and nitro compounds.

The excited molecule disposes of the acquired energy, with varying degree of efficiency, by exhibiting either physical phenomena such as fluorescence and phosphorescence or by bringing about some chemical reaction. The quantum yield of a reaction is said to be unity (which is seldom the case) when one mole of the reactant is converted into products per Einstein of light absorbed. The organic chemist is primarily interested in chemical reactions which depend on the states of excitation. The triplet and singlet excitation states differ very much in their properties. (1) The triplet state has a net angular momentum, whereas the singlet has none. (2) The triplet state has less energy than the singlet state and hence reactions of low energy can be conveniently carried out in triplet state. (3) A triplet state has a longer life. Thus phosphorescence shown by the triplet state has a longer decay time than fluorescence exhibited by the singlet state (Chart 2). Due to the longer life of the triplet state, reactions in this excitation are marked by novelty, selectivity and stereospecificity. (4) The triplet state is transferable, i.e. an organic molecule can also be excited to triplet state straightaway; the reaction of transfer can be written as follows:



This property is very useful and has enabled the development of a number of photosensitizers of varying energy. Thus it is possible to control reactions and isolate different products using different photosensitizers, e.g. benzophenone, benzil, biacetyl and β -methylacetophenone.

Photochemical reactions are, therefore, of two types: one takes place in singlet excited state and the other occurs in triplet excited state. Ordinary chemical reactions are only of the former type. Many of the differences noted in photochemical reactions from the chemical ones are thus due to excitation of the molecule to the triplet state. It may be pointed out that light reactions in the triplet state are always homolytic and as intersystem crossing is possible from triplet to singlet state, the unusual

reactions of the triplet state are followed by the usual homolytic and heterolytic reactions of the singlet state.

Types of Photochemical Reactions

Four major types of photochemical reactions have been noted so far. These are photodissociation, photoaddition, photoreduction and photoisomerization. These are described here briefly giving the mechanism wherever it has been examined and examples of some important syntheses. It may be remarked that reactions described have mostly been carried out in the presence of ultraviolet light and in solution.

Photodissociation

This phenomenon occurs in many groups of compounds; the most important ones out of them are described below.

Aldehydes and ketones — Saturated aliphatic aldehydes and ketones undergo three types of photodissociation. In simple molecules like acetone, α -cleavage takes place at room temperature to give two radicals, viz. methyl and acetyl, which could subsequently undergo either dimerization or recombine to form acetone. This reaction has been studied most from mechanistic point of view and it is considered that 70 per cent of acetone molecules get excited to triplet and the rest to singlet. At higher temperatures, even acetyl radical undergoes cleavage and methyl radical is formed (Chart 3, reaction 1).

If there is a hydrogen atom at the γ -position of the aldehyde or ketone molecule, intramolecular hydrogen transfer takes place to the oxygen atom and the resulting biradical is stabilized either by cyclization to afford a hydroxy cyclobutane derivative or by cleavage to give the enol form of the ketone and the alkene (Norris type II reaction; reaction 2). The former change takes place only to a small extent and the latter always predominates. The cyclic ketones undergo mainly α -cleavage (reaction 3), but the biradical is stabilized in either of three ways, viz. (i) by ketone formation, (ii) by formation of unsaturated carbonyl compound, or (iii) by formation of cycloalkane with the expulsion of carbon monoxide.

Recently, dibenzyl ketones have been studied and they are found to lose carbon monoxide and to form dibenzyl derivatives (reaction 4). An interesting example is the photolysis of 2,3,5,6-tetra-phenyl cyclohexane-1,4-dione, when cyclopentanone, cyclobutane, stilbene and carbon monoxide are formed (reaction 5).

Aliphatic and alicyclic diazo compounds and diazirines — Diazoalkanes undergo rapid photolysis and highly interesting biradicals are formed; they are called carbenes and can be either singlet excited or triplet excited. It is found that the triplet carbene is highly discriminating. Thus it does not get inserted in non-activated C-H bonds as a singlet methylene does. For example, triplet methylene gives generally a cyclopropane derivative with an alkene; whereas singlet methylene affords a mixture of several compounds (Chart 4, reactions 6-8). Simple carbene is used for ring expansion

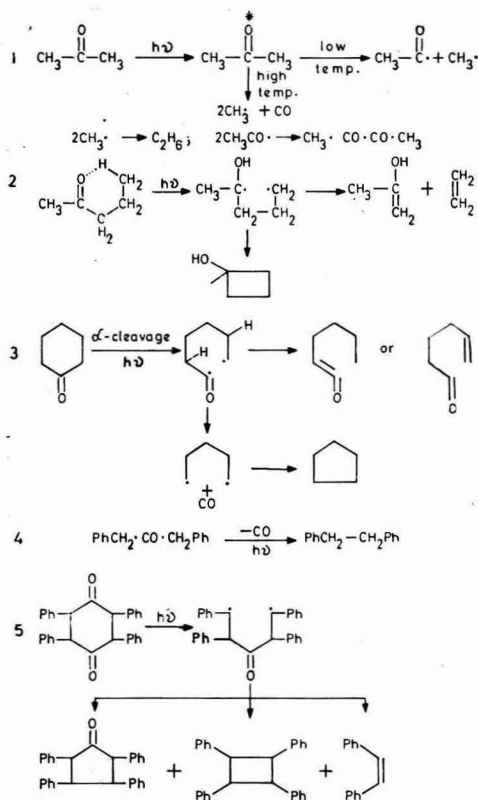


Chart 3 — Photodissociation of aldehydes and ketones

of benzene and Δ^2 -cyclohex-en-one derivatives; benzene gives 1,3,5-cycloheptatriene (reaction 9a) and testosterone undergoes insertion of methylene between keto group and the double bond (reaction 9b). Cyclic ketocarbenes are obtainable by the photolysis of cyclic α -diazoketones and they usually give ring contracted acids in aqueous medium probably through the intermediate ketene (reaction 10). Carbenes also result from the photolysis of diazirines which have recently become available (reaction 11).

The photodissociation of tosyl hydrazones of carbonyl compounds in aprotic solvents yields products which may be ascribed to the intermediacy of carbenes (Dauben *et al.*, 1962). An important application of this reaction is in the recent total synthesis of natural (\pm) thujopsene by Büchi and White (reaction 12).

Miscellaneous compounds — Various compounds having easily dissociable groups undergo photodissociation. Hypohalites having γ -hydrogen atoms undergo decomposition and subsequent cyclization to give cyclic ethers. This reaction has been particularly useful in steroids where angular methyl group at position 18 has been cyclized with the hypohalite oxygen at carbon 20 (Chart 5, reaction 13).

Similarly, the azides undergo decomposition to afford highly useful nitrene radical which is also capable of cyclizing with the γ -hydrogen atom (reaction 14). The simple carboxyazide has been obtained by the photolysis of ethyl azidoformate and this inserts readily in benzene to give N-carboxyazepine (reaction 15).

An interesting case of photodissociation is that of *o*-benzene diazonium carboxylate and *o*-iodophenyl mercuric iodide; both form a highly unstable intermediate called benzyne which rapidly dimerizes to biphenylene (reaction 16). The technique of flash photolysis has made it possible to take the absorption spectrum of benzyne which supports the assigned structure.

Photoreduction of Ketones

The triplet state of a carbonyl compound makes it a very good acceptor of hydrogen atom. Thus, in the presence of hydroxylic solvents such as isopropyl alcohol, benzophenone undergoes reductive dimerization to benzopinacol. Since quantum yields of acetone and pinacol are both nearly equal to unity, the mechanism seems to involve the use of

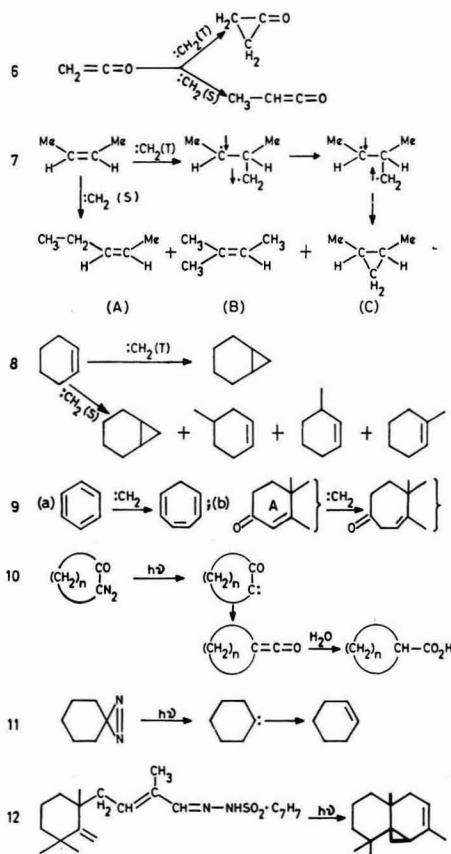


Chart 4 — Reactions of carbenes

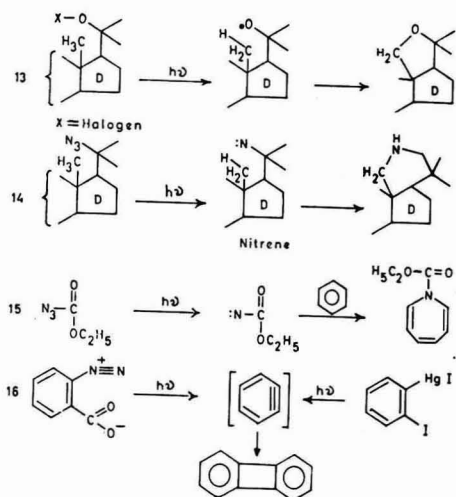


Chart 5 — Photodissociation of hypohalites, azides and other compounds

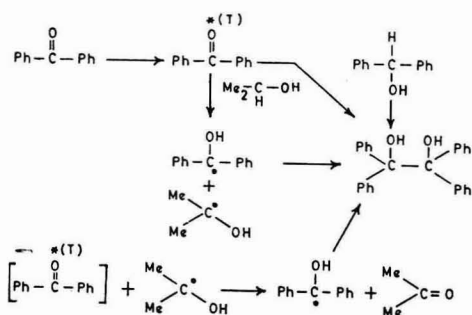


Chart 6 — Photoreduction of benzophenone

both the hydrogen atoms of the alcoholic group to convert benzophenone molecule to benzhydryl radical (Chart 6).

Photocycloadditions

A very characteristic reaction in the presence of light is 1,2-cycloaddition of unsaturated compounds to give cyclobutane derivatives. Thus photochemical means enable one to synthesize cyclobutane derivatives which are difficult to obtain by ordinary chemical means. Simple ethylenic compounds do not add by themselves, but they add to α,β -unsaturated carbonyl compounds and enol forms of β -diketones (Chart 7, reactions 17 and 18). Since the cyclobutane derivatives in the latter case are unstable, they decompose to acyclic products which sometimes can subsequently be converted into useful derivatives. Thus the addition product (A) of cyclohexene and acetyl acetone spontaneously decomposes to give (B) which undergoes cyclization in the presence of acid to give octalin derivatives (C) and (D) (reaction 18). Addition of

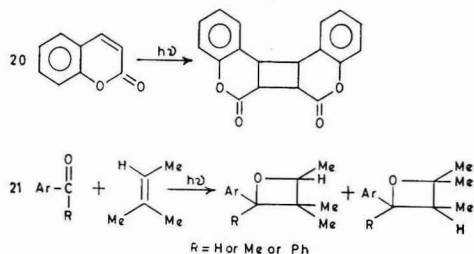
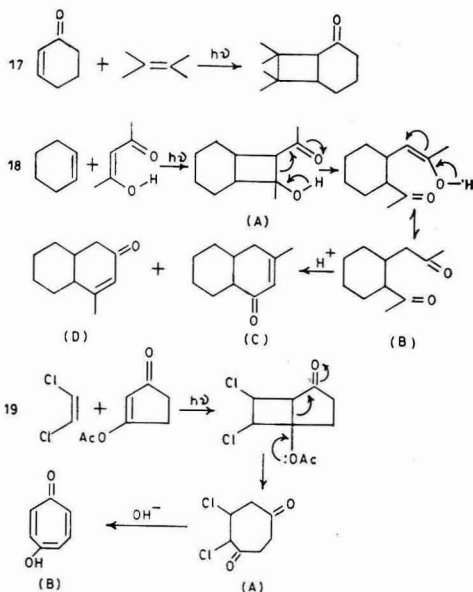


Chart 7 — Photoaddition reactions

cyclic β -diketones to ethylenic compounds help in the ring expansion. For example, Δ^2 -3-acetoxycyclopent-en-one adds to dichloroethylene to give cycloheptane-1,4-dione derivative (A) which affords γ -tropolone (B) in the presence of alkali (reaction 19).

Unsaturated carbonyl compounds also get dimerized by addition across the ethylenic bond. A well-known reaction is the photodimerization of coumarin (reaction 20).

A more interesting photocycloaddition is the dimerization of 1,3-butadiene. It is interesting from several points of view. Firstly, the products are very much different from those obtained by thermal reactions and, secondly, different photosensitizers give different products. Thus thermal reaction gives predominantly cyclohexene derivative (I); whereas photochemical reaction in the presence of acetophenone yields *trans*-cyclobutane derivative (II) and the same reaction in the presence of benzil affords a mixture of (I) and (II) in almost equal amounts (Chart 8). Further, photoaddition is highly stereoselective. This has been explained as due to the difference in excitation states; addition through a singlet excited state is a quicker process than that

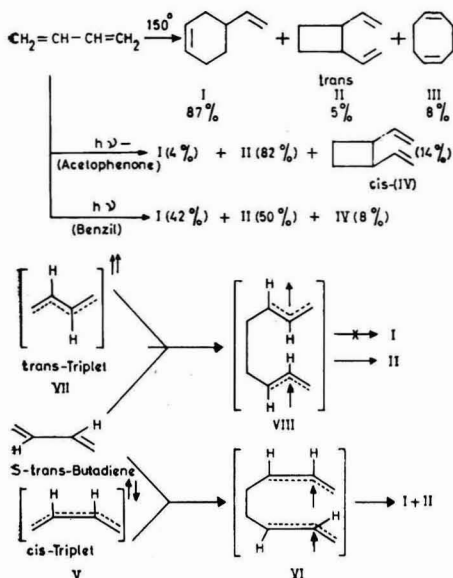


Chart 8 — Cycloaddition of 1,3-butadiene

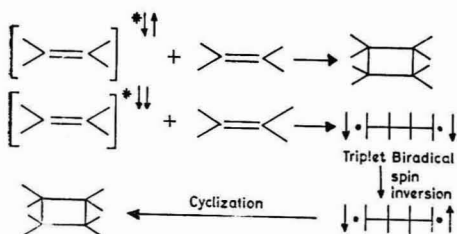


Chart 9 — Difference in cycloaddition through singlet and triplet excitations

through a triplet state (Chart 9). The formation of different products using different photosensitizers is explained on the basis of the configuration acquired by the triplet state in the intermediate stage. If the *cis* triplet (V) is formed in larger amount as in the presence of low energy sensitizers like benzil, the intermediate stage (VI) favours both the types of cyclizations (Chart 8). But if the *trans* triplet (VII) is formed in major proportion as in the presence of high energy sensitizers like acetophenone, the cyclization of the intermediate (VIII) to form only four-membered ring is favoured and the formation of six-membered ring is disfavoured.

Another unusual photoaddition reaction, called the Paterno-Büchi reaction, is the cycloaddition of C=C to C=O bonds. Thus aromatic aldehydes and ketones add to various ethylenic compounds to give oxetane derivatives (Chart 7, reaction 21).

Photoisomerizations

In the presence of light, organic compounds undergo novel types of isomerizations, many of

which are highly characteristic of only photo-reactions.

Trans-cis isomerization — In ordinary chemical reactions, *trans* form being more stable than the *cis* predominates, but in light-catalysed reactions an equilibrium state can be attained in which the *cis* isomer predominates. This reversal of the equilibrium has been studied in the case of stilbene derivatives. The difference seems to be due to the formation of a planar triplet state which is resonance stabilized (Chart 10). In this form, the original ethylenic bond acquires partially a single bond character with the result that free rotation of a phenyl group is possible. In the demotion stage the non-planar form can thus have more of the *cis* form than the *trans* form.

It may be pointed out that photo *trans-cis* isomerization is an important biological reaction taking place continuously in the rods and cones of eyes.

Valence isomerization of polyenes — Conjugated dienes and trienes undergo valency isomerization involving either ring closure or ring opening. In the former case, generally a cyclobutane derivative is formed (Chart 11, reactions 22 and 23). Both these processes take place in the irradiation of ergosterol (IX; reaction 24); the intermediate lumisterol (X) having cyclohexadiene ring gives products (XI) and (XII). The compound (XII) is pre-vitamin D₂ which on further irradiation can undergo further hydrogen shift to give vitamin D₂ (XIII). Tropolone derivatives are also known to undergo valency isomerization.

Many highly strained polycyclic systems have been made by photoisomerization of cyclic conjugated and unconjugated dienes. The most interesting examples are the isomerization of Δ^{3,5}-cholestadiene to a highly strained bicyclobutane system (reaction 25) and isomerizations of 1,5-cyclooctadiene (reaction 26), norbornadiene (reaction 27), and allyl carbene (reaction 28).

A special case of photoisomerization is that of citral (XIV) when two products, viz. photocitral A (XVI) and photocitral B (XVII), are formed (Chart 12, reaction 29). They have the same intermediate biradical (XV). Photocitral A has the same skeleton as is present in monoterpenes of ants and of catmint.

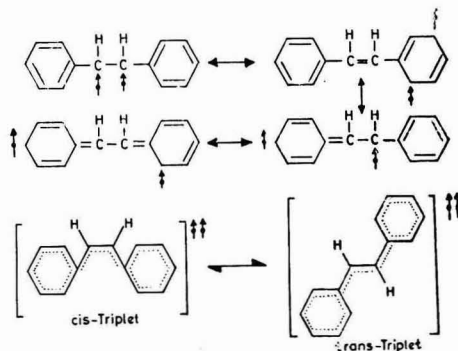


Chart 10 — Planar triplets in *cis-trans* isomerization of stilbene

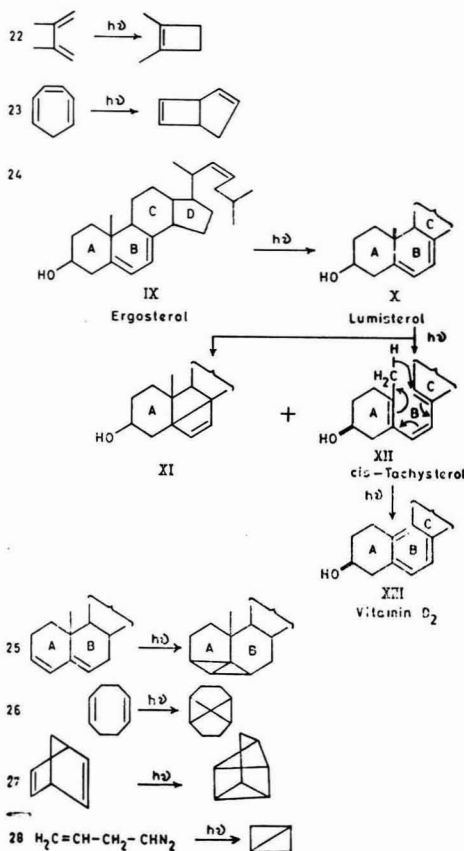


Chart 11 — Photoisomerization of polyenes

Diene-allene rearrangement—Sorbic acid (reaction 30) being a conjugated unsaturated acid undergoes photorearrangement in ether medium to give allenic acid (XXI). The reaction mechanism involves initial isomerization of the *trans-trans* acid (XVIII) to the 2,3-*cis* isomer (XIX) and subsequent rearrangement of the hydrogen atoms and double bonds involving cyclic transition state (XX).

Skeletal rearrangements of cross-conjugated dienones—A fascinating rearrangement is that of cross-conjugated dienones. The dienone moiety is present in many natural sesquiterpenes like santonin and artemisin and steroids. Simple $\Delta^{2,5}$ -4,4-diphenyl-cyclohexa-diene-one (Chart 13, XXII) forms three stable products (XXIV), (XXV) and (XXVI) which have been explained only recently by Prof. Zimmerman. The triplet state has been definitely established to participate in these rearrangements. After brief irradiation in aqueous dioxane at 25°, a bicyclic compound (XXIII) has been isolated which throws considerable light on the eventual formation of the three stable products (XXIV-XXVI). A remarkable point of the suggested mechanism (Chart 13) is that only the unusual change such as

rebonding has been postulated to occur in the triplet state, but the other reactions such as migration of a group with its electron pair to an electron deficient centre are considered to take place in the singlet state.

The photorearrangements of santonin and artemisin have been explained on similar lines. An interesting synthesis which has recently been accomplished by Barton *et al.* is that of geigerin which involves photoirradiation of 8-*epi*-artemisin acetate.

Photorearrangement of α - β -epoxyketones— α , β -Epoxy ketones undergo photorearrangement to give

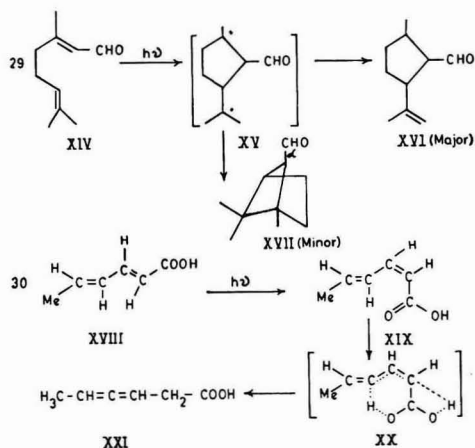
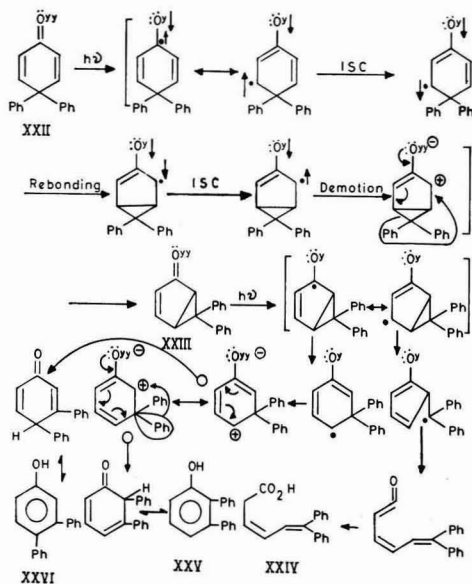


Chart 12 — Photoisomerization of citral and sorbic acid


 Chart 13 — Photorearrangement of $\Delta^{2,5}$ -4,4-diphenyl-cyclohexa-diene-one

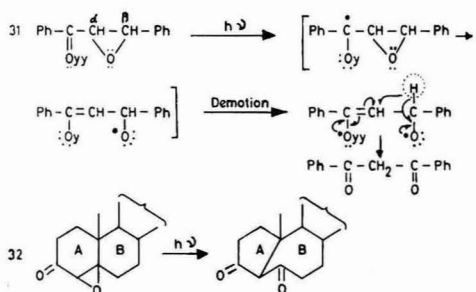


Chart 14 — Photorearrangement of α,β -epoxy ketones

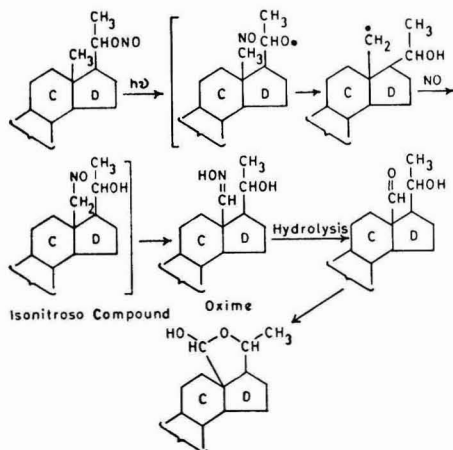


Chart 15 — Photoisomerization of nitrite esters

products other than those obtained in the acid-catalysed rearrangements. In the former, the $C_{(\alpha)}-O$ cleavage takes place and the hydrogen atom migrates from β - to α -position to afford β -diketones; whereas in the latter case $C_{\beta}-O$ cleavage takes place and hydrogen atom migrates from α - to β -position to yield α -diketones. Bodfors had obtained the corresponding dibenzoyl methanes from several chalcone epoxides by irradiation in 1917 (Chart 14, reaction 31). Recently, this photorearrangement has been used in the field of steroids to convert the six-membered rings A and B into 5- and 7-membered rings respectively (reaction 32). The mechanism has been explained in the case of simple phenyl styryl ketone epoxides by Prof. Zimmerman. In the excitation stage, γ -oxygen diradicals (A) are produced which on demotion undergoes homolytic shift of the hydrogen atom to give dibenzoyl methane (Chart 14, reaction 31).

Photoisomerization of nitrite esters—This is an important reaction from synthetic point of view and has made possible to introduce functionality at highly non-reactive angular methyl groups in steroid nucleus. Nitrite esters having γ -hydrogen atom get isomerized to γ -oximino alcohols in the presence

of light (Chart 15) and this reaction is called 'Barton reaction'. All nitrite esters present in positions 2, 6, 11 and 20 of the steroid molecule undergo such rearrangement; 11-nitrite ester gives a mixture of 18- and 19-oximino derivatives. These oximino compounds can subsequently be converted into aldehydes or hemiacetals.

Photorearrangement of phenol esters—Fries type rearrangement also occurs in the presence of light. Kobsa noted that *p-t*-butyl phenyl benzoate (Chart 16, XXVII) gives 2-hydroxy-5-*t*-butyl benzophenone (XXXI). The mechanism involves first photolysis to benzoyl (XXVIII) and phenoxy radicals (XXIX) which could then recombine in a different way to yield arenone form (XXX) of the ultimate reaction product (XXXI).

Photorearrangement of nitro compounds—Nitro compounds undergo highly facile rearrangement reactions. Generally, the oxygen atom of the nitro

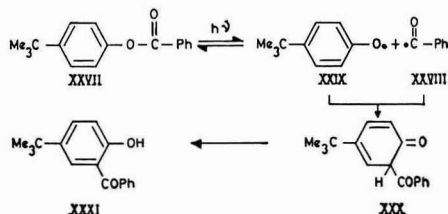


Chart 16 — Photorearrangement of *p-t*-butyl phenyl benzoate

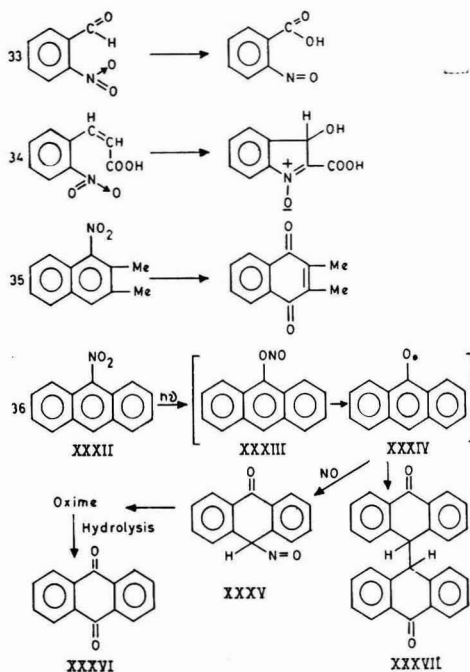


Chart 17 — Photorearrangement of nitro compounds

group is transferred intramolecularly to the other group present in the molecule. For example, *o*-nitrobenzaldehyde gives *o*-nitroso benzoic acid (Chart 17, reaction 33) and *o*-nitrocinnamic acid gives the corresponding nitron (reaction 34). When a nitro

group is present in 1-position of the naphthalene ring and in 9-position of the anthracene ring, light irradiation generally gives naphthaquinone and anthraquinone respectively (reactions 35 and 36). The mechanism has been considered by Chapman to involve initial isomerization of the nitro group to nitrite ester (reaction 36).

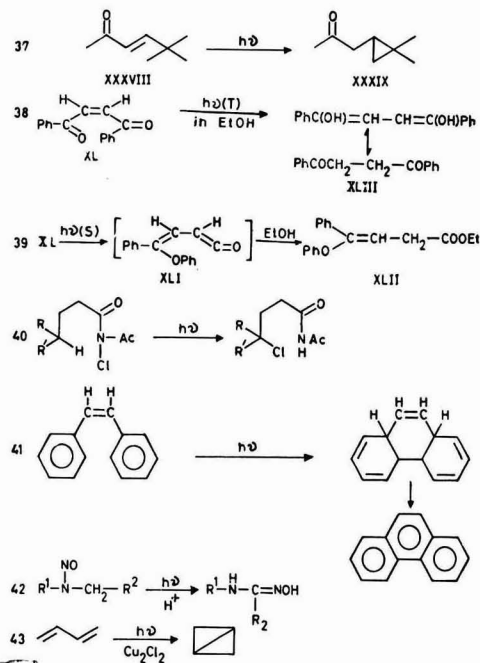


Chart 18 — Photoreactions of Δ^3 -5,5-dimethylhexene-2-one *cis*-dibenzoyl ethylene

Miscellaneous photoisomerizations — Irradiation of Δ^3 -5,5-dimethylhexene-2-one (XXXII) in ether gives a novel photochemical reaction; first, *cis-trans* equilibration occurs followed by isomerization (N. C. Yang, 1963) to give a cyclopropane derivative (XXXIII) (Chart 18, reaction 37).

An interesting difference in the reactions of singlet and triplet state is noted in the photoreaction of *cis*-dibenzoyl ethylene (reactions 38 and 39). Whereas triplet excitation in alcohol solution gives only the reduced product, dibenzoyl ethane (reaction 38); the singlet excitation causes rearrangement of phenyl group from 1- to 5-position to give ketene (reaction 39). This indicates that as singlet excited C=O group is not capable of accepting hydrogen, the other reaction, viz. rearrangement, is favoured.

The other interesting rearrangements are (a) *N*-chloro-*N*-acetyl imides to 4-chloroimides (reaction 40), (b) *cis*-stilbene to dihydrophenanthrene and subsequent aromatization to phenanthrene (reaction 41), (c) *N*-nitrosamines to amidoxime in the presence of trifluoroacetic acid (reaction 42), and (d) butadiene to bicyclo [1,1,0] butane (reaction 43).

Acknowledgement

The author wishes to express his deep gratitude and sincere thanks to Prof. T. R. Seshadri and Prof. G. S. Hammond for providing him with necessary facilities and help to attend and participate in the symposium. He is also thankful to Prof. T. R. Seshadri for going through the manuscript.

Matscience Summer School

The Annual Summer School on Recent Trends in Theoretical Physics will be organized by the Institute of Mathematical Sciences, Madras, at Bangalore during August 1965 for a period of three weeks, in which some invited foreign scientists will take part. Among these are: Dr R. J. Oakes of Stanford

University, Dr K. C. Wali of Argonne National Laboratory, and Dr V. L. Teplitz of CERN. Besides the members of the academic staff of the Institute, some active workers from India will be invited to deliver lectures and hold seminars at the school.

Present Status of Knowledge about the Viral Etiology of Human Leukemia

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THE available experimental data on human leukemia lend strong support to the concept of its viral etiology. The recent finding of human leukemia virus by the workers of the American National Cancer Institute, Bethesda, is another important breakthrough in this direction. As this area of research is assuming major importance currently, a short review of the highlights of research studies now being conducted in different parts of the world appears to be called for.

Magrassi *et al.*¹⁻³ administered to guinea-pigs leukemic materials from human subjects which resulted in a serially transmissible systemic disease, which is characterized by a decrease in the number of erythrocytes, skin atrophy and pronounced proliferation of histiocytes particularly in tissues rich in reticuloendothelial cells. The lymphoid cells were replaced by histiocytes with degenerations in liver, adrenals and kidneys. Magro⁴ demonstrated the capacity of the leukemic tissues of man to induce pathological processes in guinea-pigs. He also inoculated the bone marrow of hens suffering from erythremic leucosis into guinea-pigs which were previously inoculated with myeloid leukemic tissue from humans⁵. This inoculation resulted in the exacerbation of leukemia, which Mas Y Magro has explained as virus exaltation.

According to De Carvalho⁶, cultures of human amniotic cells inoculated with the cytoplasmic granules of leukemic cells of man produce in rabbits, immunized with these cells, antibodies which specifically reacts with the preparations of the leukemic tissues. De Carvalho⁷ also prepared nucleic acid preparations of human leukemic and solid tumors. Serial passages of these preparations in tissue cultures of human amnion cells produced cytopathogenic changes. Leukemia and pleomorphic neoplasms developed in low leukemic strains of mice inoculated with these tissue culture materials⁸.

A more detailed study demonstrating the presence, in the leukemic tissues of man, of an agent reproducing in tissue cultures, has recently been reported by Khlopin and Chistova⁹. From their experiments it was concluded that the extracts of leucocytes and blood serum of leukemic patients contain a factor which alters and disturbs cell metabolism as well as enzymatic activity.

The problem of human leukemia was also taken up by Bergoltz in 1958 in large-scale experiments, in which the leukemic effects of various fractions of human leukemic materials including cell-free filtrates were followed¹⁰⁻¹². Extracts were prepared from different organs of the leukemic patients and inoculated into low leukemic strains of mice through different routes, producing leukemia in 35 per cent of the inoculated mice. In fractionation experiments, it was found that the nuclear fraction of the leukemic tissues was the most leukemogenic, the

physical and chemical properties of which tally with the qualities required to be called it as virus^{13,14}.

De Long¹⁵ in 1960 reported that filtered and centrifuged supernatant of bone marrow cultures of few cases of acute leukemia, when inoculated into adult Swiss mice, produced leukemia in 35-90 of the inoculated mice.

Experiments of Schwartz have immensely advanced the knowledge towards the establishment of viral etiology of human leukemia. Injections of the cell-free filtrate of brain of patients, died of acute leukemia, greatly accelerated the appearance of leukemia in 40 per cent of the inoculated mice of the leukemic strain AKR^{16,17}. Leukemia did not develop in control groups inoculated with filtrates prepared from non-leukemic human brains. Heated filtrate of leukemic human brains also gave negative results. Passage of the filtrate of the leukemic brain through brains of mice greatly increased the activity of the agent and after a number of passages, the filtrate of the mouse brain caused leukemia in C_H mice. When cell-free brain filtrates from human leukemia were inoculated into Swiss mice and passaged serially every three days, leukemogenic activity could still be demonstrated, when cell-free pooled brain filtrate prepared from the last batch of Swiss mice was inoculated into AKR mice.

The antigenic properties of the leukemic human brain filtrates were later studied by Logothetis along with Schwartz and others¹⁸. Fourteen human volunteers were injected with cell-free brain filtrate prepared from a pool of brains obtained from patients died of acute leukemia. Serum from these volunteers was drawn to afford significant protection to AKR mice that were challenged with active cell-free brain filtrates of leukemic humans. This protection was not afforded by pooled normal serum or by serum obtained from volunteers prior to immunization.

Greenspan and Schwartz¹⁹ have provided evidence suggesting a relationship between the mouse leukemia and human leukemia by determining the passive cutaneous anaphylaxis in guinea-pigs using immunized human serum and leukemic mouse tissues. Very recently they²⁰ have further extended this study and demonstrated that the extracts of leukemic tissues of man elicit an antibody reaction in both man and rabbits. This antibody reaction has exhibited, by techniques of passive cutaneous anaphylaxis, immunodiffusion, microprecipitin and immunofluorescence. These immunologic studies demonstrate specific antigenic differences between normal and leukemia tissue extracts. They have postulated that the difference between normal and leukemic extracts is the consequence of the presence of viruses or the alterations caused by them.

Domochovsky and his associates²¹ have carried out electron microscopic study of leukemia in man.

In ultra-thin section of the lymph nodes of the patients with various forms of leukemia, inclusions have been detected which contained virus-like bodies. These bodies were not found in lymph nodes from normal individuals. Virus-like bodies have also been observed in the blood of leukemic patients, which are rarely visible in electron microscope in the blood of healthy men²²⁻²⁶. Many other workers²⁷⁻³³ in the different parts of the world have also worked with human leukemia to throw more light on its viral etiology.

Recently, De Carvalho³⁴ reported the results of his immunological experiments with tissues from human leukemic patients. Hyper-immune gamma globulins were prepared from the serums of animals receiving purified antigens from a variety of cancer and leukemic tissues of human subject. These antigens were separated from normal antigens by precipitation of the latter by antibodies against normal tissues. Several batches of these animal gamma globulins with titers ranging from 2⁻² to 2⁻⁶ were administered to 15 leukemia and 16 cancer patients. Of the 15 leukemic patients, 11 had previous hormone and chemotherapy that had resulted in remissions of different degrees and duration in 7. Thirteen of the patients had remissions on immune gamma globulin lasting from 4 weeks to 29 months. Among these were some with no previous treatment and some who had no remission on chemotherapy.

From all the above observations it appears that various groups of workers in different countries have brought forward evidences indicating the presence in tissue of patients suffering from leukemia, of a virus-like agent which may induce animal leucosis and other pathological conditions. The virus-like particles which are isolated from the human leukemic tissues are not necessarily the cause of the leukemia. The phenomenon of oncotripism clearly explains that many viruses may parasitize leukemic tissue. The mouse leukemic viruses of Friend³⁵ and Graffi³⁶ were both isolated originally from Ehrlich carcinoma and that of Moloney³⁷ from Sarcoma 37, but since there is no evidence that these tumours were caused by these leukemogenic viruses, it is assumed that these viruses were growing there as 'passengers'.

The leukemia which has been induced in animals by injecting cell-free filtrates of leukemic human brain may not be due to virus present in the leukemic tissues. The cell-free filtrate may stimulate the latent leukemogenic virus which may be present in the animals and produce leukemia.

From the trend of experiments which are going on in the different parts of the world, one is very much tempted to remark about the feasibility of the viral etiology of human leukemia.

Immunologic methods have been highly successful in prophylaxis of most of the acute viral diseases of man and thus it may be expected that leukemogenic virus infection may also be prevented by vaccines.

While much useful ground has been covered already and considerable experimental and clinical evidence is forthcoming on human leukemia, it would be premature to opine that the problem has been solved finally. As is to be expected, the findings in experimental mouse leukemia are at a

much more satisfactory level than on human leukemia and more intensive and broad-based studies with human patients in suitably equipped research hospitals are called for. In India, efforts in this direction is rather meagre and trained workers in a suitable environment are woefully lacking. Indian medical scientists have, therefore, been able to make comparatively little worthwhile contributions on an international level in this area so far. It is to be hoped that more basic studies on human leukemia would soon be possible in the Chittaranjan National Cancer Research Centre, Calcutta, and in other centres in India in the not too distant future.

Summary

The researches being conducted in different parts of the world on human leukemia are reviewed with particular reference to its viral etiology.

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Nitrate Metabolism in Plants & Microorganisms

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THE properties of the element nitrogen are unique in more respects than one. Thus, though gaseous nitrogen constitutes 79 per cent by volume of the atmosphere and envelops the entire earth, the only inorganic nitrogen compound found to occur in nature in significant quantities is Chile saltpetre. The name 'nitrogen' itself was suggested from *nitrogenium* meaning 'originating saltpetre'. The element is conspicuous for its chemical inertness and at ordinary temperatures is almost incapable of combining with any other element except lithium. Again, animals die in an atmosphere of nitrogen because it cannot sustain life. But in organic combination it is of outstanding importance to living matter. As a constituent of proteins and nucleic acids, nitrogen takes part in all activities of living matter and is the corner-stone of life's structural requirements.

The metabolism of inorganic nitrogen is of utmost biological importance because the ultimate source of nitrogen for all forms of life is inorganic nitrogen. The interesting view has been advanced by Nason¹ that organic evolution has led to a particular nutritional pattern among living beings with respect to the utilization of nitrogen. Nearly all plants and many microorganisms which can reduce molecular and oxidized nitrogen to ammonia may be considered as representing the foundation of a typical ecological pyramid. Built on this base are all the other forms of life, especially animals, which are devoid of the ability to reduce nitrogen and are, therefore, dependent on the exogenous supply of reduced forms of nitrogen in organic combination.

A peculiar feature of the reactions involved in the metabolism of inorganic nitrogen is that they are of the oxidation-reduction type. This is because the nitrogen atom is capable of existing in a number of oxidation states ranging from +6 to -3. With the exception of the +6 oxidation state all the other oxidation states have been implicated in inorganic nitrogen metabolism. Strangely enough, in spite of the involvement of so many different oxidation states and the fundamental and overwhelming importance of the subject, it was only during the past decade that important advancements were made in the elucidation of the enzymatic pathways and mechanisms of the metabolism of inorganic nitrogen.

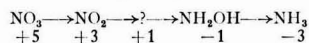
Nitrate Metabolism

Higher plants and microorganisms absorb and assimilate various types of nitrogen compounds with varying degrees of efficiency. But normally, nitrate is the main source of nitrogen for most plants and a number of microorganisms. Their nitrogenous constituents, however, apart from nitrate stored in some species of plants, contain the element almost exclusively in the reduced form, though a few organic compounds containing oxidized nitrogen are also known to occur in higher plants². It is obvious, then,

that the nitrate nitrogen absorbed has to be reduced before it is assimilated. It has been recognized that there are two broad pathways available for this reduction. One is the 'inorganic pathway' which involves the step-wise reduction of nitrate nitrogen to the lowest oxidation state represented by ammonia before it is incorporated into an organic molecule. The second possible pathway involves the incorporation of the nitrogen into an organic molecule at an oxidation state higher than that of ammonia. The two pathways, however, are not mutually exclusive³.

Conversion of Nitrate to Ammonia

If it is assumed that two-electron transfers are involved in the step-wise reduction of nitrate, then an undetected intermediate with an oxidation of +1 for the nitrogen atom has to be postulated between nitrite and hydroxylamine.



This could be the hypothetical nitroxyl (NOH), its dimer hyponitrous acid (H₂N₂O₂) or nitramide (NO₂.NH₂).

The hypothesis of step-wise reduction arose from the work of Meyer and Schulze⁴ and was given prominence by Chibnall⁵ and Burström⁶. The evidence in favour of this may be summarized as follows. (i) Many investigators⁵⁻⁷ have been able to detect the presence of nitrite in plants. The unicellular alga *Ankistrodesmus braunii* accumulates large amounts of nitrite under well-defined experimental conditions⁸. *Scenedesmus quadricauda* and *Chlorella* accumulate nitrite below pH 7 (Ref. 9). Other algae also accumulate nitrite under a variety of experimental conditions¹⁰. A number of bacteria readily reduces nitrate to nitrite which accumulates in the medium^{11,12}. (ii) The occurrence of free hydroxylamine and oximes in plants and microorganisms has been reported by many workers^{7,13,14}. (iii) The formation of ammonia as a result of nitrate reduction has been observed in plants and microorganisms^{15,16}. *Chlorella* can be made to accumulate ammonia under specific experimental conditions¹⁷. *Anabaena cylindrica* also accumulates ammonia¹⁸. The formation of N¹⁵H₂ from N¹⁵O₃ in the leaves of higher plants has been demonstrated^{19,20}. (iv) The incorporation of ammonia into glutamic acid and aspartic acid through the agency of glutamic dehydrogenase and aspartase has been demonstrated^{15,16}. (v) Perhaps the strongest arguments in favour of this hypothesis have come from studies on nitrogen fixation. Burris^{21,22} has summarized the experimental evidence in favour of the 'ammonia hypothesis'. Essentially, all of the isotopic nitrogen taken up by cell-free preparations of nitrogen fixing systems from many microorganisms can be recovered as ammonia by distillation from mildly alkaline solution and more than 90 per cent of the freshly fixed nitrogen is present as ammonia^{23,24}.

Alternate Pathway

It must be emphasized that the 'inorganic pathway' cannot explain all the known facts about inorganic nitrogen assimilation. In fact, arguments adduced in favour of it are capable of alternate interpretations⁹. Thus, the accumulation of nitrite, hydroxylamine, ammonia, etc., cannot be taken as evidence that these compounds are involved in the pathway of nitrate assimilation. These may be the products of nitrate reduction; but direct evidence to show that these are not secondary products arising from, or in equilibrium with, the intermediates on the main pathway is lacking.

The specificity of the analytical methods employed to detect these intermediates is questionable. With a method specific for the estimation of hydroxylamine, Wood²⁵ has been unable to detect free hydroxylamine in deproteinized extracts of fresh leaves or roots.

Yet another argument against this pathway is the toxic nature of the postulated intermediates. Thus, hyponitrite and hydroxylamine are highly toxic to tobacco plant and *Aspergillus niger*²⁶.

On the basis of experiments on symbiotic nitrogen fixation by *Rhizobia* in legume root nodules Virtanen^{15,27} suggested that the key intermediate is hydroxylamine which is directly incorporated into an organic molecule by the formation of oximes with α -keto acids. The oximino acid on reduction would yield the amino acid. The evidence in favour of this hypothesis is summarized below. (i) The strongest argument in favour is the accumulated body of evidence for the production of oximes, especially oximino dicarboxylic acids, in the tissues of higher plants²⁸, in the excretory products of *Azotobacter*²⁹ and inoculated legumes^{25,30} and in yeast¹³. (ii) The enzymatic reduction of oximes to amino acids by oximase and the transfer of oximino (=NOH) groups from one keto acid to another by transoximase have been demonstrated in microorganisms and a variety of other biological systems³¹⁻³³.

This hypothesis suffers from most of the disadvantages of the other pathway, because up to the hydroxylamine step there is no difference between the two. The argument may always be advanced that the existence of oximino dicarboxylic acids is no certain proof of the operation of the pathway because these may be formed by side reactions. In fact, Virtanen²⁷ himself had earlier suggested that the oximes are formed in non-enzymatic side reactions and that hydroxylamine is chiefly reduced to ammonia. Again, the existence of oximase and transoximase does not materially advance the claims of this pathway, because enzyme systems reducing nitrate and the intermediates of the ammonia pathway are also known to exist and, in fact, have been subjected to more detailed study.

The possible existence of an alternate pathway of hydroxylamine reduction has been suggested from studies on *Neurospora* mutants^{3,34} (Fig. 1). The nitrate mutants, A16, 1063, UVS2 and M13-579, which grow well on nitrite and ammonia but not on nitrate, have nitrite reductase and dehydrogenase systems but no nitrate reductase. The strain UVS2, however, can grow on nitrate at elevated pH. The fact that in this strain growth on nitrate closely

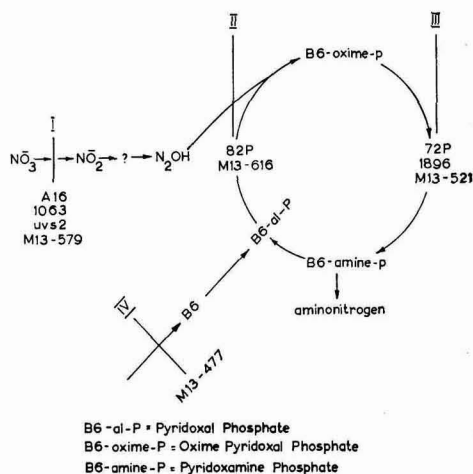


Fig. 1 — Postulated scheme for nitrate assimilation in *Neurospora*

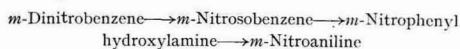
parallels the synthesis of nitrate reductase enzyme may be taken as support for the view that the first step in nitrate assimilation is its reduction to nitrite. The nitrite mutants 82P, M13-616, 72P, 2003, 1896, M13-521 and M13-477 fail to grow on nitrate or nitrite as the sole source of nitrogen. These could be placed into five heterokaryon groups indicating that at least five different steps are under enzymatic control. When grown on ammonia, strains 82P and M13-616 accumulated hydroxylamine in the medium. The mutant M13-477 which grew on ammonia did not grow on nitrate or nitrite unless pyridoxine was added to the culture medium. This and other reports in literature on growth experiments on pyridoxine requiring mutants of *Neurospora* indicate the possibility of the involvement of pyridoxine in nitrate utilization. In the scheme proposed (Fig. 1) pyridoxine is supposed to operate through the direct enzymatic condensation of free hydroxylamine with pyridoxal phosphate to form the oxime which is then reduced to the amine, which, in turn, undergoes transamination reactions to regenerate pyridoxal phosphate. Block II is placed because strains 82P and M13-616 accumulate hydroxylamine. Strains 72P, 1896 and M13-521 are presumed to be blocked at the bound-hydroxylamine level of reduction because these accumulate nitrophenyl hydroxylamine. It is not possible to fit one of the heterokaryon groups (strain 2003) in this scheme. Also, wild type *Neurospora* extracts fail to reduce the oxime of pyridoxal phosphate though the oxime could substitute for pyridoxine in the pyridoxine mutants³⁵.

The Organic Pathway

The possible existence of the 'organic pathway' in which nitrate is reduced after direct incorporation into organic molecules has been suggested because of the ability of various tissues, microorganisms and isolated enzyme systems to reduce organo-nitro

compounds. Lipschitz³⁶ reported the reduction of *m*-dinitrobenzene to *m*-nitrophenylhydroxylamine in muscle tissue. Smith and Worrel³⁷ demonstrated the ability of various bacterial suspensions to reduce chloramphenicol to the arylamine. Saz *et al.*³⁸⁻⁴² showed that cell-free extracts of *Escherichia coli* contained a nitro reductase which could reduce chloramphenicol and various other nitro compounds to the corresponding arylamines using NADH as electron donor. The enzyme has been shown to be a flavo-protein dependent on Mn²⁺ ions for maximal activity. The differential response of the enzyme to aureomycin in resistant and sensitive strains has been related to the firmness of binding of the flavin and metal to the enzyme protein. The binding is reported to be less firm in the sensitive strains so that the metal is susceptible to the chelating action of the antibiotic. Arora *et al.*⁴³ showed that the nitro reductase in cell-free extracts of *Vibrio cholerae* is inhibited by isonicotinic acid hydrazide but not by metal chelating agents or chlorotetracycline which inhibited the enzyme in *Esch. coli*. A nitro reductase which is inhibited by *p*-chloromercuribenzoate and metal binding agents was partially purified from *Aspergillus niger* by Higgins⁴⁴. The reduction of chloramphenicol by a soluble system from *Streptococcus hemolyticus* has been reported⁴⁵.

Little⁴⁶ found an enzyme in *Neurospora*, which produces nitrite from nitro-aliphatic compounds. The enzyme is induced by nitrate and to a less extent by nitrite. Experiments with *Neurospora* mutants have indicated that the groups into which they fall on the basis of *m*-dinitrobenzene reduction agree with the heterokaryon types formed on the basis of nitrate assimilation^{3,34}. But the reduction of dinitrobenzene did not appear to be under genetic control because all the mutants could synthesize the nitro reductase when grown on ammonia. de la Haba⁴⁷ could, however, produce genetic blocks in *Neurospora* and obtain mutants which do not grow on ammonia but could still utilize nitrate or nitrite. An enzyme that catalyses the reduction of *m*-dinitrobenzene to nitroaniline has been partially purified from *Neurospora* and characterized as a flavoprotein⁴⁸. If two-electron steps are assumed the reduction reaction will have the steps indicated below:



m-Nitrophenyl hydroxylamine has been crystallized and identified as an intermediate in the reduction of dinitrobenzene *in vivo*, but it does not serve as substrate *in vitro*⁴⁹.

The tumour-inducing plant pathogen *Agrobacterium tumefaciens* could reduce *p*-nitrobenzoic acid but not nitrobenzene or chloramphenicol using reduced vitamin K₃ as electron donor⁵⁰. Cain⁵¹ isolated *Nocardia* and *Pseudomonas* which could metabolize nitrobenzoic acids. *o*- and *p*-Nitrobenzoic acids under aerobic conditions could serve as the sole source of nitrogen and carbon for *Nocardia*. An enzyme has been purified over 200-fold from *Nocardia V*, which causes the reduction of *p*-nitrobenzene by NADH⁵². Ultracentrifugal studies with the purified enzyme indicate that the entire sequence of reactions is catalysed by one protein⁵³.

The metabolic significance of nitro reductases is open to criticism. Nicholas⁵⁴ considers these enzymes only as a detoxication mechanism. A number of enzymes like xanthine oxidase, diaphorase, L-amino acid oxidase, cytochrome *c* reductase, several nicotinamide coenzyme-linked flavoproteins and molybdoproteins have been found to catalyse the reduction of various nitroaryl compounds like picric acid, nitroprusside, methemoglobin, etc.^{49,55}. This has led to the suggestion that organo-nitro compounds like dyes and ferricyanide are mere non-specific, unphysiological electron acceptors of flavoproteins. Detailed investigations are needed to clarify these points and elucidate the metabolic significance, if any, of the nitro reductase system in bacteria.

Nitrate Reduction and Photosynthesis

The stimulation of nitrate reduction on illumination has been long observed. Warburg and Negelein¹⁷ suggested this to be due to increased permeability of the cells to nitrate under the action of light. That photochemical processes could result in the reduction of nicotinamide coenzymes and thus activate nitrate reduction was shown by Evans and Nason⁵⁶.

Similar results have been obtained by Jagendorf⁵⁷ also. Ferredoxin has been implicated as the electron donor for the light-dependent reduction of nitrate by isolated chloroplasts from spinach⁵⁸. VanNiel *et al.*⁵⁹ have observed that at low light intensity the uptake of carbon dioxide, but not the evolution of oxygen, is decreased in *Chlorella* by the presence of nitrate. This was ascribed to the competition by both nitrate and carbon dioxide for the photochemically produced hydrogen donors. At light saturation, however, addition of nitrate enhances oxygen evolution. Kessler⁶⁰ concluded that though photochemical reduction of nicotinamide coenzymes is likely to be one of the important factors for the enhancement of nitrate reduction in the light, it could not explain all the available data. The production of high energy phosphate is another important factor. It would appear that the 'assimilatory power' produced by the primary reactions of photosynthesis could be used not only for the reduction of carbon dioxide but of nitrate also⁶¹. Stoy⁶² has shown that photochemically reduced riboflavin is a more efficient electron donor than NADH for nitrate reduction by an enzyme purified from wheat leaves. He had earlier implicated a yellow pigment in the reduction of nitrate by detached leaves⁶³. Hageman and Flesher⁶⁴ have observed that when young corn seedlings are placed in complete darkness for 48 hr, about 90 per cent of the nitrate reductase activity is lost which is quickly regained when the plants are returned to light. Both light and nitrate are, therefore, needed for the synthesis of the enzyme in quantities required for normal growth.

Vanecko and Varner⁶⁵ using nitrite-infiltrated wheat leaves have shown that in the photoreduction of nitrite the electron donors are produced by the photolysis of water. It is concluded that nitrite is reduced to the amino level and incorporated into proteins. Kessler's observation⁶⁰ that the reduction of nitrite by intact cells of *A. braunii* is markedly

accelerated by exposure to light under anaerobic conditions has been extended by Huzisige and Sato⁶⁶ who found that the intact cells of *Euglena gracilis* show a doubling in the rate of nitrate reduction on exposure to light. These authors⁶⁷ isolated from spinach a 'photosynthetic nitrite reductase' which is required in addition to grana for the photochemical reduction of nitrite. Nason¹ suggested that 'photosynthetic pyridine nucleotide reductase' may be involved in photosynthetic nitrite reducing systems. Recent reports indicate that this may be so. It has been shown that ferredoxin functions in the nitrite and hydroxylamine reductase systems of *C. pasteurianum* and spinach chloroplasts⁶⁷⁻⁷⁰. A fast rate of photochemical hydroxylamine reduction has been reported in *Anabaena cylindrica*⁷¹. The effect of light on nitrate reduction is a complicated phenomenon, the light having to supply electron donors, high energy phosphate and possible carbon compounds⁶¹. The situation is further rendered complex by the observation that the requirements of the different steps of nitrate reduction for these factors appear to be different.

Micronutrients and Nitrate Reduction

The part the micronutrient elements play in nitrate reduction has been the subject of many investigations. The most important nutrient element in nitrate assimilation is molybdenum. Long before effective methods for the rigorous purification of culture media were evolved, Bortels⁷² could spotlight the importance of the metal for nitrogen fixation. Steinberg⁷³ has shown that *A. niger* needs molybdenum only when grown on nitrate. He suggested that the metal might activate the reduction of nitrate because it is an integral part of the enzyme. It has been later shown that molybdenum deficiency results in the accumulation of high concentrations of nitrate in the foliage of plants grown on nitrate^{74,75}. Molybdenum is rapidly transported to the leaves⁷⁶, and it is in the leaves that the disappearance of nitrate occurs most when the deficient plant is supplied with the metal. Spencer and Wood⁷⁷ found that when molybdenum was supplied to deficient tomato grown on nitrate, nitrite appeared in the leaves 2 hr after the supply. These experiments revealed that the metal was primarily needed for nitrate reduction.

The role of molybdenum in nitrate reduction has been elucidated by Nicholas and Nason^{78,79} who showed it to be an electron carrying component of the nitrate reductase of *Neurospora*. That molybdenum is the metal constituent of the enzyme is shown by: (i) close parallelism between the nitrate reductase activity and molybdenum content in various protein fractions; (ii) decrease in molybdenum content concomitant with decrease in enzyme activity during dialysis against cyanide; (iii) specific reactivation of the dialysed enzyme on addition of molybdenum; and (iv) decreased enzyme activity of the mold when grown in molybdenum-deficient nitrate medium. Subsequently, nitrate reductases from soybean leaves^{80,81}, *Esch. coli*^{82,83} and *Pseudomonas aeruginosa*⁸⁴ have all been shown to contain molybdenum. Nicholas⁸⁵ has suggested that all nitrate reductases may have molybdenum as the terminal electron carrier.

A significant contribution to the elucidation of the metabolic role of molybdenum has been made by De Renzo^{86,87} who demonstrated that tungstate inhibits, *in vivo*, the formation of intestinal xanthine oxidase induced by feeding molybdenum to deficient rats and that the inhibition is of the competitive type. The growth of *A. niger*⁸⁸ on nitrate medium has been found to be inhibited by tungstate and this is annulled by increasing the concentration of molybdenum in the medium. With ammonia as the source of nitrogen tungstate has no effect. The same effect of tungstate on growth of *Azotobacter* under conditions of nitrogen fixation and nitrate assimilation has been observed by Takahashi and Nason⁸⁹. According to Bulen¹² this approach to the study of the function of molybdenum has the advantage that it obviates the necessity of depleting the cells of molybdenum (as is done in a deficiency study) and also avoids the introduction of secondary effects resulting from growth under deficiency conditions. But, according to Nason⁹⁰, such evidence cannot be taken as final proof of the essentiality of molybdenum.

In a detailed study on the incorporation of labelled tungstate and molybdenum into *Azotobacter*, Keeler and Varner⁹¹ have observed that both metals are incorporated into proteins from which they could not be dissociated by dialysis against cyanide. It has also been observed that tungsten inhibits molybdenum uptake more than it inhibits the growth. These results have been interpreted by Bulen¹² to indicate that tungstate acts as a competitive inhibitor of molybdenum only when the observed function depended on the cellular uptake of molybdenum. But Nason contends⁹⁰ that the greater effect of tungsten on molybdenum uptake than on growth would suggest that relatively large quantities of molybdenum taken up and incorporated into proteins by the growing cells are apparently not essential for growth and replacement of molybdenum by tungsten in the protein cannot be ruled out as the most plausible mechanism of inhibition.

Tungstate has been shown to be a competitive inhibitor of molybdate in *A. niger*⁸⁸, *Neurospora*⁹² and *Azotobacter*^{12,89,91}. Tungstate interferes with nitrate utilization by *Mycobacteria*⁹³. The growth of *A. tumefaciens* in a synthetic medium has been found to be inhibited by tungstate when nitrate and not ammonium is used as the sole source of nitrogen⁹⁴. The inhibitory effect is completely prevented when molybdate is present in the medium in equimolar proportions. The growth of the organism as well as the nitrate reductase activity have been found to be depressed when the organism is cultured under conditions of molybdenum deficiency^{94,95}. Similar results have been reported with *Neurospora* and *A. niger*⁹⁶ and *P. aeruginosa*⁸⁴. It has been a general observation in all molybdenum deficiency studies that addition of molybdenum to the preformed deficient enzyme does not increase the activity of the enzyme, indicating that the element plays a role in the synthesis of the enzyme protein itself. In a similar manner, though tungstate inhibits the growth of *A. tumefaciens*⁹⁴ and *Neurospora*⁸⁹, it does not inhibit the nitrate reductase activity of the preformed enzyme when tested *in vitro*. Tungstate, however, inhibits the accumulation of nitrite in the medium

containing growing cultures of *Mycobacteria*⁹³. This may be only a reflection of the suppression of growth by tungstate and not inhibition of nitrate reductase activity.

Depressed nitrate reductase activity under conditions of iron deficiency has been observed in *A. fischeri*⁹⁷ and *P. aeruginosa*⁸⁴, in both of which ionic and haeme iron have been implicated in the transfer of electrons to nitrate. The growth and nitrate reductase activity of *A. tumefaciens*⁹⁵ are also depressed under iron deficiency.

Inorganic phosphate has been reported to stimulate the molybdenum requiring step of *Neurospora* nitrate reductase^{98,99}. This has led to the suggestion that phosphate may act by preventing the over-reduction of molybdenum by forming a phosphomolybdate complex⁹⁹.

There are numerous reports¹⁴ that deficiencies of calcium, potassium and phosphorus interrupt nitrate metabolism by their effect on nitrate absorption rather than reduction. But accumulation of nitrate in sulphur-deficient and zinc-deficient plants have been reported^{100,101}. These may be due to impairment of other metabolic functions as neither of the elements has been shown to play any role in nitrate reduction.

Enzymology of Nitrate Metabolism

Verhoeven¹¹ recognized three types of nitrate reduction: (i) 'assimilatory nitrate reduction' in which nitrate is reduced only for the formation of nitrogenous cell constituents; (ii) 'true dissimilatory nitrate reduction' in which nitrate acts, under certain conditions, as the essential electron acceptor which enables the organism to grow; and (iii) 'incidental dissimilatory nitrate reduction' in which nitrate acts as a non-essential electron acceptor.

The Japanese workers prefer to term dissimilatory nitrate reduction as 'respiratory nitrate reduction' because of its physiological similarity to aerobic respiration.

In spite of the known existence of strong reducing agents like ascorbic acid, cysteine, etc., in the cell, it has been recognized quite early that the reduction of nitrate is an enzymatic process. Since the time of Gayon and Dupetit¹⁰² who suggested that nitrite is the first product of nitrate reduction, this reaction has been subjected to extensive study. Quastel *et al.*¹⁰³ showed that *Esch. coli* reduces nitrate only in the presence of electron donors like leuco-dyes. The enzyme was called 'nitratase' by Green *et al.*¹⁰⁴ who were the first to study the reaction in cell-free system using formate in the presence of Nile blue as electron donor. Yamagata¹⁰⁵, with similar cell-free preparations, confirmed the cyanide inhibition observed earlier. During the period 1875-1914 many bacteria and fungi have been shown to reduce nitrate to nitrite and often to ammonia².

Perhaps the first demonstration of enzymatic reduction of nitrate by plant systems was made by Pozzi-Escost¹⁰⁶. Kastle and Elvove¹⁰⁷ made a detailed and critical examination of a potato-tuber extract which catalysed the reduction of nitrate to nitrite. Irving and Hankinson¹⁰⁸ have shown nitrate reduction by the tissues of several plants. Eckerson¹⁰⁹ incubated a mixture of expressed cell sap,

nitrate and glucose and showed nitrate reduction. Burström¹¹⁰ has expressed the fear that bacterial contamination during prolonged incubation (24-48 hr) perhaps vitiated Eckerson's results. This was later proved to be so²⁵. Bernheim¹¹¹ described an aldehyde oxidase system in potato which could use nitrate also as an electron acceptor. Nason⁴⁹ has suggested that this could be the same as the system reported earlier by Kastle and Elvove¹⁰⁷. Wu and Loo¹¹² obtained a nitrate reductase from soybean sprouts which could use lactate as electron donor. Cysteine has been shown to protect plant nitrate reductases from inactivation by polyphenol oxidase¹¹³.

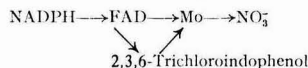
Assimilatory Nitrate Reductase

Evans and Nason^{56,114} have isolated and characterized nitrate reductases from both soybean leaves and *Neurospora crassa*. Both enzymes catalyse a reduced nicotinamide coenzyme-dependent reduction of nitrate to nitrite. The plant enzyme, which has purified 67-fold, could use NADH and NADPH as electron donors with equal efficiency, while the *Neurospora* enzyme which has also been purified 67-fold showed a high degree of specificity for NADPH. Both are shown to be molybdo-flavoproteins with FAD as the prosthetic group^{78,80,81}. It has not been possible to reverse the reaction



even by coupling with the glutathione reductase of peas. This is to be expected in view of the high equilibrium constant ($K = 10^{27}$).

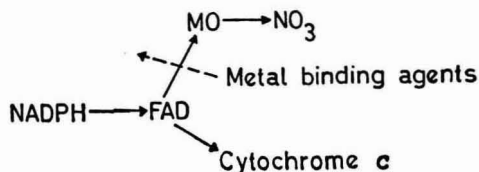
The role of flavin and molybdenum in the reaction has been elucidated by the pioneering researches of Nicholas and Nason⁷⁹ with the *Neurospora* enzyme. It has been demonstrated that flavin and molybdenum function as electron carriers in the sequence:



That flavin precedes Mo in the electron transport sequence has been demonstrated by the inability of the molybdenum-free enzyme to catalyse the reduction of nitrate by NADPH or reduced flavin, even though the flavin is reduced by the reduced nicotinamide nucleotide. Externally added molybdate has been found to specifically restore the enzyme activity. Molybdate specifically could enzymatically oxidize externally added FMNH₂ and molybdate reduced with sodium hydrosulphite could act as electron donor for the reduction of nitrate by the enzyme. These results have provided strong indication that Mo could function as an electron carrier between flavin and nitrate. Molybdenum forms compounds with the positive oxidation states of 2, 3, 4, 5 and 6. The fact that molybdate with oxidation state of +6 reactivates the Mo-free enzyme suggests this to be the more oxidized state of the metal in the enzyme. By analogy with other metalloproteins like cytochrome *c*, polyphenol oxidase and ascorbic acid oxidase in which the metal undergoes only single electron change, it was suggested that molybdenum is reversibly reduced to the +5 oxidation state during enzyme action⁷⁹. Subsequently, Nicholas and Stevens¹¹⁵ isolated and separated molybdenum

in the three oxidation states of +3, +5 and +6, and showed that Mo^{+5} is as effective an electron donor as NADPH for the enzymatic reduction of nitrate.

Purified nitrate reductase of *Neurospora* exhibit nitroethane oxidase, NADPH oxidase and NADPH-cytochrome *c* reductase activity; these enzymes are inseparable from nitrate reductase and are induced in the same manner as nitrate reductase when the mold is grown on nitrate. It is, therefore, suggested⁹⁹ that either both reactions are catalysed by the same protein with multiple reactive sites or have a common intermediate as shown below:



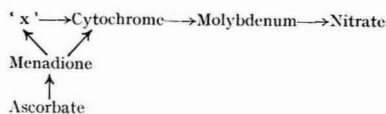
The fact that in nitrate reduction the $\text{NADPH} \rightarrow \text{FAD}$ step is three times more sensitive to *p*-chloromercuribenzoate than the $\text{FAD} \rightarrow \text{Mo}$ step has been interpreted to indicate that the transfer of electrons from NADPH to FAD is catalysed by a separate protein. The purified enzyme preparation shows on electrophoresis 3-8 bands indicating the heterogeneity of the protein⁹⁹.

Spencer¹¹⁶ purified 8-fold a nitrate reductase from the embryo of germinating wheat. The enzyme shows a specific requirement of NADH for activity. FAD has been identified as the prosthetic group but the metal involved could not be identified. The suggestion has been put forward that in higher plants NADH-specificity may be characteristic of the nitrate reductase of embryo in both the pre-germinant and early post-germinant stages. Germinating soybean seedling is also reported to contain only a NADH-specific enzyme though the one from mature leaves could use both the nicotinamide coenzymes with equal efficiency. Similar results are obtained in germinating seedlings of *Vigna sesquipedalis*¹¹⁷.

Vaidyanathan and Street¹¹⁸ have shown that the nitrate reductase activity of the extracts of excised tomato roots grown in nitrate could be coupled to alcohol, lactate, succinate and glutamate dehydrogenases. Tomato seedlings show the enzyme activity even without the addition of any dehydrogenase substrate. Root extracts contain a nitrate reductase for which ascorbate and ferrous ions are essential cofactors. This enzyme is activated by molybdenum.

An inducible, assimilatory type of nitrate reductase in *Azotobacter Vinelandii* has been studied by Taniguchi and Ohmachi¹¹⁹. Except for the particulate nature it resembles the *Neurospora* enzyme. The metal, however, has not been identified. As contrasted with this enzyme, which shows a preference for NADH, Becking¹²⁰ solubilized another nitrate reductase from *A. Vinelandii* (strain 9), which is NADP^+ -specific and can be coupled to glucose-6-phosphate dehydrogenase. The system, however, contains a powerful NADH oxidase also.

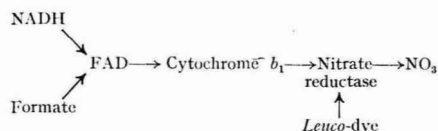
The following pathway of electron transport has been suggested for nitrate reduction in *A. tumefaciens*¹²¹:



With a particulate preparation, the involvement of both molybdenum and a *c* type of cytochrome has been demonstrated. It has been shown that menadiol or menadiione in combination with ascorbate could act as electron donor for the reduction of nitrate. Reduced nicotinamide coenzymes are ineffective. In the absence of nitrate, hexavalent molybdate is reduced to the pentavalent state which is rapidly reoxidized on the addition of nitrate. When pentavalent molybdenum is used as electron donor, ferrous ions activate the enzymatic reduction of nitrate.

Respiratory Nitrate Reductase

The first demonstration of nitrate respiration in non-denitrifying bacteria is by Quastel *et al.*¹⁰³ who showed that *Esch. coli* fails to grow anaerobically on lactate unless a suitable acceptor like nitrate was provided. Subsequent experiments of Yamagata¹⁰⁵ provide direct proof for the existence of nitrate reductase. The particulate nature of the *Esch. coli* respiratory nitrate reductase has been recognized and the metal involved has been identified as iron¹²² because of the light-reversibility of carbon monoxide inhibition. The enzyme is inhibited by cyanide and shows a cytochrome *b* type of spectrum. On the basis of these results the enzyme has been identified as cytochrome *b*. This view has been subsequently reconsidered and it has been suggested that the enzyme is not identical with cytochrome *b* but catalyses the transfer of electrons from the cytochrome to nitrate¹²³. Joklik¹²⁴ purified the enzyme from *Esch. coli* and showed that it is not inhibited by carbon monoxide. This has been confirmed by the Japanese group on reinvestigation¹²⁵. The involvement of cytochrome *b* or *b*₁ was confirmed by the inhibition of the reaction with 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO). The purified preparation catalysed the reduction of nitrate to nitrite under anaerobic conditions in the presence of reduced methylene blue, formate or NADH; but the activity in no case was more than 30 per cent of that obtained with *leuco*-methyl of benzyl viologen. The particles showed a spectrum characteristic of cytochrome *b*₁ which was rapidly reduced by NADH or even more rapidly by formate. The reduced cytochrome was reoxidized on the addition of nitrate. On the basis of these findings the following electron transport sequence has been proposed¹²⁵ for the particulate system:



Succinate, lactate and α -glycerophosphate are also effective electron donors¹²⁶.

The particulate enzyme is solubilized by treatment with isobutanol and deoxycholate¹²⁷, and the solubilized preparation shows nitrate reductase, formate dehydrogenase and NADH oxidase activities. Nevertheless, it shows no ability to accommodate formate or NADH as electron donor for nitrate reduction. Chemically reduced cytochrome b_1 or leuco-methylene blue is effective as electron donor. An unidentified, acid labile, heat stable, soluble cofactor not replaceable by FAD, menadione or ferrous iron, singly or in combination, which has been found^{127,128} necessary for the full activity of the particulate NADH or formate nitrate reductase system, is removed by the isobutanol treatment indicating its probable lipid nature. The same *Esch. coli* strain, when grown anaerobically, has been found to produce a soluble nitrate reductase¹²⁹ which is sensitive to HOQNO but could use NADH but not formate as electron donor. The enzyme is markedly stimulated by FAD, menadione and ferrous ions, and is inhibited by dicumarol which effect could be reversed by menadione. In these respects the enzyme resembles the one reported in the same organism by Wainwright¹³⁰ and Medina^{131,132}.

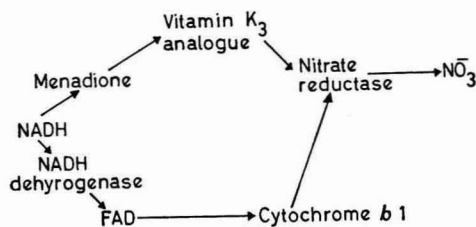
From the Yamaguchi strain of *Esch. coli*, grown anaerobically in the presence of nitrate, Taniguchi and Itagaki^{83,133} obtained a particulate formate-nitrate reductase which has been found to be devoid of NADH and NADPH oxidases and dehydrogenases except formate dehydrogenase. The enzyme is thought to be a metallo-flavoprotein containing both iron and molybdenum in addition to cytochrome b_1 . It is solubilized by treatment with proteolytic enzymes and though the enzyme has been purified over 1000-fold, it still contains one atom of molybdenum and 40 atoms of iron per mole. The protein which is ultracentrifugally and electrophoretically homogeneous shows no specific absorption peak except that at 275-280 m μ and contained no flavin or cytochrome¹³⁴. The enzyme does not catalyse the reduction nitrate with formate or NADH but reduces nitrate best with leuco-methyl and benzyl viologens and with reduced flavins with 0.6-3 per cent efficiency. This enzyme is considered to be the solubilized form of the terminal oxido-reductase which carries electrons from cytochrome to nitrate.

From the particulate fraction of *Esch. coli*, a nitrate reductase which is solubilized by treatment with snake venom and deoxycholate, has been reported¹³⁵. This preparation contains cytochrome b_1 and shows formate dehydrogenase activity also, but has lost a lipid factor required for the transfer of electron from formate dehydrogenase to cytochrome b_1 . Though the lipid has not been identified, menadione could substitute for it^{135,136}. With the solubilized preparation, it has been shown that reduced cytochrome b_1 could be anaerobically re-oxidized by nitrate in a cyanide and azide-sensitive reaction. Vitamin K_3 mediates the reduction of cytochrome b_1 suggesting that the quinone accepts electrons from formate and passes them on to the cytochrome.

The Japanese school has always been using inhibition by HOQNO as specific evidence for the pre-

sence of cytochrome b_1 . Kogut and Lightbown¹³⁷ have shown that this streptomycin antagonist inhibits succinate oxidase and NADH oxidase activity of heart muscle sarcosomes by inhibiting not only the oxidation of cytochrome b but the reduction of cytochrome c as well. Moreover, NADH peroxidase of *Bacillus subtilis*, which is a flavoprotein containing no haeme groups, is inhibited by HOQNO which is shown to be non-competitive in nature with either NADH or H_2O_2 . They have questioned the justification for using the inhibition by HOQNO on oxidative enzymes as diagnostic evidence for the participation of cytochromes. Heredia and Medina¹³² have shown that in the nitrate reductase system of *Esch. coli* the inhibition observed with HOQNO is competitive in nature and could be reversed with menadione.

Like the enzyme in *A. tumefaciens*^{50,131,132}, the menadione-dependent nitrate reductase of *Esch. coli* is inhibited by atetrin, but the inhibition is not reversed by FAD but is reversed by menadione. This has been taken to indicate the non-involvement of FAD, and Heredia and Medina¹³² suggested the existence of two pathways for nitrate reduction in *Esch. coli*.

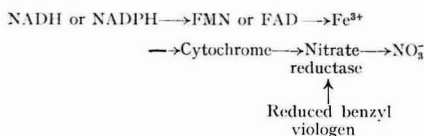


This pathway gains support from the work of Wosilait and Nason¹³⁸ who showed that the purified menadione reductase of *Esch. coli* is probably not a flavoprotein. The menadione-dependent nitrate reductase observed in *Esch. coli* by Wainwright¹³⁰ like that of *A. tumefaciens*¹²¹ is activated by ferrous ions. But while the *Esch. coli* enzyme is inhibited by cysteine the enzyme activity in *A. tumefaciens* is inhibited or activated by cysteine depending on the order of addition of the reagents⁵⁰.

Nitrate Reductase in Other Microorganisms

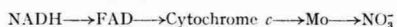
Sadana and McElroy⁹⁷ have purified a nitrate reductase from *A. fischeri* free of electron donating systems and associated cofactors. No requirement of flavin could be shown for this enzyme when reduced benzyl viologen is used as electron donor indicating that the enzyme itself is not a flavoprotein. The electron donor system, namely NADH-cytochrome c reductase, however, shows a requirement for FMN or FAD. The nitrate reductase has a cytochrome component with spectral characteristics similar to mammalian cytochrome c . The reduced cytochrome is reoxidized by nitrate. The involvement of ionic iron is indicated by inhibition with metal chelating agents and also carbon monoxide. Iron deficiency decreases the efficiency of whole cells to reduce nitrate. On the basis of these

considerations, the following scheme of electron flow has been proposed:



Molybdenum is not considered to be of any importance in nitrate reduction by this organism, because addition of molybdate is not a precondition for obtaining nitrate reductase activity. But, according to Nicholas⁸⁵, though the organism has been grown in peptone medium without added molybdenum, it does not follow that the culture is deficient in molybdenum because it is not purified. Subsequently, the essential nature of molybdenum for nitrate reduction by this organism has been demonstrated⁸⁴. It may be mentioned that the enzyme, on further purification, does not show the presence of any metal or cytochrome components but could still effect nitrate reduction using NADH as electron donor¹³⁹. The authors claim that the absorption bands obtained earlier⁹⁷ are due to cytochrome impurities.

Fewson and Nicholas⁸⁴ purified 115-fold a nitrate reductase from *P. aeruginosa* and showed it to be a NADH-specific molybdo-flavoprotein containing a *c* type of cytochrome. The following pathway of electron transport for nitrate reduction by this enzyme has been proposed:



The involvement of metal has been shown by the inhibition of enzyme activity by metal chelating agents and also by the decreased enzyme activity when the organism is cultured under conditions of molybdenum and iron deficiency. The inhibitory effect of *p*-chloromercuribenzoate as well as atebriin is reversed by FAD. The protein gives spectra characteristic of cytochrome *b* and *c* and shows NADH-cytochrome *c* reductase activity also. The cytochrome is reduced by NADH and reoxidized by molybdenum or nitrate. When either reduced cytochrome *c* or Mo^{+5} ion is used as electron donor, there is no requirement for flavin or cytochrome. That molybdenum undergoes a valency change during enzyme action is shown by electron spin resonance signals.

From a study of the properties of the particulate nitrate reductase of *Rhizobium japonicum*, Cheniae and Evans¹⁴⁰ conclude that the system is similar to the respiratory type of nitrate reductase in *Esch. coli*. The enzyme could use NADH, succinate or several reduced dyes as electron donors. When succinate is the electron donor, the system exhibits many of the properties of succinate dehydrogenase and succinate oxidase. The enzyme is inhibited by sulphhydryl binders, metal chelating agents, antimycin A and dicumarol. When NADH is used as electron donor, the enzyme is activated by menadione and shows a possible involvement of cytochrome *b*. The particulate enzyme has been subsequently solubilized both from pure cultures and soybean nodules¹⁴¹ and purified 11-fold with 27 per cent recovery. As contrasted with the particulate enzymes, neither

succinate nor NADH could serve as electron donor for the solubilized preparation. No naturally occurring electron donor has been found for the enzyme. Menadiol is ineffective as electron donor. Reduced benzyl or methyl viologen could effect nitrate reduction. Sulphydryl binders and metal chelators have no effect on the enzyme. This could possibly be the terminal nitrate reductase.

From soybean nodules Evans¹⁴² isolated a NADH-nitrate reductase requiring flavin and metal for activity. It was first thought that the role of this enzyme in nodules of legumes is connected with the metabolism of nitrate that enters the nodules from the soil. The bacterial enzyme is adaptive to nitrate¹⁴³. When soybean plants are inoculated with pure cultures of *R. japonicum* and cultured aseptically in nitrogen-free medium, nodule extracts have been found to exhibit nitrate reductase activity which, however, is only one-fourth of that of field-grown plants. From this it has been concluded that the organism growing in the nodule habitat is supplied with nitrate or some other compound which induces the adaptive formation of nitrate reductase. This has been taken to indicate that the initial step in nitrogen fixation leads to the formation of oxidized forms of nitrogen like nitrate or nitrite probably by decomposition of some initial product of fixation¹⁴³. The nodule nitrate reductase activity has been positively correlated with nitrogen fixing ability¹⁴⁴. Physiological studies¹⁴⁵ have revealed a positive correlation between nitrate reductase activity of the nodules and their haemoglobin content which itself shows a positive correlation with the amount of nitrogen fixed. Application of nitrate or ammonium to the plants and deficiency of molybdenum or iron have been found to lower the enzyme activity. This suggestion that nitrate reductase activity of soybean nodule bacteroids is induced by products or by-products of nitrogen fixation has been questioned by Bergersen¹⁴⁶ who demonstrated enzyme activity in soybean root nodules at least a week before fixation commences or haemoglobin is formed. Thus, neither the nature of induction nor the significance of the enzyme in functioning nodules is clearly understood.

The nitrate reductase of *Aerobacter aerogenes* has been investigated by Pichinoty¹⁴⁷⁻¹⁵⁰. The enzyme neither utilizes nicotinamide coenzymes nor contains cytochromes. Molecular hydrogen, which serves as the electron source, has been found to reduce nitrate stoichiometrically in the presence of benzyl viologen. The enzyme is inhibited by a number of metal binding agents. It can use reduced FMN as electron donor and the activity can be demonstrated by coupling nitrate reductase to a reduced FMN-generating system consisting of alcohol dehydrogenase, NAD^+ , NADH-FMN reductase from *S. faecalis* and FMN. Chlorate ion can act as a substrate for nitrate reductase while bromate or iodate do not. This is ascribed to a difference in ionic sizes. Many microorganisms containing the enzyme hydrogenase have been shown to reduce nitrate using molecular hydrogen⁶¹.

The existence of a respiratory type of nitrate reductase in cell-free extracts of *Mycobacterium tuberculosis* has been demonstrated^{93,151}. The enzyme is particulate in nature, NADH-dependent and contains flavin and cytochromes a_1 , *b* and c_1 . It is

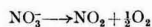
inhibited by HOQNO¹⁰¹ and high concentrations of isonicotinic acid hydrazide. The partial reversal of isonicotinic acid hydrazide inhibition by molybdenum indicates that the hydrazide inhibition results from binding to the metal.

Silver¹⁵² obtained a nicotinamide nucleotide-nitrate reductase from the yeast *Hansenula anomala* grown on nitrate. The enzyme appears to be a molybdo-flavoprotein like the *Neurospora* nitrate reductase. Granick and Gilder¹⁵³ demonstrated an obligate requirement of haeme compounds for nitrate reduction in *Hemophilus influenzae* as did Lascelles¹⁵⁴ in *Staphylococcus aureus*. Mutants of *Esch. coli* auxotrophic for haemin do not possess nitrate reductase¹⁵⁵. Nitrate reduction by Mycobacteria has been shown to be intense in young cultures. Streptomycin resistant strains do not possess the enzyme¹⁵⁶. Takagi and Maruta¹⁵⁷ have investigated the properties of the nitrate reductases of marine algae and other microorganisms.

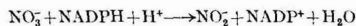
It is clear from the above discussion that the enzymological aspects of nitrate reduction had been the subject of detailed investigation. The differentiation into 'respiratory' and 'assimilatory' nitrate reductases tends to appear artificial in the light of recent developments which indicate that both may be molybdoproteins with essentially similar pathways of electron transport. Most of the bacterial nitrate reductases are particulate in nature. This would indicate that like mammalian cells, which have the mitochondria, the bacterial cells may also have rigid organelles for the purpose of electron transport.

Phosphorylation Coupled to Nitrate Reduction

Because of its physiological and enzymological similarity to oxygen respiration, nitrate respiration may involve energy-yielding reactions which are used by the organisms for growth. Nitrate reduction *per se* is endergonic and a ΔF of +20,000 cal. has been calculated¹⁵⁸ for the reaction



Evidently, when it is coupled to the oxidation of electron donors the overall reaction becomes exergonic. Thus, for the reaction



a ΔF of -38,000 cal. has been calculated by Nason⁴⁹. Takahashi *et al.*¹⁵⁹ observed the incorporation of P₃₂ into whole cells of *Esch. coli* during the anaerobic reduction of nitrate. Ohnishi and Mori¹⁶⁰ demonstrated that P₃₂ is incorporated into nucleotides under anaerobic conditions in resting cells of *P. denitrificans* during denitrification. Esterification of phosphate during anaerobic reduction of nitrate by cell-free extracts of *P. aeruginosa* was first reported by Yamanaka *et al.*¹⁶¹. The phosphorylation activity is sensitive to KCN and 2,4-dinitrophenol. Phosphorylation coupled to nitrate reduction in cell-free extracts of *P. denitrificans* has been shown by Ohnishi¹⁶². The low P: NO₃⁻ ratio of 0.23 has been attributed to dephosphorylation by ATPase and adenylate kinase present in the extracts.

Adaptive Nature of Nitrate Reductase

The adaptive nature of nitrate reductase has been indicated by the earlier studies of Pollock¹⁶³. Wainwright and Nevill^{164,165} have shown that the enzyme is adaptive in auxotrophic mutants of *Esch. coli*. A similar mutant of *Esch. coli* for haemin, however, could not be induced to form the enzyme¹⁵⁵. Straughn¹⁶⁶ observed that *Esch. coli* possesses active nitrate reductase even when grown in the absence of oxidized forms of nitrogen. He concluded the enzyme to be non-adaptive. The possibility of the formation of nitrate in the medium by the oxidation of peptone under aeration cannot, however, be excluded⁵⁴. Cell suspensions of *Esch. coli*, grown on a peptone medium supplemented with nitrate, have been found to reduce nitrate at a faster rate and without time lag in contrast to cells grown in a nitrate-free medium. The cell-free extracts, however, in both cases have been found to reduce nitrate at the same rate¹⁶⁷. It has been suggested that a permeability barrier exists in old cells towards nitrate. The possibility⁵⁵ exists that if nitrate reductase is a constitutive enzyme in *Esch. coli*, the permease system concerned with the transport of nitrate may be the adaptive one. The adaptive formation of nitrate reductase in *A. aerogenes* has been shown to be due to synthesis *de novo* of the enzyme from free amino acids^{168,169}. The enzyme synthesis was inhibited by chloramphenicol as well as molecular oxygen. The adaptive nature of *Neurospora* and *A. niger* nitrate reductases has been established⁴⁹. For *Neurospora*, nitrite is a better inducer than nitrate.

The adaptive nature of the enzyme in plants, though less convincing, is well recognized. Tang and Wu¹⁷⁰ observed that 4- to 6-day old rice seedlings show no nitrate reductase activity when grown on ammonium as the sole source of nitrogen while plants grown on nitrate show the presence of the enzyme. In older rice plants also the enzyme is adaptive. In cauliflower, the enzyme activity is high when grown on nitrate or nitrite and low when grown on ammonium¹⁷¹. Hewitt and Afridi¹⁷² could detect nitrite within an hour when detached leaf fragments from ammonium-grown cauliflower plants absorbed nitrate under vacuum infiltration. The same phenomenon was observed in intact plants also. The induction of the enzyme is prevented by inhibitors of protein synthesis. Vaidyanathan and Street¹¹⁸ found a high rate of nitrate reduction in excised tomato roots supplied with nitrate. Hardly any reduction occurs when the roots supplied with ammonium or glutamine are transferred to nitrate-containing solutions. Parthasarathy *et al.*¹⁷³ observed a gradual increase in the nitrate reductase activity of leaf extracts of the healthy sandal (*Santalum album*) from the vegetative through the flower bud formation to the flower-formation stage of the plant. In the leaves of the 'spiked' plant, which does not grow beyond the vegetative stage, the enzyme activity has been found to be higher than that in the normal plant even at the flower-formation stage. This is ascribed to the rapid synthesis of the viral protein in the spiked leaves. The prolonged incubation (17 hr) employed for the assay of nitrate reductase is, however, open to criticism.

Factors Affecting Pathways of Nitrate Reduction

Walker and Nicholas¹⁷⁴ showed that submerged felts of *Neurospora* grown at low oxygen tensions dissimilate nitrate and in this condition, nitrate reductase requires both iron and molybdenum. At a later stage of growth, when the felts break the surface of the culture solution and emerge into a more aerobic environment, there is no longer any iron requirement. These results have essentially confirmed the earlier reports^{175,176} that the oxygen tension of the medium determines the alternative pathway of electron transfer by the organism. Similar results have been obtained with a halophilic *Achromobacter* species¹⁷⁷. The necessity of haeme compounds for anaerobic reduction of nitrate by *Staphylococcus epidermidis* has been demonstrated¹⁷⁸. The suggestion has been made¹ that in aerobically grown cells the molybdo-flavoprotein functions primarily in the nitrate assimilatory process, whereas in anaerobically grown cells the cytochrome nitrate reductase chain serves in terminal respiration.

The position of nitrate reductase in relation to the electron transport chain is worth consideration. Though the researches of Nicholas and Nason⁷⁹ point to the conclusion that *Neurospora* nitrate reductase is a protein capable of transferring electrons from NADPH to nitrate, the heterogeneity of the protein as well as its intimate association with NADH-cytochrome *c* reductase led Kinsky and McElroy⁹⁹ to suggest that both reactions are catalysed by the same protein or have a common intermediate, namely FAD. This indicates that nitrate reductase like polyphenol oxidase, cytochrome oxidase and sulphite reductase is a terminal enzyme engaged in transferring electrons from a respiratory component to nitrate. This view is supported by the researches of the Japanese school^{179,180}.

Evidence in favour of the above views has been forthcoming from genetic studies as well. Nitrate reduction has been shown to be under genetic control in maize¹⁸¹ and in *Chlorella*¹⁸². Sorger¹⁸³ reported that at least two genes are concerned with the reduction of nitrate by *Neurospora*; the first for carrying electrons from NADPH to FAD and cytochrome *c* and the second from FAD to molybdenum and then to nitrate. The fact that ammonia represses not only nitrate reductase but also NADPH-cytochrome *c* reductase in *Neurospora*¹⁸⁴ indicates the intimate association between the two enzymes. In *Aspergillus nidulans* nitrate reductase has been shown to be determined by at least six genetic loci. Five of these have been suggested to be involved in the synthesis of a cofactor which also acts as a repressor^{185,186}. In *A. tumefaciens*¹²¹ lyophilization of the cells destroys the nitrate reductase activity without impairment of the molybdenum reducing ability of the cells. All these results indicate the multi-enzyme nature of nitrate reduction.

Nitrate Reductase in Animal Tissues

Bernheim and Dixon¹⁸⁷ observed that liver preparations of various species can reduce nitrate. Nitrate has been shown to function as an electron acceptor in place of methylene blue or oxygen.

Nitrate reduction appears to be a general property of molybdo-flavoproteins since it has been shown^{188,189} that xanthine oxidase and aldehyde oxidase can reduce nitrate anaerobically. Omura and Takahashi^{190,191}, however, have shown that the nitrate reductase activity of animal cell preparations may not necessarily be due to the presence of other molybdo-flavoproteins. The existence of enzymes connected with the metabolism of nitrate, nitrite, etc., has been demonstrated in the silk worm¹⁹². Anaerobic incubation of minces of fresh pig muscles with sodium nitrite, but not potassium nitrite, has been shown to produce nitric oxide by a heat-sensitive reaction¹⁹³. Hyponitrite has been determined quantitatively as the reaction product of nitrite reduction by fowl liver¹⁹⁴. Further evidence for the presence of the enzyme and its metabolic significance in animal tissues is necessary before any conclusions could be drawn¹.

Reduction of Nitrite

A nitrite reductase was first reported in cell-free extracts of *P. aeruginosa* by Yamagata¹⁰⁵ and later by Taniguchi *et al.*¹⁹⁵ in *Bacillus pumilus*. However, the use of leuco-methylene blue as electron donor in these experiments made the physiological interpretation of the results difficult. The detection of nicotinamide nucleotide-nitrite reductases in extracts of *Neurospora* and soybean leaves^{56,114} led to their partial purification and characterization. Nason *et al.*¹⁹⁶ showed that nitrite is reduced to ammonia in both systems. The soybean enzyme has been purified about 75-fold¹⁹⁷. The enzyme shows an absolute requirement for a heat-stable, dissociable, organic cofactor present in soybean leaf extracts. The enzyme activity is inhibited by several metal binding agents and is stimulated by Mn²⁺ ions. There is disappearance of nitrite with concomitant oxidation of NADH; but the product has not been identified.

The nitrite reductase of *Neurospora* has been shown to be dependent on iron and copper for maximal activity¹⁹⁸. The particulate enzyme has been solubilized and purified over 50-fold^{95,198} and shown to be a FAD-enzyme with NADH, a more effective electron donor than NADPH. Inhibition by metal binding agents indicates the involvement of a metal. The enzyme activity in extracts of mycelia deficient in Mg, Fe, Cu or Zn is significantly lower, while a deficiency of Mo or Mn is without effect. Addition of Cu to extracts of Cu-deficient felt and Fe to extracts of Fe-deficient felt has been found to regenerate the activity. There is no reactivation on addition of Mg to extracts of Mg-deficient felts. Cuprous ion is effective as electron donor in the enzymatic reduction of nitrite, while Fe²⁺ ions or reduced cytochrome *c* are ineffective. The enzyme is considered to be a copper protein.

Spencer *et al.*¹⁹⁹ reported that the nitrite reductase of *A. agile* is an adaptive metallo-flavoprotein which reduces nitrite to ammonia. From *Esch. coli* strain Bn, Lazzarini and Atkinson²⁰⁰ obtained a NADPH-specific nitrite reductase which has been found to be sensitive to cyanide. The preparation also reduces hydroxylamine and cytochrome *c* at the expense of NADPH. These activities paralleled the nitrite reductase activity during a 190-fold purification.

No other cofactor requirement could be demonstrated. Experiments using labelled nitrite have shown that free hydroxylamine is not an obligatory intermediate in the reduction process. It has been suggested, therefore, that the overall reduction of nitrite to ammonia is catalysed by a single enzyme without the intervention of free intermediates.

According to Taniguchi *et al.*¹²⁵, though *Esch. coli* is unable to reduce nitrate beyond the nitrite stage in anaerobic cultures, it reduces nitrate to nitrite, hydroxylamine and ammonia in well-aerated cultures. However, a halotolerant *Micrococcus* has been found to reduce nitrate to ammonia in successive steps under anaerobic conditions. It has been suggested that the nitrite reductase of this organism is a flavoprotein. Reduced methylene blue is a good electron donor for the reduction. NADH has no effect while reduced FAD is somewhat effective. The enzyme is strongly inhibited by hydroxylamine.

Pichinoty and Senez²⁰¹ showed that suspension or extracts of *Desulphovibrio desulphuricans* can reduce nitrite and hydroxylamine to ammonia using molecular hydrogen. Cytochrome *c*₃, by virtue of its ability to restore the activity inhibited by hexadecyl trimethyl ammonium bromide, has been implicated as a component of the enzyme systems. The use of hydrogen by the same species for the reduction of nitrite to ammonia has been reported by Krasna and Rittenberg²⁰² also. McNall and Atkinson²⁰³ isolated a strain of *Esch. coli* which uses molecular hydrogen for the reduction of nitrate and nitrite to ammonia. The organism can utilize hyponitrite, hydroxylamine or nitrous oxide as the sole source of nitrogen²⁰⁴. The ability of certain algae to use hydrogen for the reduction of nitrite has been reported by Kessler⁶¹.

The adaptive nature of nitrite reductase in tomato root has been observed by Vaidyanathan and Street¹¹⁸. Roots grown on ammonia or glutamine do not contain the enzyme which is present in roots grown with nitrate or nitrite. Hageman *et al.*²⁰⁵ obtained an enzyme system from leaves of vegetable marrow, *Cucurbita pepo* and *Zea mays*, which catalyses the rapid and quantitative conversion of nitrite to ammonia. The system can also reduce hydroxylamine. NADH and benzyl viologen or reduced benzyl viologen can act as electron donors. The rate of reduction of hydroxylamine is much lower than that of nitrite. This is interpreted to indicate that hydroxylamine may not be an intermediate in the pathway of the assimilatory reduction of nitrate.

Najjar and Chung²⁰⁶ obtained for the first time cell-free extracts of *B. subtilis* and *P. stutzeri* which could reduce nitrate and nitrite to gaseous products consisting of nitrous oxide, nitric oxide and nitrogen. With ammonium sulphate fractions of *P. stutzeri* extracts, it has been shown that NADH and NADPH could serve as electron donors for the reduction of nitrite to nitric oxide. The involvement of flavin, cytochrome, iron and copper was also shown. Walker and Nicholas²⁰⁷ purified a nitrite reductase from *P. aeruginosa* more than 600-fold and the enzyme has been found to reduce nitrite to nitric oxide. This enzyme has also been shown to be a flavoprotein containing both iron and copper as well as cytochrome *c*. NADH or NADPH, however, did not serve as electron donor, whereas reduced 1,4-naphthaquinone,

pyocyanine, methylene blue or flavins have been found to be good electron donors.

Yamanaka *et al.*^{208,209} purified a cytochrome oxidase from *P. aeruginosa* which catalysed the reduction of nitrite under anaerobic conditions. Reduced cytochrome *c* isolated from the organism can act as electron donor. Hydroquinone, leuco-pyocyanine, and leuco-phenazinemetosulphate also act as electron donors. The reduction product has been shown to be nitric oxide. It has since been reported²¹⁰ that in this organism cytochrome oxidation and nitrite reduction are catalysed by the same protein. Iwasaki and Mori²¹¹ obtained a cell-free preparation from *P. denitrificans* which reduces nitrite to nitrogen in the presence of lactate and to nitrous oxide in its absence. Hyponitrite is not utilized but hydroxylamine stimulates nitrogen evolution. It is, therefore, suggested that in denitrification a portion of nitrite is reduced to hydroxylamine which, in turn, reacts with the remaining nitrite to produce nitrous oxide. Another possibility suggested is that in the presence of lactate and lactic dehydrogenase, nitrite is reduced to nitric oxide which then reacts enzymatically with hydroxylamine to produce nitrogen. Iwasaki²¹² separated two inactive fractions from the extract which could be recombined to reconstitute the denitrification system. One fraction contains cytochrome *c* and the other, which is considered to be enzyme, is coloured red due to the presence of 'cryptocytocrome *c*'. On further purification by electrophoresis the 'cryptocytocrome *c*' fraction has been found to consist of cryptocytocrome *c* and a non-haeme blue protein which catalyses the reduction of nitrite with cytochrome *c* and dimethyl- β -phenylene diamine²¹³. Both have been crystallized and the blue protein has been shown to contain copper^{214,215}. It is suggested that cryptocytocrome *c* contains two haemes per mole. Its biological function, however, is not yet clear.

Using a partially purified nitrite reductase from a halotolerant *Micrococcus* Asano²¹⁶ observed that nitrite is stoichiometrically converted to nitrogen. The NADH-nitrite reductase activity is stimulated by FAD and menadione and inhibited by amytal, quinine, dicumarol, antimycin A and carbon monoxide. The system contains cytochrome *b*₄ as electron carrier. The preparation possesses powerful hydroxylamine reductase activity. Subsequently²¹⁷, the enzyme has been resolved into a particulate component and a soluble one, both of which are required for activity. The activity of the soluble component parallels the hydroxylamine reductase activity. The soluble component is activated by copper and the particulate by iron. Neither component is of cytochrome nature.

Hyponitrite Reductase

Medina and Nicholas^{218,219} reported the presence of NADH-dependent hyponitrite reductase in extracts of *Neurospora*. It has been suggested that the enzyme is a metallo-flavoprotein and ammonia has been identified as the product. The preparation also shows nitrite and hydroxylamine reductase activity. Inhibition by 1,4-naphthaquinone and HOQNO suggests the participation of haeme compounds. Inhibition by dinitrophenol is assumed to indicate

the involvement of phosphorylation in the reducing step. This, according to Nason¹, is a premature conclusion. Hyponitrite reductase has the same characteristics as nitrite reductase and, the claim²¹⁹ that the two enzymes are different proteins based on a 32 per cent decrease in the nitrite reductase activity under zinc deficiency, must await confirmation⁵⁵. The activities of both the enzymes are depressed under iron and copper deficiency. This is taken to indicate that these metals are components of the enzymes. Under manganese deficiency, hydroxylamine reductase activity is depressed which indicates a role for the metal in the enzyme. Nason and Takahashi⁵⁵ have questioned these claims since by the same logic nitrite reductase should be a zinc enzyme also.

The presence of enzyme systems that reduce hyponitrite to hydroxylamine with FAD as cofactor and NADH as electron donor in yeast and animal tissue has been reported^{220,221}. In contrast to nitrite reductase from the same sources this enzyme is sensitive to *p*-chloromercuribenzoate²²².

Extracts of nitrate-grown tomato roots have also been found to reduce hyponitrite to ammonia, but prolonged incubation is required to obtain measurable quantities of ammonia¹¹⁸. In the system studied by Hageman *et al.*²⁰⁵, hydroxylamine does not accumulate or limit the rate of reduction of nitrite to ammonia. Hyponitrite is not reduced by a system containing all the enzymes for the successive reduction of nitrate to ammonia¹²⁵.

Hydroxylamine Reductase

Woods²²³ and Yamagata¹⁰⁵ were the first to observe hydroxylamine reductase in bacteria. It was later shown by Egami's group¹²⁵ that cell-free extracts of bacteria reduce hydroxylamine to ammonia. The natural electron donor has not been identified. Klausmeier and Bard²²⁴ reported an ammonia dehydrogenase from *B. subtilis* which reversibly catalyses the formation of ammonia from hydroxylamine. On reinvestigation²²⁵ it has been found that ammonia is not enzymatically converted to hydroxylamine by the system which has only hydroxylamine reductase with an equilibrium constant of 10 (Ref. 35). Subsequently, a NADH-dependent hydroxylamine reductase has been identified in *Neurospora*²²⁵ and soybean leaves^{196,197}. The fungal enzyme is a flavo-protein while the plant enzyme, which could not be separated from nitrite reductase, is activated by Mn²⁺ ions. Hydroxylamine is stoichiometrically reduced to ammonia by the fungal enzyme while the product could not be identified with soybean enzyme. The product of hydroxylamine reduction by the enzyme obtained from *Azotobacter*¹⁹⁹, which is specifically activated by Mn²⁺ ions, has not also been identified.

An enzyme that catalyses the reduction of hydroxylamine by *leuco*-methylene blue has been obtained from a halotolerant *Micrococcus*¹²⁵. The enzyme is activated by Mn²⁺ ions. Kono and Taniguchi²²⁶ purified the enzyme 200-fold and showed the presence of a *c* type of cytochrome and manganese in it. A reaction mechanism involving the transfer of electrons from cytochrome *b*₄ to hydroxylamine via cytochrome *c* and manganese has been proposed for the enzymatic reduction of hydroxylamine. This

organism and other typical denitrifiers like *M. denitrificans* and *P. aeruginosa* possess active hydroxylamine reductase even when strongly denitrifying.

Walker and Nicholas²²⁷ purified 47-fold a hydroxylamine reductase from actively denitrifying *P. aeruginosa*. The enzyme, which reduces hydroxylamine to ammonia using reduced pyocyanine or methylene blue as electron donor, has been shown to be a flavo-protein-containing manganese. Only cobalt could substitute for manganese with 10 per cent efficiency.

A requirement of cytochrome has been shown²²⁸ for the hydrogenase-dependent reduction of hydroxylamine to ammonia by extracts from sulphate reducing bacteria. It has been concluded by Senecz and Pichinoty^{229,230} that in *D. desulphuricans* the hydroxylamine reducing mechanism involves reduction of cytochrome *c*₃ by hydrogenase. The reduced cytochrome is non-enzymatically reoxidized by hydroxylamine. The ability of haeme proteins to serve as electron donors in the non-enzymatic reduction of hydroxylamine has been observed by many workers¹.

Mager²³¹ suggested that in *Esch. coli* the NADH-sulphite and hydroxylamine reductases may be the same protein. Michaelis constant for sulphite is about one-hundredth of that for hydroxylamine. This is interpreted as to indicate that sulphite reduction represents the true physiological function, while the reduction of hydroxylamine is an incidental property of the same protein. Kemp *et al.*²³² obtained a NADPH-nitrite reductase from *Esch. coli* (strain Bn) which appears to be identical with Mager's sulphite reductase. The inability of ammonia or amino acids to repress the enzyme activities is interpreted as to indicate that the physiological function of the enzyme is sulphite reduction.

Vaidyanathan and Street¹¹⁸ observed manganese-activated reduction of hydroxylamine to ammonia in tomato roots in the presence of lactate, succinate or formate. Manganese, however, has no effect on the reduction of hydroxylamine by the system of Hageman *et al.*²⁰⁵. But the preparation catalysing the peroxidation of hydroxylamine is activated by the metal.

Evolution of Inorganic Nitrogen Pathways

It is recognized that organic substances could have been formed on the earth even before the appearance of life by purely abiogenic means in the waters of the then existing seas and oceans²³³. According to Oparin²³⁴, because of the reducing atmosphere prevalent in primitive earth conditions, cyanogen and ammonia could have played major roles in the evolution of life and metabolism on the earth. Recent reports have lent considerable support to this hypothesis^{235,236}. Several suggestions²³⁷⁻³⁹ have been put forward recently about the evolutionary development of the different pathway of inorganic nitrogen metabolism and their impact on the economy of the cell. These are, at best, rational speculations. Nason¹ is of opinion that anaerobic heterotrophs were the forerunners of the aerobes because of the dependence of the latter on the accumulation of oxygen by photosynthesis. Assimilatory nitrate reduction, which, in his view, may be considered quite primitive, would, in addition to reducing nitrate for the production of proteins, have facilitated

fermentation by preventing the accumulation of reduced nicotinamide coenzymes. The subsequent evolution of pigmented organisms would account for the phenomenon of photochemical nitrate-assimilation. The evolutionary formation of cytochromes led to respiratory nitrate reduction which later on evolved into the respiratory pathway.

One serious disadvantage of this hypothesis is the assumption that nitrate was present under primitive earth conditions. Under primitive conditions nitrogen could have been present only in the reduced state, namely as ammonia. Therefore, it would be reasonable to conclude that it is only a close study of nitrification and not nitrate assimilation or respiration that may successfully unravel the mystery of the evolutionary development of the pathways of inorganic nitrogen metabolism and its relation to cell economy.

Conclusion

It is clear from the foregoing review that biochemists and microbiologists are at present fully seized with the importance of the inorganic nitrogen metabolism. The controversies on pathways of nitrogen metabolism and the criticisms offered not only on the broad conclusions drawn but also on even the techniques employed in experimentation give a good measure of the contemporary importance attached to the subject.

The subject of nitrate reduction has been investigated in detail and considerable insight into the mechanism of function of nitrate reductases has been gained. The properties and physiological function of nitro-reductases and the presence and importance, if any, of nitrate reductases in animal systems are two fields that invite enquiry.

Schreiner and Rinfret²⁴⁰ made an interesting observation that the purified nitrate reductase of *N. crassa* is stimulated *in vitro* appreciably by several polymers especially methyl cellulose and polyvinyl pyrrolidone. This polymer effect has been extended to mean that many macromolecules possess physico-chemical characteristics which render them capable of enhancing *in vivo* the catalytic activity of enzymes by providing suitable environment. If this were so, it would be interesting to study whether the substrate specificity of enzymes concerned in the metabolism of nitrate is subject to influence by macromolecules. Such studies may help in placing the observations of Mager²³¹ and Kemp *et al.*²³² in the proper perspective.

It must also be emphasized that while the enzyme that reduces nitrate to nitrite has been studied in detail, those concerned with the further metabolism of nitrite still remain to be investigated in detail.

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Desalination of Sea & Brackish Water

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THE need for fresh drinking water in large quantities in many parts of the world at reasonable prices is being increasingly felt at the present time, as existing natural sources are only barely adequate. The certain increase of population envisaged by A.D. 2000 will make the natural fresh water sources quite insufficient to meet the increased demand. It is imperative to project for conversion of sea water into fresh drinking water at low cost and further, to install specially designed plants for conversion of brackish water into drinkable fresh water, again at low cost, in various parts of the world where inland sources of brackish water exist. Viewed as a world problem this is particularly important in respect of under-developed and over-populated countries, for example, African and Asiatic countries. That the problem has been actively pursued as one of great importance is clear from the European conference on desalination held some time ago in Greece, and the proposal of the US Government Office of Saline Water (OSW) to spend \$ 75 millions on research and development in this field in the next six years as well as the numerous projects to be started shortly in many countries in Europe and in Asia, for example, India.

Commercial Projecting

Conversion into drinkable water means bringing down saline content of the feed or treated water to less than 500 p.p.m. from anywhere up to about 3500 p.p.m. in sea water. Commercial projects developed up to the present time adopt one of the following: (1) distillation processes, (2) membrane or electrodialysis processes, (3) separation by freezing, and (4) chemical and physico-chemical processes. A brief account of these processes and discussion of possible developments in production methods are given in this paper.

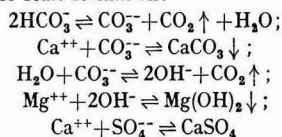
Distillation Processes

In these, whether in the boiling system or flash system, water is separated and removed from the dissolved salts and saline water by evaporation and recovered by condensation. Thermodynamic energy considerations of the evaporation and condensation form basic factors in regard to both

efficiency and cost of process. Problems of heat transfer, corrosion and scaling, recovery and utilization of heat of condensation are important for consideration. As energy source, nuclear fission, waste heat from industrial operations, solar radiation and ocean temperature difference can all be used for the distillation process. Research work has shown that heat transfer can be improved on the liquid side by (a) increasing velocity of liquid pumped through the tubes, (b) using rapidly rotating surface causing dropwise condensation, (c) coating metal with water-repellent material for the same purpose as in (b), and (d) use of coaxially grooved surface for easy flow of condensate. Other improvements of process technique which have been made are transverse vibration of inner tubes, acoustic vibrations in the water of frequency 1000-5000 c/s. and use of high conductivity metals like oxygen-free bronze.

Scale Formation and Corrosion

The extent of fouling of heating surface by formation of scale is probably the controlling factor in the economy of distillation processes. Temperature and concentration of feed water that can be used in a distillation process are limited by formation of calcium sulphate scale. As water is removed in evaporation, dissolved salts concentrate and as limit of solubility is reached, precipitation and formation of scale from the saline water on any heat transfer surface with which it is in contact occur, causing rapid loss of thermal efficiency and deterioration of the equipment. It has been put forward that the following mechanism of reaction accounts for scale formation:



Though scale formation depends on temperature and concentration of feed, it does not depend on whether the sea water comes to boil or not. The mechanism given above indicates that hydroxide and carbonate scales can be prevented by pH control using an acid like HCl or H₂SO₄ or a salt like FeCl₃.

But this involves an increased risk of accelerating corrosion and higher process cost. Use of seed crystals in the salt solution or sludge circulation of 1 per cent calcium sulphate helps to reduce scaling considerably. Use of polyacrylic acid in the feed prevents scale formation by making metal surfaces non-adherent to scale and by breaking off through shear stresses the alkaline and sulphate scale as it builds up. Turning off feed momentarily at 1 hr intervals and having a feed system where temperature drops as concentration of salt increases, evaporation at low temperature under high vacuum, and prevention of CO₂ escape are other methods which have been used to minimize scale formation.

Like scale formation, corrosion is a common difficulty in distillation processes, chief factors associated with it being dissolved oxygen, temperature, composition and flow velocity of feed, and galvanic action due to contact between dissimilar metals. Chloride in particular is a strong corroding agent and its presence must be avoided. Protective coatings and use of special alloys like monel metal, carbon alloys for plant and equipment, and deaeration of feed, are methods commonly used to minimize corrosion.

Commercial plants using distillation process are in operation at several places in the world. A large distillation plant has been working at Kuwait for some years. Two new and large-scale plants (million gallons per day units) have been recently established in the USA at Freeport, Texas, and San Diego, California. Costs of production at these plants by this process range from about \$ 1.0 to \$ 2.0 per 1000 gal.

Electrodialysis Methods

The separation of colloids and extremely small-sized dissolved particles (ions) from the solute by use of membranes of suitable size, spacing, fineness and ion selectivity is a well-known method (dialysis) and in recent years adoption of several refinements and improvements in regard to all these factors has greatly enhanced its efficacy. In electrodialysis methods used for desalting of water, membrane separation is given added flow and momentum by application of direct electrical current to move ions out to the synthetic anionic and cationic ion-exchange membranes arranged alternately in such manner that the salt ions accumulate in a central compartment.

The ohmic losses of energy in electrically caused ion movement in the brine solution constitute the largest part of energy consumption in electrodialysis methods. These methods are, therefore, most satisfactorily applied at present to low salinity feed waters. If salinity of natural sources of water does not exceed 1000 p.p.m., costs of fresh water production by electrodialysis are lower than in distillation. Electrodialysis for desalination of sea water on large scale has been recently found possible through use of permselective special quality membranes. The economics of a commercial project for desalting of brackish water (of which large natural sources exist in many parts of India, e.g. Rajasthan, South India and Assam) depends essentially upon (a) the design of the electrodialysis unit, particularly its water compartment with reference to its position, volume,

flow rate, membrane size and spacing, and (b) the quality of the membrane material, viz. its low electrical resistance, low osmotic permeability, high physical strength and good ionic selectivity. Electrodialysis methods hold very good promise for commercial projects in regard to brackish water, and specific projects in appropriate regions of brackish water sources are worthy of high priority consideration. Combination of electrodialysis process as a first stage and distillation as final stage of an integrated plant, installed in appropriate regions having brackish water sources with more than 500 p.p.m. and up to about 1500 p.p.m. saline content, may well be most satisfactory for a commercial project.

Separation by Freezing

This process is now rated along with distillation and electrodialysis in importance and makes use of the principle that when a salt solution is cooled below crystallization point, pure water separates out as ice: the ice formation proceeds from outside to inwards of the solution. But the ice entrains some salt solution of higher concentration than in the original. Relative merits of this process as compared with distillation process are (a) separation of water (as ice) requires less consumption of energy than in evaporation as latent heat of ice is much less than that of steam, (b) the difference of temperature from room temperature involved in the processing is less, (c) consequently scaling and corrosion cause operating difficulty to a much less extent, and (d) simpler equipment may be used and better control of the purity of water may be secured.

The most serious problem of the process is the removal from the crystallized ice of occluded brine liquor and the adherent surface salt. To overcome the difficulty several methods have been adopted successfully, which involve expense to a higher or lower extent. In one method sea water is evaporated under vacuum at -2°C. resulting in ice formation. The corresponding vapours are condensed in direct contact with a suitable absorbent and the heat of the condensation is utilized for melting ice. Supplementary heat introduced is removed by a refrigerator. Water absorbed is withdrawn in a multiple effect evaporating plant thus regenerating condenser liquid. In a novel method used to remove the brine from the ice, air is blown through a hollow stirrer into the freezing solution, which causes ice to form as fine snow. The snow can be relatively easily removed from the mother liquor by centrifugation. The costs incurred in the cleaning and separation of the brine to secure fresh water, and some risk inseparable from using inflammable hydrocarbon as refrigerent are handicaps to commercial plant operation of this process. However, in many countries large-scale plants operating by this process are being constructed now.

Chemical and Physico-chemical Processes

The formation of solid hydrate when sea water is reacted with propane gas, methyl chloride or fluorocarbons and subsequent release of fresh water by melting the hydrate forms the basis of a chemical process for desalination of sea water. Since this

process operates at a moderately low temperature (10°C.), scaling is avoided and energy is conserved.

In electrochemical desalination, ions are removed from saline water by electrically induced absorption at very low voltage on carbon electrodes. For maximum efficiency each electrode should be able to remove only either cations or anions from the solution and give up the same to a reject solution on reversal of the applied voltage. Carbon chars, blacks, are the most attractive present-day cation responsive electrode materials and quaternary polyvinylimidazole yields electrodes of fairly high capacity for anions.

Other Processes

There are some other processes which, though not accepted as commercial so far, may be used for desalination with varying cost considerations and operating features. Ion-exchange resins have been used for desalination of water containing less than 1000 p.p.m. and their use is affected by the high regeneration costs. Osmotic process is similar to electro dialysis in the membrane segregation and removal of the ions from the brine, but instead of using a direct electric current for the movement of ions, the process uses the difference in concentration between brine solution and the water to be demineralized as the source of energy for ion movement. The poor economy of producing consumable power by this process and the necessity of having both brine and brackish water at the same location seriously limit its applicability. In the 'reverse osmosis' process, by the application of pressure greater than the osmotic pressure in the reverse direction, fresh water is made to flow through the membrane from sea water side. Apart from the difficulty of finding a membrane which has high desalination capacity and durability at the pressures used, the process has the inherent limitation of large-scale movement of water present in large quantities against frictional forces, whereas in electro dialysis only the salt present in a smaller amount is removed.

Economics of Desalination

Based upon present data the following general propositions may be stated: (a) the larger the size of a plant under any selected process, the lower is the cost of production of fresh water at a desalination plant; (b) fuel and/or power costs form a dominant factor in plant operation; and (c) there is a clear trend for the cost of fresh water of natural sources to steadily go up due to increased demand, scarcity of natural sources, and higher cost of storage and distribution—a factor which favours desalination projects.

A fundamental factor in the selection of the process to be adopted and installation of a commercial desalination plant in any desired region is the estimation of the costs of production of fresh drinking water. The nature and capacity of the source of saline water, its cost delivered ex-plant, operation costs and costs for delivery at distribution point—these have to be computed working back from the optimum cost of fresh water at delivery point, which is usually predetermined by the economic factors

of the region concerned. Thus the optimum rate may well be Re 0.75 or \$ 0.15 per 1000 gal. in India, even lower in parts of Africa or Asia, \$ 0.30 per 1000 gal. in USA, etc. However, the aggregate costs of production of fresh water by desalination at the present time lie far above these rate ceilings, and planned efforts to cut costs have secured small progressive reductions. Latest estimated cost of 1000 gal. of water obtained by desalination in different plants is: flash desalination plant (San Diego, estimated capacity 14,000,000 gal./day), \$ 0.60; electro dialysis plant (Buckeye), \$ 0.50 and (Webster, capacity 2,000,000 gal./day) \$ 0.45; 10 per cent reduction in cost, for example, may be secured at selected places by using natural gas or fuel instead of oil. The use of atomic reactor as heat and/or power source for desalination process operations appears to be an economical proposition; reduction of costs by as much as 20 per cent may be effected as confirmed by the results obtained in recent work both in USA and USSR and the project study in Israel.

Efforts made in recent years to secure a heavy cut in cost of desalination and production of fresh water show that it is increasingly difficult to effect a further substantial reduction by improvements and refinements of the established processes though marginal cost cuts can be achieved. On present studies the adoption of a combination of distillation and electro dialytic processes in a commercial plant would perhaps be the most satisfactory arrangement.

Possible Developments

It has been suggested that study of the habits and gland activities of certain sea birds which are able to convert sea water into fresh water for their requirements through their metabolism and gland activities may give some knowledge regarding economical process for conversion of sea water.

Another valuable clue to a profitable line of action may be gained by study of the existence of some natural sources of fresh water close to sea water which may have resulted from conversion through chemical and/or bacterial actions occurring by virtue of the natural conditions existing at these places. It has been known, for instance, that through operation of microbiological organism sulphur has been removed from sea water containing sulphates and deposited as colloidal sulphur and relatively fresh water occurs inland, for example, close to the shores along the coast of Orissa and Andhra Pradesh in India. Another instance of such a possibility may be seen in the water which is pumped out in large quantities from its natural overlay on lignite beds in Neyveli near the south-east coast of India, near Pondicherry and Cuddalore. The relative freshness of the water is possibly due largely to the effects arising from its existence over lignite beds since long time. It would seem that if saline water is pumped through a bed of some depth of alternate layers of coal or carbon and zinc accompanied by aeration by blowing air or oxygen through the bed along with the water, the water that is collected from the top of what in effect is a natural primary electrolytic cell will have much less saline content than the feed water. Detailed

studies of this method and investigations of the chemical actions which facilitate the conversion appear to be worth pursuing.

It is also known that the mother liquor obtained after removal of salt contains a higher than natural abundance of D_2O and as such can be used as a feed for a plant producing D_2O with advantage. The possibility of coupling a heavy water production plant and a desalination plant for economical purposes deserves some study.

Summary

The production of fresh, potable water from saline and brackish water by different methods is described briefly. The limitations of the production processes employed, the improvements that may be possible in them, the economics of desalination and the necessity of investigating the naturally exist-

ing saline-to-fresh water conversion phenomena in selected regions are discussed.

Acknowledgement

The author wishes to thank Prof. E. Weingaertner for introducing him to the subject of desalination of sea water by solar evaporation and Miss M. Jones for assistance in preparing this article.

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International Centre for Theoretical Physics

The first scientific research and educational institution to be set up on an international basis was formally inaugurated in Trieste (Italy) on 5 October 1964 by the Director-General of the International Atomic Energy Agency (IAEA). Scientists from sixteen countries are currently engaged in research and teaching at the centre. The centre's main object is to foster the advancement of theoretical

physics through training and research. The subjects will include high energy and elementary particle physics, plasma physics, low energy physics and solid state physics.

Aided by the International Atomic Energy Agency, Unesco and the Italian Government, the centre will work in close collaboration with the European Organization for Nuclear Research.

REVIEWS

RECENT ADVANCES IN AEROSOL RESEARCH — A BIBLIOGRAPHICAL REVIEW by C. N. Davies (Pergamon Press Ltd, Oxford), 1964. Pp. ix+80. Price 30s.

Interests on aerosols are spread very widely over meteorology, military science, biology and health physics. The research papers on it are not well integrated as these are published in diverse and even unexpected journals. Therefore, the indexing of abstracts are also not in a satisfactory state. To remedy the situation this review has been written.

It is mainly concerned with presenting important contributions, without discussion or criticism, to purely scientific aspects and not applications. It focuses attention on the period 1957-62, the subject matter being divided into nineteen chapters under different headings, such as Acoustic, ultrasonic and shock wave effects, Adhesion of particles, Chemical reactions, Coagulation and diffusion, Evaporation and condensation, Nucleation and growth, Optics of aerosols, Radioactivity, Sedimentation and fluid resistance and Turbulent flow and deposition of aerosols.

The author has faced the task of including in a book of 75 pages only a very wide spectrum of topics, so that even a little desirable background to form a basis for these topics could not be provided. However, the compilation of recent work indicating the progress in various aspects would be a valuable source material for scientists to readily obtain information on recent progress in their respective fields.

A. B. BISWAS

REACTION KINETICS: Vol. 1 — HOMOGENEOUS GAS REACTIONS; Vol. 2 — REACTIONS IN SOLUTIONS by Keith J. Laidler (Pergamon Press Ltd, Oxford), 1963. Vol. 1., Pp. x+136. Price 30s. Vol. 2, Pp. x+232. Price 30s.

A number of books have appeared during the last three decades on reaction kinetics, but all these are essentially meant for the advanced level. The two volumes under reference, presented by an outstanding physical chemist at a level suitable for students who have some training in physical chemistry, bring an amazing wealth of information in accessible form to the user, whether an advanced student or a university teacher. The simple style and continuity of presentation clearly reflects the experience of a professor who teaches the subject to university students.

Volume 1 comprising four chapters deals with the kinetics of homogeneous gas reactions. The first chapter gives an excellent introduction to chemical kinetics and provides the essential background for the proper understanding of the subject. Rate expressions for reactions of different types and various orders (static and flow systems) have been derived and their methods of determination outlined. The second chapter furnishes a sound theoretical background by treating in a simple way the general principles involved in the construction of potential-energy surfaces of elementary reactions and discussing

the significance of Arrhenius law, kinetic theory of collisions, theory of absolute reaction rates and kinetic isotropic effects. The last two chapters explain the different theories of activation of unimolecular reactions and also discuss the mechanisms of some typical elementary and complex inorganic and organic reactions.

Volume 2 deals with the kinetics of reactions in solutions. The special features of reactions in solution, the various factors like the medium, pressure and structure of the reactants that influence the rates of different types of reaction and the energy-entropy relations in response to the changes in above are elegantly discussed in the first chapter. The Hammett and Taft equations which account for the influence of polar substituents on the rates have also been briefly discussed. In the last chapter, the importance of kinetic parameters in arriving at the appropriate mechanism has been admirably elucidated by a discussion of typical reaction mechanisms which have been investigated in detail.

The volumes are intended for the student with a general chemical background and can be readily recommended to advanced post-graduate students and college and university libraries. They should also prove useful to research scholars and teachers who require a broad survey of the subject.

The volumes are well printed, clearly illustrated and also provided with a good bibliography of about 50 books and monographs on the subject. Appendix I on correlation and the method of least square at the end of Vol. 1 is very valuable.

The only disappointing feature for students in India is the cost; most individuals are likely to find the price (60s. net), perhaps not too unreasonable in view of the very specialized nature of the subject, beyond their means.

H. S. VENKATARAMAN

WATER AND SOLUTE-WATER INTERACTIONS by J. Lee Kavanan (Holden-Dey Inc., San Francisco), 1964. Pp. 101. Price \$ 5.50

This book is a concise survey of the interactions of water molecules and forms part of a treatise on 'Structure and function in biological membranes' to be published later. Topics treated are the structure of ice, structure of liquid water, effects of solute on liquid water parameters, proton mobility and ion-water interaction. Theories pertaining to these topics are discussed with emphasis on the concepts. No attempt is made to present these theories in mathematical form. This approach is to be commended since the use of impressive mathematical formulations usually obscures the physical picture and gives the impression of having given a complete solution to the problem. Examples cited are invariably from biological fields. These serve to illustrate how the unique properties of water make it the matrix of all living organisms. The author has successfully condensed much in this slim volume. This book will be appreciated by research workers in physics and

chemistry for whose benefit it is published and especially by electrochemists whose appreciation of the role of water is not second to that of the biologists.

M. A. V. DEVANATHAN

INTERPRETATION OF THE ULTRAVIOLET SPECTRA OF NATURAL PRODUCTS — International Series of Monographs on Organic Chemistry: Vol. 7, by A. I. Scott (Pergamon Press Ltd, Oxford), 1964. Pp. x+443. Price 84s.

The application of physical techniques in the study of organic chemistry has advanced rapidly in recent years. With the availability of precise instruments capable of easy manipulation, these techniques are now accessible to almost all organic chemists and are used as a routine measure in research as well as in industry.

Ultraviolet absorption spectroscopy was one of the earliest physical techniques which the organic chemist employed to his great advantage. Examination of a large number of organic compounds enabled him to arrive at some broad-based generalizations connecting the chromophore present with the particular wavelength of absorption. This led to progress which is now too well known to need mention. And in spite of the advent of infrared spectroscopy, NMR, etc., ultraviolet spectroscopy still continues to hold its place of importance to the organic chemists.

A practising organic chemist requires a book on this subject which is not burdened with theoretical discussions, but provides him with sufficient information and guidance to enable him to interpret his recorded spectra and deduce correct inferences therefrom.

To this end Scott's present book is an admirable one. It not only provides a valuable introduction to the subject of ultraviolet spectroscopy but also incorporates a very wide range of examples drawn from natural products whose ultraviolet spectra have been critically discussed and attention directed to the source of the recorded spectra. The practising chemist will find a rich collection of a wide variety of recorded spectra of diverse natural products all in one place. It is just the kind of book that an organic chemist needs in his daily practice.

Beginning with a very brief introduction to the definition and concepts of ultraviolet spectroscopy, the first six chapters are largely devoted to a tabulated survey of the absorption characteristics of different types of chromophores frequently encountered in the study of organic natural products. This is followed by chapters on the application of ultraviolet spectroscopy to analysis of natural products, application of spectral data to investigations of gross molecular structure and the interpretation of the ultraviolet spectra of some complex molecules, all richly illustrated with numerous examples. The inclusion of a chapter on the application of spectroscopy in the analysis of steroids has indeed been very fitting, since much of the progress in the field developed as a sequel to investigations in the steroids.

The book unfortunately is not without serious printing mistakes. To cite only one case, on page 345 the same structural formula is printed for the two isomeric diols, CXXXI and CXXXII. They, however, do not rob in any way the value of this excellent book which no doubt will prove useful as a text-book at the

advanced level and also as a dependable guide for the organic chemists.

P.C.M.

BIOLOGICAL EFFECTS OF DEUTERIUM by J. F. Thomson (Pergamon Press Ltd, Oxford), 1963. Pp. vii+129. Price 45s.

In eight chapters, the book provides a wealth of experimental data on the effects of deuterium on isolated enzymes, viruses and unicellular and more complex organisms up through mammals. Notably, the review brings out the diversity in the responses of various species to deuteration. Since replacement of hydrogen by deuterium is essentially an environmental alteration, like change in temperature, food supply or radiation, one would expect species differences in the capacity to compensate for alterations in physiological functions. Thus, many algae and bacteria could survive and multiply in high concentrations of D_2O , while other closely related unicellular organisms are unable to do so unless they possess special survival features such as cyst formation; the capacity of higher animals to tolerate D_2O is even further reduced.

Basic questions on the mechanisms of toxic action of deuterium and of adaptation or acclimatization have, however, remained unanswered. Nor is it explicable how deuterium-sensitivity correlates with radio-sensitivity. Dr Thomson's comprehensive survey of the literature, which has not been attempted earlier, will, undoubtedly, be of help in efforts at elucidation of the diverse ways in which metabolic processes may be disturbed or cell divisions affected by deuterium through solvent, substrate, kinetic or other surface effects.

P. FATERPAKER

PROBLEMS OF THE BIOCHEMISTRY OF THE NERVOUS SYSTEM edited by A. V. Palladian; translation by F. S. Freisinger; translation edited by H. Hillman & R. Woodman (Pergamon Press Ltd, Oxford), 1964. Pp. xii+350. Price 80s.

The present book is a collection of articles by Soviet scientists presented at a Conference on Biochemistry of the Nervous System at Kiev in 1957. As pointed out by the editors of the translation, this book provides a useful introduction to the problems, the approaches and the techniques of Soviet biochemistry. The articles presented in this book indicate that the Soviet biochemists have given a particular stress on functional biochemistry of the nervous system.

The book is divided into eleven chapters each dealing with different aspects of the metabolism of the nervous system. Most of the chapters comprise a number of papers having a common interest and scope.

The first chapter deals with the metabolism of protein in the nervous system under the various physiological conditions.

The second chapter contains articles on the metabolism of phosphorus compounds, methods of their isolation and the study of their turnover rate in the brain. These chapters are followed by various chapters dealing with the carbohydrate metabolism and formation of ammonia in brain during various functional changes. Separate chapters have been devoted to the metabolism and function of adrenaline

in cerebral tissue with special reference to high energy compounds. A few papers have also been presented dealing with the histochemical approaches in the studies on neurochemistry.

The ninth chapter presents data on the effect of ionizing radiation on the metabolism of the nervous tissue. The last but one chapter deals with the changes involved in the cerebral tissue under increased pressure, high oxygen tension and also during the infection with poliomyelitis virus.

The last chapter deals with the summary of the contribution to the biochemical congress at Brussels with special reference to the work done in brain biochemistry in the laboratories of the USSR.

This book will serve as a useful introduction to the Soviet work, particularly in the field of neurochemistry.

B. K. BACHHAWAT

SYMPOSIUM ON FOODS: PROTEINS AND THEIR REACTIONS edited by H. W. Schultz & A. F. Anglemier (AVI Publishing Co. Inc., Westport, Connecticut), 1964. Pp. x+472. Price \$ 3.50

This is the third in the series of symposia on foods held at the Oregon State University in 1963. The publication of the proceedings of the symposia does yeoman service in purveying expert and up-to-date knowledge by specialists in the field for the benefit of interested scientific workers. That way, the present publication is welcome to research workers in the field of proteins.

The topics presented for discussion include microheterogeneity, structural organization and conformation in the protein molecule so important from the point of view of biological activity, concepts of denaturation, the nature of bonding in protein complexes (with protein, lipid and carbohydrates), and physico-chemical considerations in proteins from milk, egg, meat and fish and in seeds. The concluding chapters relate to little known aspects of the nutritional and immunological aspects of proteins, metabolic antagonists and food allergens. Each chapter, representing an individual contribution, is accompanied by conference discussion, a feature which was missing from the publication on enzymes, the first in this series. And specially useful is an extensive bibliography — characteristic of a research paper — at the end of each chapter. Instead of the conventional summing up by the chairman, this symposium concludes with a panel discussion which is a projection into the future trends in protein research.

An enormous amount of rich and thought-provoking material is offered in a condensed form. The publication coming from the hands of two capable editors and from a reputed publishing company is excellent in printing and get-up.

M.S.

SOME PROBLEMS IN THE THEORY AND ASSESSMENT OF TURBO-JET ENGINES by K. V. Khol'shevnikov; translated into English by W. E. Jones; translation edited by B. P. Mullins (Pergamon Press Ltd, Oxford), 1964. Pp. xiii+133. Price 35s.

The book gives a concise mathematical approach to the problems of performance and design of turbo-jet engines. It is divided into two main chapters.

The first chapter begins with the definitions of thrust, specific thrust and specific fuel consumption. It then elaborates the expression obtained into various parameters. Since the specific fuel consumption is an indication of the merit of an engine, methods for obtaining the optimum values of the independent variables to give the minimum specific fuel consumption conclude the first chapter.

The second chapter goes a step further and links them with the design limitations of the turbine and compressor for rated flight conditions, these being structural and aerodynamic limitations, the former controlled by the peripheral speed and the latter by the output and stalling characteristics. The author introduces here a complex parameter which relates all these properties with the thermodynamic characteristics obtained in the first chapter. The application of this parameter to design problems is further explained. The appealing aspect of this parameter is its generality in covering both turbo-jet and turbo-prop engines.

The book, because of its mathematical nature, adopts an elaborate notation. This notation is quite different from that adopted by British and American authors in books dealing with the same subject. Hence an extensive study of the notation is necessary before a comprehensive reading of the book is undertaken. However, the mathematics used is mainly algebra and can be understood by technical college students, without much effort.

This monograph will be a valuable addition to a mechanical engineering library and is valuable as a reference work for performance engineers in the turbine industry.

Y. V. G. ACHARYA

FATIGUE RESISTANCE OF MATERIALS AND METAL STRUCTURAL PARTS edited by A. Buch (Pergamon Press Ltd, Oxford), 1964. Pp. viii+312. Price 100s.

This book is a collection of papers on various aspects of fatigue of metals, presented at the international conference held in Warsaw in May 1960. The papers included in Section A deal with special problems covering interesting and controversial subjects such as cumulative damage theory, correlation of fatigue limit with ultimate strength of structures and various other properties, and fatigue cracks and fractures. In Section B on materials and technological problems, some fifteen papers on influence of inclusions, sintered materials, hydrogenated carbon steels, surface treatments, effects of some alloys in low-carbon steels and fatigue characteristics of some special materials have been discussed. Section C of the book relates to constructional problems and the papers in this section include some reports of experimental investigation on fatigue of welded structures and vibrating systems.

Although results of experimental investigations on many of these problems have been reported in various publications before and after 1960, the publication of these reports in 1964 is quite welcome specially when so many reports are collected in a single volume. Some of the theoretical and mathematical discussions presented in Section A contain good reviews of some not-so-well-known work. In the opinion of the

reviewer special mention may be made of Serensen's paper on 'Fatigue damage accumulation', Buch's paper on 'The influence of fatigue on the tensile strength, elongation, electric resistance and microhardness of steel' and Lapinski's paper on 'Measurement and calculation of fatigue stresses in jet-propulsion engine blades'.

B. KARUNES

FIRST SUPPLEMENT TO AGARD MULTILINGUAL DICTIONARY edited by A. H. Holloway (Pergamon Press Ltd, Oxford), 1963. Pp. ix+334. Price 70s. The original dictionary was published by AGARD in 1960. This is a valuable adjunct to that dictionary.

This supplement differs from the original dictionary in that the terms are defined in English only. This has been done to keep down the bulk. This makes the dictionary a little more difficult to use for translation from languages other than English, though the appropriate index in the particular language, which has been provided, may be used and the term looked up in the dictionary, whose number is given in the index. In the original dictionary, this cross-reference is not needed. But the supplement is easy to use for those who know the English terms. Since English has become an international language, it is presumed that this is not a serious disadvantage as all aeronautical scientists have generally a knowledge of English.

The original dictionary gave equivalents in German, Spanish, French, Italian, Dutch, Russian and Turkish. Another language, namely modern Greek, has been included in this supplement.

This supplement adds 1287 terms. Most of the definitions are based on the US-NASA dictionary. It is claimed some of the definitions have been revised removing irrelevant material. All the figures included in the supplement are based on those in the NASA dictionary. The latter dictionary contains some 700 and odd more terms. But these have not been included in this dictionary as it was felt that some of them were slang terms or variations of other terms.

The supplement is very useful as a reference book by itself and also in conjunction with the original volume and will be a good addition to any aeronautical library.

Y. V. G. ACHARYA

BOOK NOTES

NUCLEAR PHYSICS — AN INTRODUCTION by W. E. Burcham (McGraw-Hill Book Co. Inc., New York), 1963. Pp. xiv+739. Price \$ 12

This book written by an expert fills a long-felt need. Intended for a graduate student with an elementary knowledge of modern physics, this book falls somewhere in between the exhaustive *Atomic Nucleus* by R. D. Evans and *Nuclear Physics* by I. Kaplan. Although the stress is on experimental aspects, sufficient theory is given to make the treatment self-contained. The inclusion of recent developments such as the Mössbauer effect enhances the usefulness of the book. A set of problems at the end of each chapter would have further enhanced the value of the book to graduate students.

M. K. RAMASWAMY

NOBLE METAL THERMOCOUPLES: Booklet 7550/2 (Johnson, Matthey & Co. Ltd, 73-83 Hatton Garden, London, EC 1)

An up-to-date publication on noble metal thermocouples which also covers standard and special purpose types has been issued by Messrs Johnson, Matthey & Co. of London. The pamphlet describes the thermocouples in the standard range, 1000-1850°C., and contains data on e.m.f.-temperature relationships. For measuring temperatures up to 2000°C. the iridium: 40 per cent iridium-rhodium (Feussner) couple and for temperature measurement under conditions of neutron irradiation 0.1 per cent molybdenum-platinum: 5 per cent molybdenum-platinum couple are described. The advantages of certain protective sheath materials for use at various temperatures are discussed. The booklet is available free on request from the company.

MIT RADIATION LABORATORY SERIES (Boston Technical Publishers, 5 Bryant Road, Lexington, Massachusetts, USA)

A series of 28 volumes covering fundamentals of microwave and radar theory and technology are now available in inexpensive hard-bound editions (about 600 pp., \$ 5 per volume). These texts were developed as a result of extensive work at the Massachusetts Institute of Technology (MIT) during World War II in the development and perfection of microwave and radar technology and equipment.

PUBLICATIONS RECEIVED

THEORY OF CRYSTAL DISLOCATIONS by A. H. Cottrell (Gordon & Breach Science Publishers, New York), 1964. Pp. ix+91. Price \$ 2.50 (paper); \$ 4.50 (cloth)

PHYSICS-ELECTRONICS TITLES — BIBLIOGRAPHY-AND KEYWORDS INDEX (Boston Technical Publishers Inc., Massachusetts), 1964. Pp. vii+455. Price \$ 12.50

ORGANIC CHEMISTRY by Hans Beyer (Asia Publishing House, Bombay), 1964. Pp. xv+763

SOLUBILITIES OF INORGANIC AND ORGANIC COMPOUNDS: Vol. 2 — TERNARY AND MULTI-COMPONENT SYSTEMS, Part 2, by H. Stephen & T. Stephen (Pergamon Press Ltd, Oxford), 1964. Pp. 947-2053. Price £ 12 10s.

METHODS IN MICROANALYSIS: Vol. 1 — SIMULTANEOUS RAPID COMBUSTION by T. A. Kuck; translated by Phyllis L. Bolton & Kurt Gingold (Gordon & Breach Science Publishers, New York), 1964. Pp. xv+640

PROCESS CONTROL by Peter Harriott (McGraw-Hill Book Co. Inc., New York), 1964. Pp. xvii+374. Price \$ 13.50

FOOD PROCESSING OPERATIONS: Vol. 3, by Maynard A. Joslyn & J. L. Heid (AVI Publishing Co., Westport), 1964. Pp. ix+556. Price \$ 20.75

BIOCHEMISTRY OF PHENOLIC COMPOUNDS by J. B. Harbourne (Academic Press Inc., New York), 1964. Pp. x+618. Price 126s.

MECHANICAL WORKING OF METALS (Introductory Monographs in Material Sciences) by F. A. Crane; General Editor, A. R. Bailey (Macmillan & Co. Ltd, New York), 1964. Pp. ix+81. Price 13s. 6d. or Rs 10.80

Observations have been reported for the first time of scattering of the laser beam by the plasma produced by focusing a laser beam itself to a point. The observations are consistent with scattering by the free electrons in the plasma, under conditions in which the spectral distribution of the scattered radiation is governed by cooperative interactions between the ions and electrons. The wavelength of the scattered radiation is observed to be shifted slightly with respect to that of the laser beam and this shift has been interpreted as a Doppler shift due to motion of the plasma during the initial phase of the spark. The experimental arrangement consisted of a lens which focused the beam from a Q-spoiled ruby laser to a point and a light trap which absorbs the rays diverging beyond the point of focus. Radiation from the point of focus (where a spark was produced) was observed at right angles to the direction of the beam using a spectrograph equipped for both photoelectric and photographic recording. In the visible region of the spectrum, the radiation from a spark in air consisted of an intense continuum upon which were superimposed a few N II lines. The radiation scattered from the laser beam appeared as a sharp line close to the laser wavelength, but shifted from it by about 3 Å. This line was absent when the gaseous breakdown was prevented, thus showing that the line was not due to scattering by the air itself. The scattered radiation was found to occur only after gaseous breakdown had taken place. When the laser beam was recollimated after the spark and then refocused on to the spark in a direction at right angles to both the primary beam and the direction of observation, a second scattering signal was obtained. The wavelength shift of the scattered radiation was found to depend upon the point of observation of the spark along the axis of the lens. The assumption that the shift in the wavelength of the scattered radiation is due to the Doppler effect caused by the motion of the

plasma as a whole towards the lens condensing the laser beam during the initial stage of the spark is justified since the velocity of the luminous front evaluated from the streak photograph ($\approx 10^7$ cm./sec.) can account for the observed shift (3 Å.) [*Phys. Rev. Lett.*, **13** (1964), 227].

Selective energy absorption of electrons through a crystal

When a beam of electrons passes through a solid, the electrons lose some energy and the energies of the emerging electrons lie about a mean energy at random. Recent experiments conducted at the Brookhaven National Laboratory and the Bell Telephone Laboratories, New Jersey, however, gave evidence that when electrons pass through a single crystal in a direction parallel to one of the main axes of the atomic lattice, the pattern of energies of the emerging electrons is not random but is biased towards the higher energies.

In these experiments, a parallel beam (within 0.05°) of 3 MeV. electrons was passed through a single crystal of silicon mounted so that it could take up any orientation with respect to the beam. The electrons that emerged at energies above those at which an appreciable number would have been expected, if absorption had been random, were counted. When the beam was aligned with a particular axis — the (110) axis — an extremely high count was recorded. Similar but less pronounced effects were observed in the directions that did not coincide with a particular lattice axis but lay in 'planes of symmetry'.

The new phenomenon is explained by the hypothesis that the electrons are channelled into the gaps between the planes of atoms (thus eliminating head-on collision with the atoms), after which only resonance has appreciable effect in reducing their energy. The results obtained confirm this hypothesis. In the experiments the average loss of

energy in passing through the crystal at random was found to be 0.7 MeV., whereas it was less than 0.35 MeV. when the beam was aligned with the crystal [*Phys. Rev. Lett.*, **13** (1964), 530].

Modulation of light waves with gallium phosphide diodes

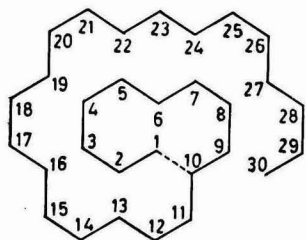
A new method of modulating light waves using gallium phosphide diodes has been devised at the Bell Telephone Laboratories. In this method, phase modulation of light waves is achieved by focusing polarized light on one edge of the gallium phosphide *p-n* junction diode, and applying a varying reverse bias to the diode as the light travels along the plane of the diode junction. Even though gallium phosphide diodes are basically phase modulators, they can be modified to serve as amplitude and polarization modulators. The main features of the gallium phosphide modulators are: (i) they can achieve large phase differences with small voltages, (ii) can be operated at room temperature, (iii) the length of the light path is short, (iv) high frequency modulation is feasible, and (v) the transmission range of the diodes extends from the green to the near infrared region.

The phase modulation in gallium phosphide diode arises from the phenomenon known as the linear electro-optic effect or Pockels effect in which the index of refraction of piezoelectric field is applied to them. In the case of gallium phosphide diodes, the Pockels effect takes place in the *p-n* junction region when reverse bias is applied. The gallium phosphide in the region of the *p-n* junction under these conditions changes from an optically isotropic medium to a birefringent medium. As a result of the birefringence, velocities of the different polarized components of the light wave are altered, thus causing a phase modulation to each of the polarized components. In effecting phase modulation,

light which is focused on the diode $p-n$ junction is decomposed into two equal polarized components having their electric vectors at an angle of 45° to the plane of the junction. The two components are then guided along and confined to the plane of the junction by discontinuities in the index of refraction on both sides of the junction plane. In the experiments made at the Bell Telephone Laboratories, a mercury arc and a helium-neon laser were used as light sources, and phase differences were measured with a standard polarizing microscope and an Ehringhaus compensator. Phase differences up to 140° in the case of 5460 Å. radiations were observed on the application of 31 V. to a gallium phosphide diode. This means that up to 88 per cent of the light intensity entering the diode can be amplitude modulated [*News from Bell Telephone Laboratories, New York*].

Hexagonal structure of proteins

Peptide and protein molecules have been pictured as either α -helixes or pleated sheets. Neither of these approaches, however, provide for the structural arrangement of the 30 per cent (approx.) weight of bound water in proteins. Also, the models based on these concepts must be severely distorted to represent some of the smaller peptides. Dr Donald T. Warner of Upjohn's research laboratory has suggested a hexagonal arrangement (1) of the carbonyl oxygen atoms in proteins and peptides. This arrangement places the oxygen atoms in a pattern corresponding to the oxygen lattice of the ordered water structure. The new structure helps to explain how water plays such an important role in the structure and activity of proteins and polypeptides.



Space-filling molecular models of some simple peptide rings have been prepared, in which the carbonyl oxygen can be laid out in the form of regular hexagons—a single hexagon for a hexapeptide, a pair of rings (similar to naphthalene) for a decapeptide. All the oxygens are in a single plane on one side of the models with the side chains neatly making up most of the other side. There is no strain in these models. The spacing of these oxygen atoms is almost identical to the spacing of the alternate (second neighbour) oxygens in a water lattice. Both are hexagonal, are in one plane, and the distance between the oxygen atoms is nearly 4.8 Å. in both cases.

With this similarity of spacing of the oxygen atoms, a water layer could be linked to a peptide layer by collinear hydrogen bonds. This would provide maximum bond strength. It also suggests a way of bringing water into a protein molecule in a structurally useful way. The same hexagonal structure in both water and protein could theoretically stabilize both of them.

Based on this theory a space-filling model of the B chain of insulin has been constructed. The B chain has 30 peptide residues, thus 30 carbonyl oxygens. Starting with the decapeptide structure, the chain is spiralled around it, building up new hexagons on its edges. This way, the 30 carbonyl oxygens are located at the corners of nine contiguous regular hexagons arranged in a honeycomb. There is no distortion in this model. This shows that the hexagonal approach can be applied to large as well as small peptides.

Tobacco mosaic virus (TMV) protein contains 158 amino acids in a straight chain with an N-acetyl terminal group and a total of 159 carbonyl oxygens. Since TMV is too large a molecule, its space-filling model is difficult to prepare. Using a graphical model, Dr Warner has shown that all the 159 carbonyl oxygens of TMV can be fitted at the corners of a honeycomb of 65 hexagons [*Chem. Engng News*, 42 (No. 43) (1964), 53].

New synthesis of guanidines

The synthesis of guanidines is usually carried out by the Rathke

reaction of amines with S-methyl isothiuronium salts and although high yields are obtained by this method the method has a restricted application as the availability of the intermediate amines is limited by the reduction procedures which are often involved in their preparation. A new synthesis developed at the Research Division of Pfizer at Sandwich, Kent, uses alcohol intermediates as the starting materials. These are converted to tosyl esters and displacement of these groups by free guanidine in a variation of the well-known nucleophilic displacement reactions of tosylates gives the guanidine derivatives.

The new method is illustrated by the preparation of 2-guanidinomethyl-1,4-benzodioxan, the sulphate of which has been reported to have valuable hypotensive properties. To dry *tert*-alcohol (170 ml.) is added sodium hydride (50 per cent dispersion in oil; 3.8 g.; 0.08 mole), followed by guanidine hydrochloride (8.1 g.; 0.085 mole). The suspension is heated under reflux and stirred for 30 min., the sodium chloride is filtered from the hot solution, and the filtrate added to the 2-tosyloxymethyl-1,4-benzodioxan (13.0 g.; 0.04 mole) in *tert*-butanol (30 ml.), and the mixture heated under reflux for 7 hr. The solvent is removed under reduced pressure and the residue is extracted with hot water. The aqueous extract gives the product as the tosylate salt (9.4 g.; 76 per cent based on unrecovered starting material). The reaction has been used successfully on a variety of other ring-substituted 2-tosyloxymethyl-1,4-benzodioxans [*Chem. & Ind.*, (1964), 1806].

Ferridoxin in primary carbon dioxide fixation

A new carbon dioxide fixation reaction has been discovered in photosynthetic bacteria *Chromatium*, by the workers of University of California, Berkeley. In the presence of ferridoxin and hydrogen gas, cell-free extracts of *Chromatium* have been observed to reductively couple carbon dioxide to a C_2 compound (acetyl coenzyme A) to form a C_3 compound (pyruvic acid).

It is believed that carbon dioxide adds to a C_5 compound, ribulose

1,5-diphosphate, which splits into two molecules of a C_3 compound, phosphoglyceric acid. The formation of pyruvic acid via a C_2 plus C_1 reaction named as pyruvate synthetase reaction provides a more direct route to a C_3 compound from carbon dioxide. These findings provide the first evidence that the pyruvate synthetase system may be the major pathway for assimilation of carbon dioxide in a photosynthetic organism which is also consistent with the earlier postulation about the mechanism for the synthesis of a C_3 compound from C_2 and C_1 units.

All ferridoxins have an oxidation-reduction potential close to that of hydrogen gas (-420 millivolts) which makes them the most electronegative electron carriers known in cell physiology. This assimilation of carbon dioxide can make use of the strong reducing potential of ferridoxins directly without the mediation of pyridine nucleotides.

The pyruvate synthetase system was prepared from *Chromatium* grown under artificial light. Cells were harvested and made into a paste. They were suspended in buffer and were disintegrated by subjecting them to sonic oscillation and centrifuged. The cell-free supernatant fluid contains pyruvate synthetase system and ferridoxin. Ferridoxin was removed by adsorption on a DEAE cellulose column. Acetyl phosphate, ferridoxin, coenzyme A, semicarbazide and manganese chloride were added to the extract and the mixture was placed in dark along with hydrogen gas and labelled (C^{14}) carbon dioxide. Pyruvic acid accumulates only if trapped by semicarbazide. In the presence of diphosphopyridine nucleotide and glutamine, the main products of carbon dioxide fixation are aspartic acid, glutamic acid and alanine. The reaction requires the presence of ferridoxin or acetyl phosphate or acetyl coenzyme A. Use of acetic acid instead of acetyl coenzyme A as the C_2 acceptor for carbon dioxide requires adenosine triphosphate (ATP) or adenosine diphosphate (ADP) and inorganic phosphate; acetic acid functions as carbon dioxide acceptor only in the presence of light. Thus photophosphorylation becomes essential to manufacture ATP from ADP and inorganic

phosphate. With ATP supplied, *Chromatium* can convert acetic acid to acetyl phosphate or acetyl coenzyme A [*Chem. Engng News*, 42 (No. 38) (1964), 48].

Ja Oxford — a new haemoglobin

Abnormal haemoglobin variants, 'haemoglobin Norfolk' and 'haemoglobin JBaltimore', are known to exist in English families. In a survey undertaken on 1971 non-related outpatients in Oxford and Peterborough, a new haemoglobin variant named as 'haemoglobin Ja Oxford' has been found in one person.

The abnormal haemoglobin was isolated and the peptide chromatogram showed the absence of peptide α TpIII. Two new peptides could be demonstrated instead which were in the neutral area and both contained tryptophan. One of the two new peptides was isolated by utilizing the fact that the neighbouring peptides in the neutral area contain histidyl and, therefore, can be made to move towards the negative pole on electrophoresis at pH 3.5 and could finally be purified from the remaining peptides in the neutral area by 20-h chromatography. The other tryptophan containing peptide showed the presence of histidine, tyrosine and arginine on staining.

The electrophoretic properties of haemoglobin Ja Oxford and haemoglobin Norfolk are clearly different. The hybridization experiments show that the abnormality has been carried by the α -chain [*Nature, Lond.*, 204 (1964), 269].

Progress Reports

Virus Research Centre, Poona

The Virus Research Centre, Poona, maintained jointly by the Indian Council of Medical Research and the Rockefeller Foundation of the United States of America, with its two field stations, one at Sagar in Shimoga district of Mysore State and the other in the Department of Microbiology, Christian Medical College and Hospital, Vellore, has completed ten years of its existence. Though the centre is primarily devoted to the study of the epidemiology of arthropod-borne virus occurring in India, it also serves

as a research and training centre for workers both from India and abroad.

The research centre has undertaken several investigations on viruses other than arboviruses, such as rabies, Newcastle disease (Ranikhet), herpes, Coxsackie and other enteroviruses, and is currently engaged in unravelling several hitherto unresolved problems in relation to two major diseases, viz. Kyasamur Forest Disease (KFD) and Japanese B Encephalitis (JBE). The observations made in the study of KFD suggest that (1) the disease may be maintained in the forest as an enzootic with a natural transmission cycle among small mammals through the agency of ticks, (2) the monkeys act more as sentinels than as disseminators of KFD, and (3) the importance of bovines as amplifiers of the tick population is being increasingly appreciated. A study of the human and monkey cases indicates that KFD seems to be slowly spreading south-west of hitherto known infected areas. The preparation of vaccine to protect the human populations is in progress at the Haffkine Institute, Bombay. So long as the epidemiology of this disease is not completely understood and the effective control measures are devised, KFD has to be regarded as a public health problem of unassessable importance. The studies on JEB were directed mainly in investigating the prevalence of the disease in and around Vellore and determining the relative roles of the three species of mosquitoes which together form the '*Culex Vishnui*' complex. A comparative study of the binomics and seasonal prevalence of the three species of the *C. Vishnui* group is in progress.

At the Sagar field station, studies were undertaken on the march, persistence, recurrence and increment of virus manifestations in the KFD infected area by: (1) surveillance for detection of human cases; (2) surveillance for detection of monkey deaths; and (3) examination of monkey carcasses for viral, pathological and parasitological findings. Studies are being made on virus behaviour in the areas of virus activity as revealed by human cases for monkey deaths and they include: (a) search for virus in ticks collected

by dragging, leaf-picking, and brushing; (b) study of the spatial distribution of infected ticks by establishing several collecting stations separated by a conventional distance; (c) intensive search for nymphal forms during monsoon in order to see whether any of them persisted through the wet season; (d) trapping of small mammals and search for virus in their organs; and (e) collection of ticks from such mammals which resulted in isolation of viruses from ticks, and the peddy bird virus, belonging to the psittacosis group. Survey studies undertaken in Sagar area indicated the presence of 14 species of *Haemaphysalis* ticks.

At the Vellore field station studies were undertaken on the epidemiology of Japanese encephalitis in South India. Virus isolations were made from 22 human and 6 mosquito isolates. Immune sera for all isolates were prepared in adult mice.

A survey of human sera from Kerala conducted in the Poona laboratory indicated that the group B activity in this region is only moderate. To facilitate the work of the field workers in identifying the developmental stages of ticks before processing them for virus isolation, a schematic key has been prepared by the laboratory.

Central Leather Research Institute, Madras

The annual report of the Central Leather Research Institute, Madras, for the year 1962-63 records the varied activities of the different units. In the Raw Hides and Skins unit, a new type of organism, different from the common 'red heat' type bacteria, has been isolated from salted goatskins. The complete amino acid composition of dugong collagen has been determined by paper chromatography. A method for selective blocking of the hydroxyl groups of collagen by acetylation without affecting the amino group has been developed.

The nature of the carbohydrate of the gallotannin isolated from dhawa leaf was investigated in the Vegetable Tannage unit, the carbohydrate core has been deduced to be pentagalloyl glucose. Among the projects completed in the Mineral and other Tannages units

are the estimation of methylol group in formaldehyde tanned leather, production of heat resistant leathers, estimation of ionically bound phthalate in basic phthalate masked cerous liquors, studies of different tannages and production of good cricket and hockey ball leathers by various tanning processes. Specificity studies on *A. parasiticus*, with special reference to its mode of action on the peptide chain has been taken up with crystalline bovine serum albumin. A method has been developed for the preparation of dry bright wax emulsions with carnauba wax. A new method of dyeing goat hair of shades other than jet black into a fast and non-bleeding jet black colour has been developed in the Byproducts unit.

Announcements

■ *Indian Association of Biological Sciences* is a new association formed during the combined 51-52 session of the Indian Science Congress Association held at Calcutta in January 1965. A Steering Committee consisting of Prof. B. R. Seshachar, Prof. T. S. Sadasivan, Prof. Sivatosh Mookerjee, Dr B. Mukerji, Dr H. B. Tewari, Dr B. S. Chauhan (Treasurer) with Prof. P. Maheshwari as Chairman was elected by the meeting to formulate the detailed scheme of the association. The main aim of the new organization will be to create a common forum of Indian biologists and try to integrate the research activities of biologists working in the various fields such as medicine, agriculture, veterinary science, botany and zoology and in the border line fields of biochemistry, biophysics, biometrics, etc. The subscription has been tentatively fixed at Rs 10.00.

Suggestions may be communicated to Prof. T. S. Sadasivan, Director, University Botany Laboratory, Madras 5, or Prof. P. Maheshwari (Chairman), Professor and Head of the Department of Botany, Delhi University, Delhi 7.

■ *International Conference on Crystal Growth*—The conference will be held at Boston, Massachusetts, USA, during 20-24 June 1966. The scope of the conference will include molecular mechanisms

of crystallization, growth of metals and semiconductors, growth of non-metals, techniques, and properties associated with growth parameters. Contributions are invited from workers in the field. The proceedings will be published as a special issue of the *International Journal of the Physics and Chemistry of Solids*. The last date for preregistration and for submission of the title of an intended contribution is 1 September 1965 and for extended abstracts 1 February 1966. Inquiries may be addressed to the Conference Secretary, International Conference on Crystal Growth, 40 Acorn Park, Cambridge, Massachusetts, USA.

■ *Endeavour Prizes*—Prizes totaling 100 guineas are offered by Imperial Chemical Industries Ltd, publishers of the international scientific review *Endeavour*, for essays submitted on scientific subjects. The prizewinners will also receive invitations to attend the British Association meeting at Cambridge from 1 to 8 September 1965. The competition is restricted to those whose twenty-fifth birthday falls on or after 1 September 1965. The prizes will include a first prize of 50 guineas, second prize of 25 guineas and third prize of 15 guineas. Two special prizes of 5 guineas each will be awarded to competitors who have not passed their eighteenth birthday on 1 September 1965. The subjects for the essays are as follows: (1) Physics of the Moon, (2) Physiological basis of memory, (3) Molecular sieves, (4) Chemicals from oil, (5) Mechanisms of enzyme action, and (6) Strength of materials. The essays must be in English, typewritten and should not exceed 4000 words in length. Entries should be addressed to: The Deputy Secretary, British Association for the Advancement of Science, 3 Sanctuary Buildings, Great Smith Street, London, SW 1, and the envelope should be marked 'ENDEAVOUR PRIZE Essay'. The last date for receipt of entries is 1 June 1965. The essays must be submitted without signature. The competitor's full name and address and date of birth should be disclosed in sealed covering letter attached to the essay and addressed to the Deputy Secretary of the British Association.

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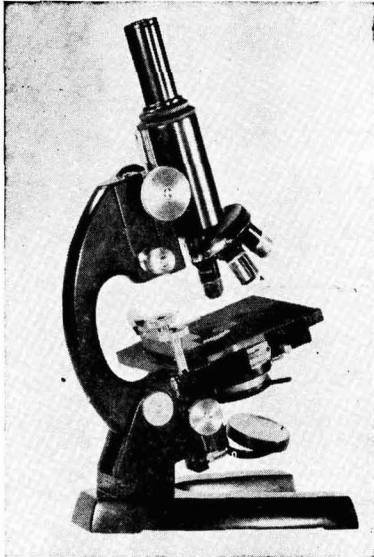
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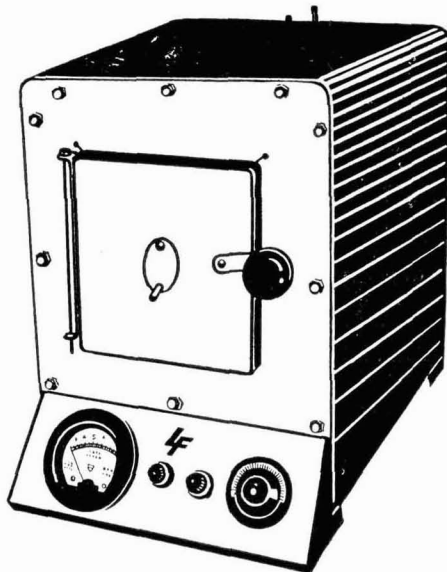
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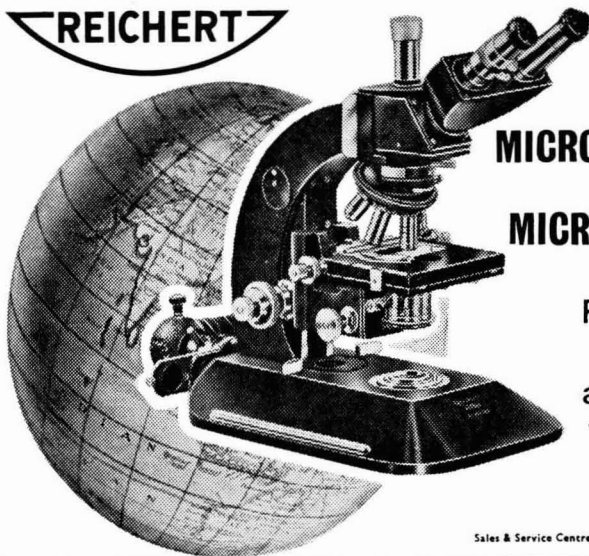
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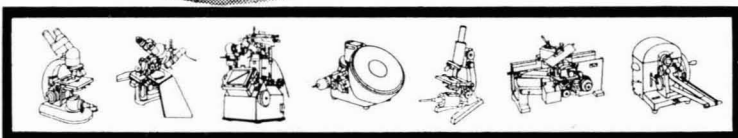
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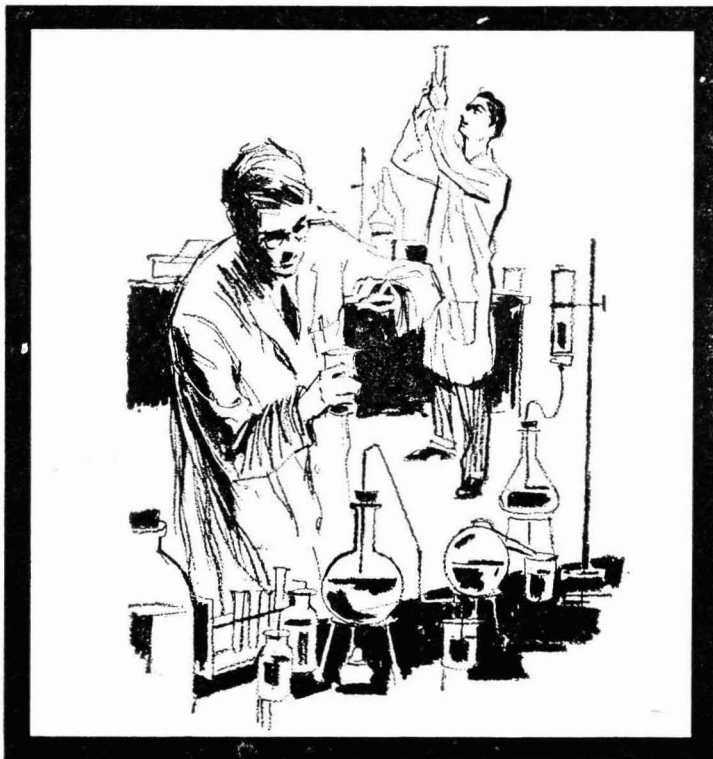
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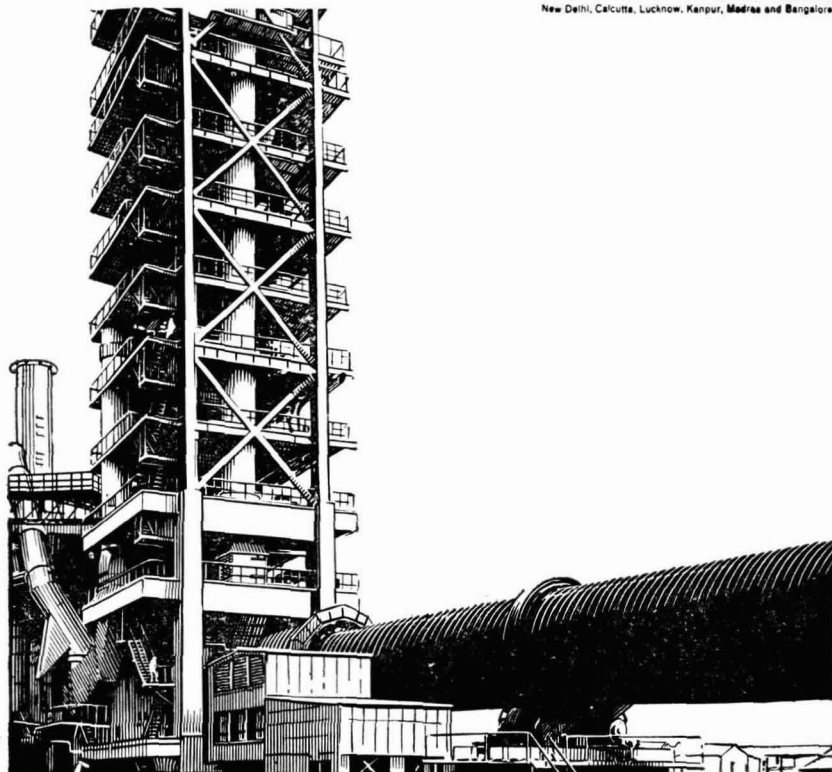


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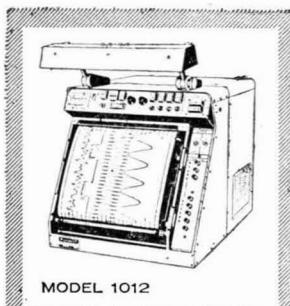
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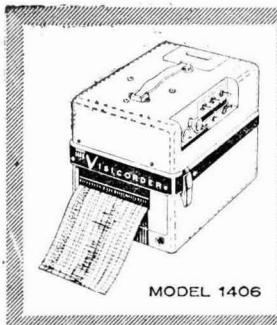
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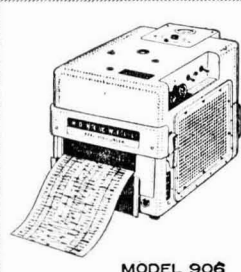
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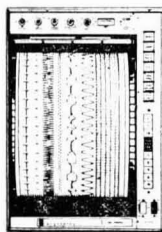
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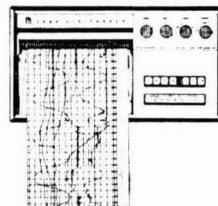
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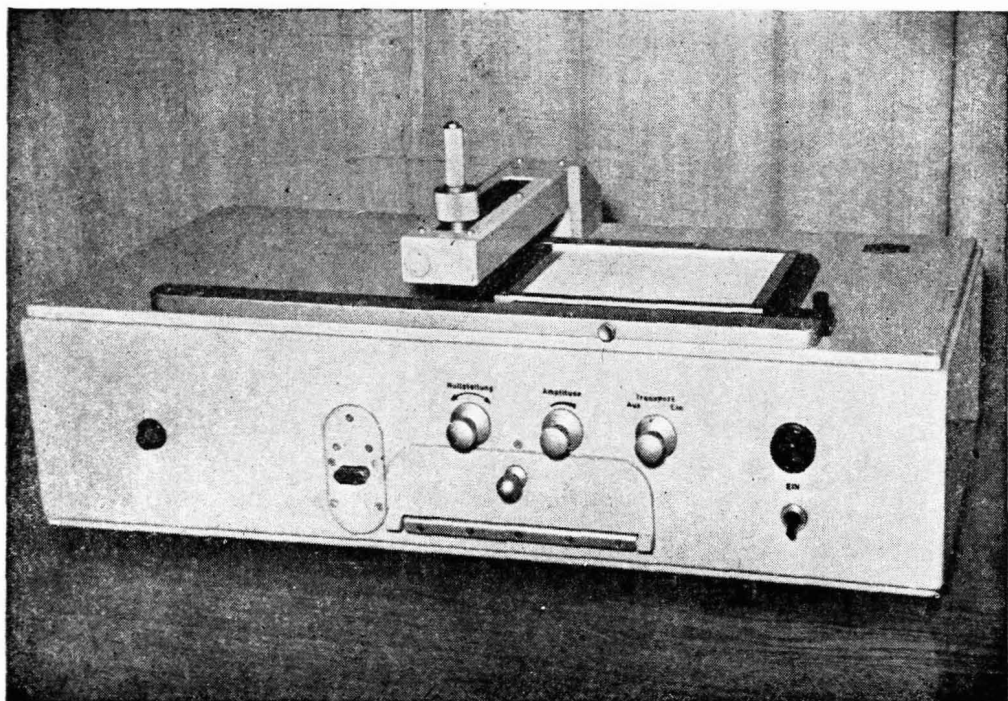
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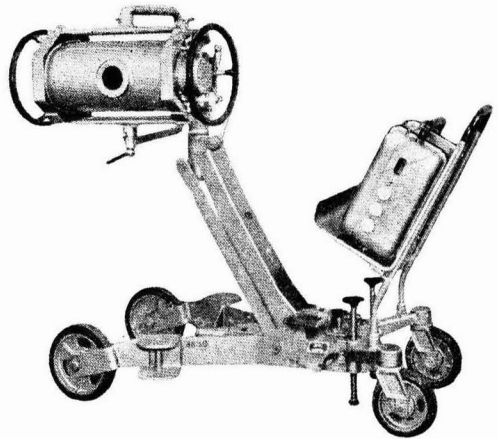
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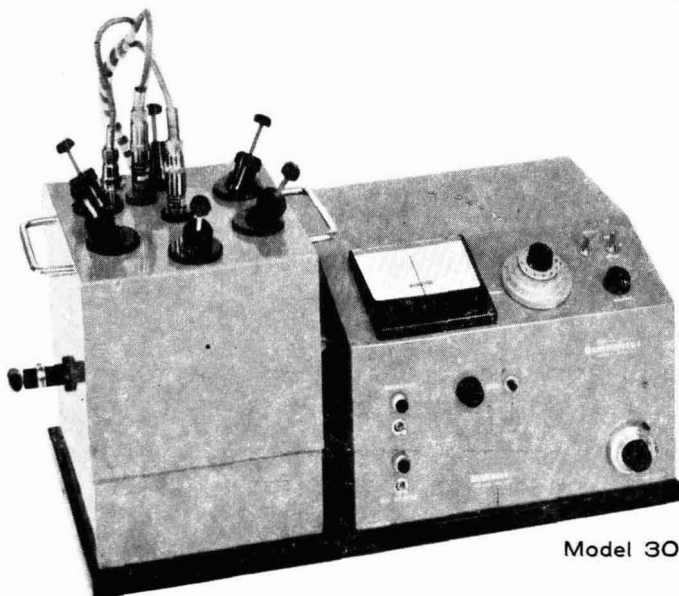
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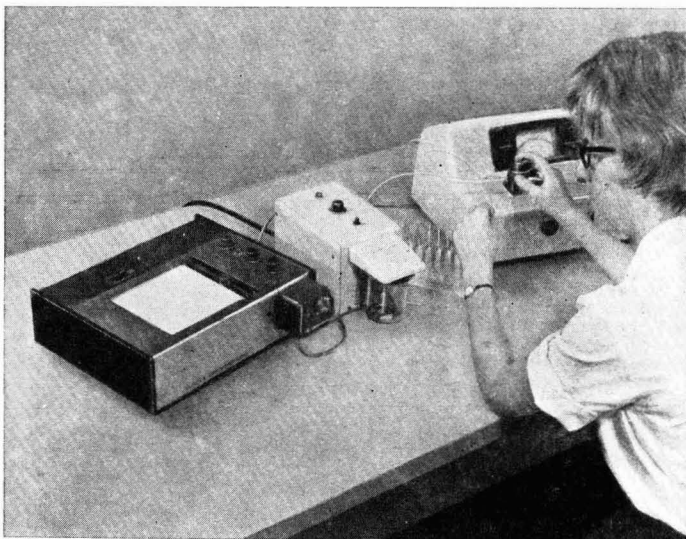
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