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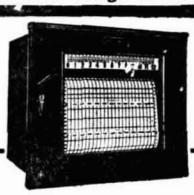

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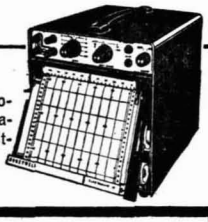

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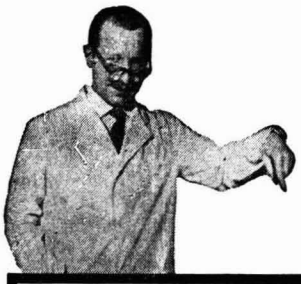

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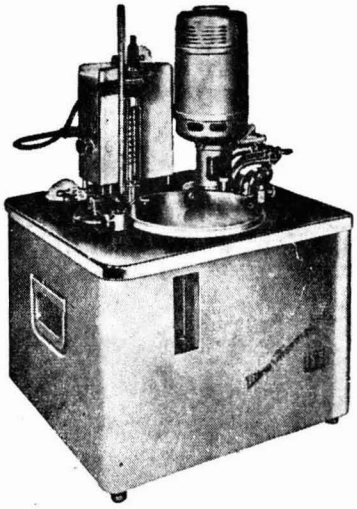

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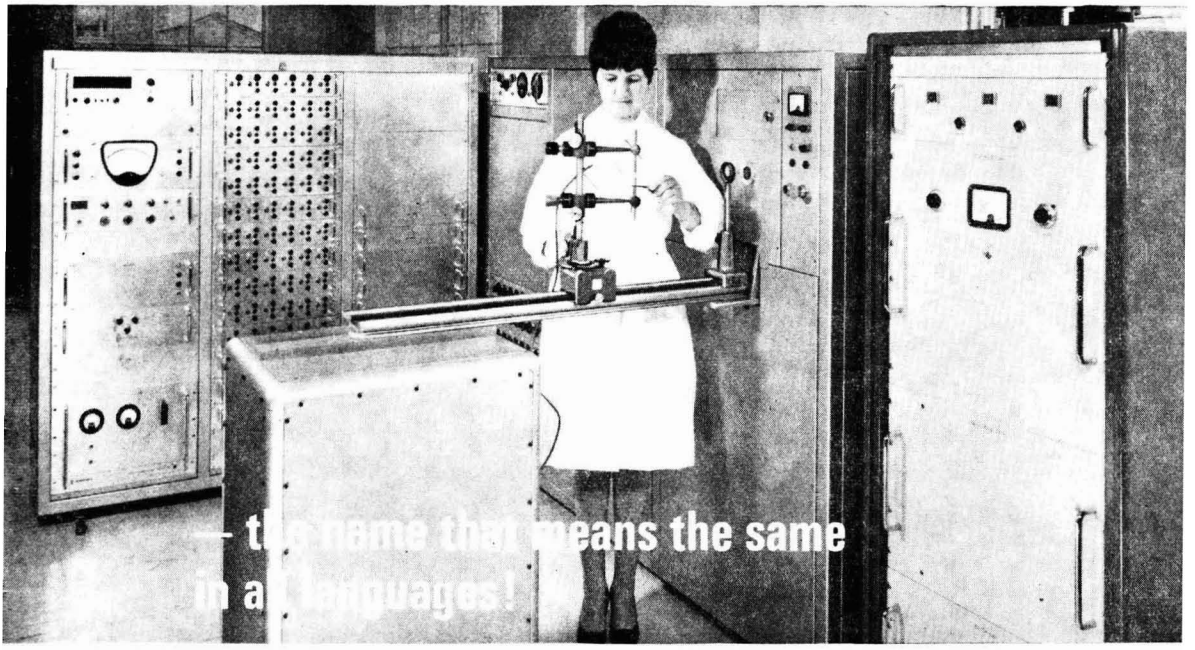

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Current Topics

Fifty Years of Nutrition Research in India

MALNUTRITION is, today, the most important problem affecting more than half of mankind. The problem is assuming alarming proportions, particularly in the less developed countries, where the population is continuing to increase at a high rate. In the technologically advanced countries, nutritional deficiencies have been largely eliminated, and the need for research into nutritional deficiency diseases is mainly of academic interest. Therefore, the less advanced countries have to undertake research on a large variety of nutritional problems faced by them. Reviewed in the light of the above considerations, the achievements of the Nutrition Research Laboratories, Hyderabad, which celebrated its Golden Jubilee during 26 September-2 October 1969, are significant.

From a modest beginning as a small Beriberi Disease Enquiry Unit set up at Coonoor in the year 1918 — almost coinciding with the emergence of the subject of nutrition as a full-fledged science — the institution has grown into one of the leading nutrition research centres of the world. The laboratories has to its credit pioneering work on deficiency diseases like kwashiorkor, marasmus, anaemias, pellagra, endemic dropsy, beriberi, phrynoderma, nutritional oedema, fluorosis, etc., which are endemic in the developing countries. A striking feature evident from the record of the activities and achievements of the laboratories is the dynamic and pragmatic approach adopted in tackling these diseases. A typical example is the work done on the etiology of pellagra, a widespread disease of the Deccan region, traditionally linked with the intake of *jowar*. Systematic and painstaking work at the laboratories, involving the analysis of several hundred varieties of *jowar*, has made it possible to trace the disease to amino acid imbalance in certain varieties of *jowar* (excess of leucine) rather than to *jowar* intake itself. The answer suggested for the problem is selective propagation of low leucine strains of *jowar*, rather than a drastic change in the dietary habits of a large section of the population. The work done on lathyrism, a paralytic condition caused through the excessive intake of the pulse, *Lathyrus sativus*, has led to equally striking results. The disease has been attributed to the presence of a toxic principle in the pulse, for the removal of which a simple procedure — soaking of the grain in warm water — adaptable under village conditions has been developed. A finding of still greater significance is that the concentration of the toxic principle varies widely in different strains of *L. sativus*. As in the case of *jowar*, the possibility of selective propagation of strains of *L. sativus*, low in the toxic principle, has been indicated.

In fulfilling its overall responsibilities towards the improvement of the nutritional status of the people, the laboratories has made important contributions in several areas through extensive survey and clinical work. Notable among the problems investigated are protein-calorie malnutrition and vitamin A deficiency in school children and iron-deficiency anaemia in pregnant women. For overcoming protein-calorie malnutrition, recipes based on judicious combinations of locally available and relatively inexpensive vegetable materials like groundnut, pulses and other cereals have been worked out. The finding that a single massive dose of vitamin A given once a year can largely overcome vitamin A deficiency in children has attracted worldwide attention. It is gratifying that the Government of India have included a pilot project in the Fourth Plan concerning large-scale administration of two doses of 200000 IU of vitamin A to children at 6 monthly intervals. Work on iron-deficiency anaemia in pregnant women has established that the condition can be prevented and controlled by the daily administration of tablets providing 30 mg of elemental iron and 500 μg of folic acid during the last 100 days of pregnancy.

Assessment and periodic review of the requirements of calories and different nutrients — proteins, B-group vitamins, vitamin C, iron and calcium — of the people in different physiological groups, and constant review of food composition tables form too major continuing activities of the laboratories. Nutrient compositions of over 850 foodstuffs available in India have been worked out.

An imaginative line of research initiated recently concerns the upgrading of newly developed high yielding strains of various foodgrains in respect of their nutritive value. In collaboration with the Indian Agricultural Research Institute, New Delhi, the laboratories has been conducting a systematic screening of all the newly developed strains to ensure that apart from disease resistance and improved yield, the nutritive value of the strains is also taken into consideration before they are released for propagation.

Another important topic of research taken in hand recently concerns aflatoxins, the toxic principles produced in foodgrains (particularly groundnuts) through fungal contamination, capable of causing serious damage to the liver and producing cancer in several organs. The approach being followed is radical in that stress is distributed equally on the improvement of harvesting practices and the evolution of strains resistant to fungal attack. An improved strain of groundnut (US 26) has been evolved; even when the fungus grows on this strain, the toxin is not elaborated.

Improving the nutritional status of the people and combating malnutrition are the most important tasks of the laboratories and these will be its continuing responsibility. The various measures and programmes which the laboratories has suggested to ensure this will demand very efficient and effective extension programmes. Machinery of a size commensurate with the magnitude of the task has to be set up in addition to providing adequate funds.

A significant feature of the work of the laboratories in recent years is the collaborative approach to the solution of the problems with other bodies promoting research. This collaborative approach can pay rich dividends and should be assiduously cultivated and extended to other bodies like the Defence Science Organization, the Council of Scientific & Industrial Research, and the Department of Atomic Energy.

During the past fifty years the laboratories has tackled and solved many important nutritional problems of the nation and one can look forward confidently to the successful solution of the problems of the future.

International Conference on Ion-exchange in the Process Industries

N. KRISHNASWAMY
Central Salt & Marine Chemicals Research Institute, Bhavnagar

THE Society of Chemical Industry, UK, organized the above conference in the auditorium of the Mechanical Engineering Division of the Imperial College of Science and Technology, South Kensington, from 16 to 18 July 1969. Over 300 delegates made up of scientists and industrialists from different parts of the world participated in the three-day conference in which two sessions were held simultaneously both in the mornings and afternoons. Dr Neil Iliff, President of the Society of Chemical Industry, opened the conference.

Session 1: Preparation and Properties of Organic Resins

In the morning session presided over by Dr K. W. Pepper, Dr T. R. E. Kressman described the properties of some modified polymer networks and derived ion exchangers with reference to the kinetics of exchange and equilibria. A knowledge of these data revealed the structure of the modified polymers. After describing the various methods for the pore structure determination of macroreticular ion-exchange resins, Dr R. Kunin projected electron micrographs for various resins. It was indicated that it was now possible to obtain pyrogen-free water with these resins and also that they were useful for virus purification. Dr J. A. Mikes reviewed the developments in the field of porous ion exchangers since 1951, by projecting electromicrographs of various stages in polymerization and critically examined the methods of their evaluation and discussed some useful methods. After describing in detail the standardized procedure to be adopted to obtain IR spectra of ion-exchange resins, Dr D. H. Freeman discussed the use of IR spectroscopy in recognizing the extent and nature of crosslinking of poly(styrene divinyl benzene) resins. The interpretation of different wave peaks observed in the IR spectra was discussed and the possibility of drawing up microstandards for ion-exchange resins was pointed out. Prof. H. P. Gregor described the preparation of an oleophilic ion-exchange resin and its application in biological separations. The kinetics of separation was found to be dependent on the manufacturing conditions which affect the structure.

In the afternoon section of Session 1 held under the chairmanship of Dr G. E. Boyd, Prof. K. M. Saldadaze described the structure and properties of ion exchangers, chiefly based on styrene divinyl benzene copolymers. Copolymerization of styrene with different grades of divinyl benzene (*para*, *meta* and technical) was studied and the products obtained were compared in terms of stability, etc. By understanding the mechanism of formation of defects within the resin, it was aimed at overcoming these and obtaining ion-exchange resins of the desired quality. The influence of the matrix structure on the exchange capacity was brought out. The synthesis and study of anion exchangers based on vinyl pyridine and alkylvinyl pyridine with different grades of divinyl benzene was described by Prof. A. B. Pashkov. The properties of the different products were compared and shown to be affected by the nature of the monomer employed. Mr G. O. Roberts, discussing the effect of chemical and physical structure on anion-exchange equilibrium in quaternary ammonium ion exchangers, brought out the influence of matrix on the exchange kinetics by studying resins of different structures. The effect of different substituents on the equilibrium characteristics of various resins was studied and the basis for the resistance to organic fouling by macroporous solvent modified resins explained. Prof. J. E. Salmon explained the significance of the results obtained from studies on the structural features of ion-exchange equilibria; the values of ΔH obtained over a range of temperatures were compared with results of calorimetric measurements for the same resin. The observation that finely powdered forms of resin exhibit higher rates of exchange with possible mechanical filtration properties and

hence could be used for improving the quality of treated water was made by Dr F. Martinola when discussing the properties and application of powdered ion-exchange resins. Besides, since powdered resins were normally not regenerated and reused, these lend themselves for adoption by unskilled operators. The necessary prerequisites for ion exchangers to be used in continuous systems, such as good resistances to crushing, abrasion and attrition, resistance to osmotic shock and maximum rate of exchange with ease of regeneration, were discussed by Dr P. Grammont. He stressed the need for incorporation of these characteristics, as many of the existing resins did not exhibit them. Discussing the thermal stability of ion-exchange resins, Prof. G. R. Hall enumerated the conditions at which different resins decomposed. In order to establish the mechanism of decomposition, the products of decomposition were analysed and the exchange capacities were determined after heat treatment. The effect of different degrees of crosslinking on thermal stability was brought out and it was observed that resins with lower degrees of crosslinking were more useful at higher temperatures. Dr H. T. Fullam considered the thermochemical instabilities in anion-exchange processing; the nitrate form of various anion-exchange resins was exhaustively studied and various conclusions drawn. Based on these, safety procedures to be adopted while processing nitrate forms of anion exchangers were recommended.

Session 2: Developments in the Design of Ion-exchange Plant

This session was held simultaneously with Session 1, under the chairmanship of Dr N. K. Hiester. Dr G. S. Solt reviewed the new techniques under development in different countries for carrying out ion-exchange processes aimed at reducing cost and increasing efficiency of ion-exchange reactions and pointed out that the CI (or Asahi) process was finding wide acceptance as is evident from plants operating on this principle in different parts of the world. This process was discussed in detail by the next speaker, Dr J. Bouchard, who enumerated the advantages of this continuous process. Dr R. C. Clayton presented a systematic analysis of continuous and semicontinuous ion-exchange techniques and described the stages of development of a continuous system by enumerating its requirements; the performance data for the pilot plant showed that a continuous softener compared favourably with a conventional fixed bed softener. The features and requirements of a continuous ion-exchange process were reviewed by Dr D. G. Stevenson who also traced the progress in the design of a commercial scale plant. The Chemseps continuous counter-current ion-exchange contactor which uses the 'downflow' system and its application to demineralization process were described by Dr I. R. Higgins; the use of low-cost lime for regeneration and lower requirements of process water in complete demineralization steps make the process very attractive. Dr M. J. Slater compared the hydrodynamics of two continuous counter-current ion-exchange contactors and examined the data, with special reference to voidage and resin hold-up in each. Mr F. L. D. Cloete and his collaborators presented a systematic analysis of the performance of a pilot plant contactor in order to provide a useful design procedure for predicting the performance of a full scale plant. The distribution of residence time of the resin in a counter-current continuous ion-exchange column was described for a system of moving bed contactor by Dr J. C. R. Turner. The effect of fluidization of the bed on the residence time was brought out by the data obtained on actual experimental runs.

Session 3: Developments in the Design of Ion-exchange Processes

This session was held under the chairmanship of Mr A. A. Lemon. After reviewing the essential features of the application of ion-exchange resins in mineral industry, Mr D. R. George described the use of both solid and liquid ion exchangers to recover sulphur and sodium carbonate from brines, sea water and to recover potassium from the Great Salt Lake deposits. The feasibility of the technique for these new applications was brought out by the exhaustive data presented. In the combined ion-exchange-solvent extraction (Eluex) process for ammonium diuranate production, Mr J. W. Fisher presented the results obtained with a semi-continuous mini-plant apparatus simulating plant conditions and showed the suitability of the technique for the purpose. The design and cost for ion-exchange softening for a sea water evaporation plant were presented by Dr G. Klein, who after explaining the schematic flow sheet pointed out its potential scope for preventing scale in evaporators and indicated the low cost of pretreated water supplied to the evaporators. Fractional separation of various feed mixtures by continuous ion-exchange was shown possible by Dr M. Streat and the technique was claimed to be easy to adopt as a conventional solvent extraction technique. Dr D. Glasser described an apparatus for fractional ion-exchange separation in which the resin was enclosed in an annular section on the lower surface of a rotating disc. Data on the separation of various ions with very similar properties from mixtures under various experimental conditions were presented and a simple steady state mass transfer equation was derived to describe the behaviour of the column of resin.

Session 4: Separation and Radioactive Waste Treatment

This session was held simultaneously with Session 3 under the chairmanship of Mr R. H. Burns. Mr J. Inczedy described the planning of ion-exchange chromatographic separations using complex equilibria and presented data on the separation of (1) bivalent cations by a cation-exchange resin using a complex forming agent as eluant and (2) organic aromatic acids, using a column of anion-exchange resin and nickel(III) ions as the complexant eluant. The use of a shielded ion-exchange facility as an adjunct to a reactor fuel reprocessing plant for the recovery and purification of valuable elements from the nuclear industry was discussed by Dr E. J. Wheelwright who also reviewed several separation

flow sheets in this regard. Dr L. H. Baestle described the ion-exchange properties of zirconium and titanium phosphates, with particular reference to their use in the separation of nuclear reaction products. The ion-exchange properties of a volcanic tuff and its application to selective adsorption of radionuclides were described by Dr W. Lutze. After presenting the composition of the material and some data on fundamental properties, such as apparent self-diffusion coefficient, the construction of a pilot plant for decontamination on a semi-technical scale using this material was described.

Session 5: Sugar Processing by Ion-exchange

Dr T. V. Arden was the chairman of this session. The use of anion-exchange resins for syrup decolorization in sugar refining was discussed by Dr K. J. Parker who pointed out that colour removal by the resin was a selective process and hence it is necessary to choose the proper resin structures to achieve maximum decoloration. Mr E. Waleriaiiczyk described the problems of decolorizing beet sugar solutions and reported the purification effected by combined carbon and ion-exchange resin treatment to be efficient; it increases the usefulness of the resins. The prerequisites for a successful and economical method for decolorizing beet sugar with 'Wofatit' adsorption resins on an industrial scale were set down by Mr J. Pilot who described the existing plants for this purpose. A modified 'Desal' process for the treatment of sugar beet diffusion juice and the various advantages resulting from the process were highlighted by Dr R. Kunin.

Session 6: Miscellaneous

Selected topics were discussed at this session, which was held under the chairmanship of Prof. H. P. Gregor. In discussing the thermal effects in ion-exchange reactions with organic exchangers, Dr G. E. Boyd presented experimental data on the enthalpy and heat capacity changes in various systems and indicated the possibility of reversal of selectivity, depending on the nature of the ionogenic group and the temperature of the reaction. Dr D. Willis described the thermal regeneration of weak electrolyte resins in mixed bed systems under the 'Sirotherm project'; the theoretical significance of the data collected and the practical application of the study were discussed. Some ion-exchange processes of energy transduction within biological membranes were discussed by Dr D. E. Weiss using a model sodium pump; the possible technological potentials of resins with variable crosslinking for separation purposes were indicated. A theoretical model based upon irreversible thermodynamics was proposed and discussed by Dr M. G. Rao for the ternary ionic diffusion rates in ion-exchange resins. Mr F. Wolf covered the theory and application of ion-exchange catalysis in an exhaustive presentation. It was pointed out that a phenol formaldehyde type resin showed better catalytic properties than a styrene divinyl benzene sulphonic acid resin; this finding was corroborated by Prof. H. P. Gregor and Dr N. Krishnaswamy from their experimental evidences. Prof. H. P. Gregor suggested that as the matrix or compound became more aromatic, its catalytic activity increased. The various cost reducing factors in electrodialysis were discussed by Mr C. Chapman who described the details of a commercial electrodialysis plant with a new design and incorporating all the factors. This was followed by Dr O'Brien's demonstration of a film where the polarization phenomenon at membrane surfaces was brought out in the studies conducted by Prof. K. S. Spiegler in USA.

While Session 6 was considering selected topics, there was a group discussion on the present situation with respect to continuous ion-exchange plant and its development potential. This discussion between the industrialists, pure scientists and the engineers in charge of implementing the designs was very popular and highlighted the current interest and trend in continuous ion-exchange plants.

Session 7: Developments in Inorganic Exchangers

This session was held under the chairmanship of Dr C. B. Amphlett. Dr A. Clearfield presented the detailed structure and properties of the α phase of zirconium phosphate type ion exchanger, and indicated the finding of two other phases, viz. β and γ. Their ion-exchange behaviours were discussed in relation to the α phase. The preparation and properties of four new inorganic exchangers of the zirconium phosphate type, by adopting changes in the concentration of acid used and the reaction temperature were described by Dr E. Torracca. He pointed out that in this field which was considered till now as exhaustively studied, it was possible to obtain newer samples and further careful researches might yield more products of value. Dr G. Alberti compared the ion-exchange properties of amorphous and crystalline insoluble acid salts of tetravalent metals and polybasic acids. While reporting the preparation of a product exhibiting the highest cation-exchange capacity reported so far, he pointed out the clear difference in exchange properties of crystalline and amorphous exchangers and explained these to be due to steric effects. A similar difference in hydrolysis effect was noticed. The use of the exchanger in membrane form was also described. While presenting the properties of hydrous oxide inorganic exchangers, Dr N. Krishnaswamy pointed out the difference in the properties of ammonia and sodium hydroxide precipitated samples. The construction and use of an electrodialysis cell employing inorganic membranes for desalting were discussed with relevant details. Dr H. S. Sherry described the ion-exchange properties of the synthetic zeolite, Linde T, prepared according to a patented procedure. Studies on alkaline earth-alkali metal ion-exchange reactions on this zeolite have revealed that it prefers potassium to divalent ions — conforming to a theory based on selectivity. Cation diffusion kinetics in synthetic zeolites of types X and A were discussed by Dr E. Hoinkis. With the help of a model, the behaviour was explained and the effect of self-diffusion on ion-exchange behaviour was demonstrated and discussed.

The second half of Session 7 was held under the chairmanship of Prof. J. E. Salmon. Dr L. V. C. Rees discussed the computer programme developed for determining the kinetics of ion-exchange and compared the experimental and computed kinetics of ion-exchange. The need for more exhaustive accurate data was pointed out. Self-diffusion and ion-exchange processes in synthetic zeolites were reported by Dr A. Dyer and the behaviour of the exchanger was shown to be related to the structure. Dr H. W. Levi also described the self-diffusion and exchange diffusion of cations in vermiculite and showed that the self-diffusion of certain cations was governed by the hydration of the cations and for certain others only by the electrostatic forces.

Session 8: Water and Effluent Treatment

In the first section of this session held under the chairmanship of Dr E. L. Streatfield, Dr D. E. Weiss considered some ion-exchange processes for partial demineralization, wherein a counter-current method using strong acid and weak base resin helped in demineralization with least requirement of regenerants. Also the preparation and properties of an anion exchanger with barium ferrite acting as a magnetic aid for inducing flocculation were described. The demineralization of tertiary sewage effluent was described by Mr J. G. Grantham with data obtained from a semi-automatic pilot plant operated over several months. This work has shown the ability of modern ion-exchange resins to withstand fouling or poisoning. The possible effect of detergents on the life of the resins and methods to overcome this were discussed. Dr J. Seidl described the removal of organic substances from industrial and drinking waters by adsorption resins and pointed out the inherent difficulties encountered, such as variation in the composition of the wastes and lack of knowledge of the structure of natural organic components of surface waters. The author stressed the need for exhaustive studies to completely understand and solve the problem. The relative merits of different methods of ion-exchange treatment of condensates were discussed by Mr R. Vincent with data from various plants in existence. Results from experiments on the filtration and demineralization of the condensates were compared and discussed. In order to obtain biologically pure water by ion-exchange, Prof. L. Saunders studied various methods for sterilizing ion-exchange columns and adsorbents and used porous macroreticular resins and membrane filtration to obtain the desired degree of purity.

In the second half of the session presided over by Dr M. Streat, the reactivation of phosphoric acid and anodizing liquors by ion-exchange was described by Mr J. Pilot. Also an expression was derived to assess whether such reactivation was economically justifiable for different acids under the operating conditions studied. Ion-exchange in the treatment of effluent from metal finishing processes was discussed by Mr A. E. Evington, with particular reference to the observations made at a plant. After enumerating the difficulties encountered and the remedial measures adopted, the use of the deionization process was advocated, since reuse of water was possible and products with cleaner appearance were obtained from the plating shop by the use of deionized water. Dr C. Calmon described the behaviour of ion-exchange in ultra-pure water systems. In an exhaustive treatment of the sources of various contaminants and the studies on different types of resins, the performance of mixed beds was reported and the real causes for poor performance in some cases were traced.

The concluding lecture of the conference was delivered by Dr R. Kunin. In this, he traced the developments in the field of ion-exchange since the last conference of the Society of Chemical Industry in 1954. After briefly reviewing the developments topic-wise, the speaker brought out the phenomenal growth of interest in this field due to the high pitch of research in the synthesis of improved ion-exchange materials. Though the conference did not include the aspect of ion-exchange membranes, the speaker brought out the advances in that field also. He expressed the hope that with the developments in progress everywhere, this technique will have a bright future and it would enable economical treatments not hitherto possible by other techniques. He also reminded the audience that the various varieties and types of ion-exchange resins are always useful, as each suits a particular application and newer developments do not make the resins in use now obsolete.

The conference, truly international in character, served a very useful purpose and the Society of Chemical Industry in UK has played a very useful role by organizing the conference.

Proton Magnetic Resonance (PMR) Study of Hydrates

S. V. MURTHY*

THE exact location of the protons in the crystal structures has not been possible through the conventional methods of structural crystallography like X-ray diffraction, electron diffraction, etc. This limitation is due to the fact that the hydrogen atom is the lightest atom possessing only one scattering electron and exerts a negligible effect on X-ray scattering. In the X-ray diffraction method, the H-position is only tentatively assigned after the heavy atom structure has been determined, from considerations of hydrogen bonding. This is only approximate and the hydrogen bond knowledge also is very insufficient.

The technique of proton magnetic resonance (PMR) is, on the other hand, a powerful tool for the location of the protons, since the proton possesses a strong magnetic moment [2·7926 nuclear magnetons (nm) as compared with 2·2171 nm for ^{23}Na, 2·2696 nm for ^{81}Br, etc., and other heavy nuclei] and hence gives strong PMR spectra which enable their positions to be determined accurately. Neutron diffraction studies give more accurate data than NMR in the light nucleus location, but they have the limitation that the dynamics of the proton cannot be studied by them, whereas PMR is an ideal tool for these investigations.

The study of the proton systems in which the protons occur in pairs is very convenient. Due to the local field produced at a resonating nucleus by the neighbouring magnetic nucleus, the resonance is produced at different values of the external field and the resonance spectrum shows structure. Owing to the interaction of each neighbour, the resonating nucleus gives rise to a pair of lines equally disposed about the centre and the measurement of the line-widths from the centre enables one to calculate the internuclear distance and also the orientation of the internuclear vector if a single crystal is used. With polycrystalline samples, only the internuclear distance can be evaluated. The hydrates provide a very good example of proton pairs and hence are highly suitable for PMR studies. This was first suggested by Pake[1] in 1948.

Theory

We shall now briefly describe the theory behind the PMR study of hydrate water molecules. We shall assume for simplicity that the lattice is rigid and free from internal motions. Considering directly the interaction between two nuclear magnetic dipoles (of spin $\frac{1}{2}$), so that there are only two possible orientations of each with respect to the external field, viz. parallel and antiparallel, corresponding to $m = \pm\frac{1}{2}$, the field at one nucleus due to the other, in the direction of the applied external field, will be given by the classical theory as

$$H = \pm\frac{\mu}{r^3}(3\cos^2\theta - 1) \quad \ldots \quad \ldots \quad \ldots \quad \ldots(1)$$

where θ is the angle between the line joining the two nuclei and the direction of the external field, r is the internuclear distance, and μ is the magnetic moment of either nucleus. Hence, the actual field seen by the nucleus will be

$$H \pm \frac{\mu}{r^3}(3\cos^2\theta - 1) \quad \ldots \quad \ldots \quad \ldots \quad \ldots(2)$$

This shows that a single resonance line will now be split up into two by the action of the nearest spin; the other neighbours will cause a broadening of the two split up lines. Hence, we expect for a given proton pair and the corresponding pairs (same θ) in all other unit cells in a single crystal, two resonance lines equally spaced about the resonance field strength H by an amount $\pm\mu/r^3$ $(3\cos^2\theta - 1)$.

A quantum mechanical evaluation of the local field at a nucleus due to the neighbour in a pair gives an additional factor 3/2 into Eq. (1) if the two nuclei are identical. Pake treated the magnetic dipole-dipole interaction between the two nuclei in a pair as a perturbation of the energy levels of the nuclei in the applied field H. By a first order perturbation calculation, he found the new energy levels and from them showed that the resonance peak should occur at field strengths given by

$$H^* \pm 3/2\ \frac{\mu}{r^3}(3\cos^2\theta - 1) \quad \ldots \quad \ldots \quad \ldots(3)$$

Using this quantum mechanically modified equation, we can examine the variation with orientation of the displacement of the peaks from the centre and find the angular disposition of the nuclear pairs in the unit cell and also the pair separation r. This measurement of the internuclear distance may be made with some precision, since an error of 6% in measuring μ/r^3 causes an error of only 2% in r itself. Pake's analysis also shows that for unlike nuclei, the factor 3/2 is absent in Eq. (3).

A physical explanation of the purely mathematically derived factor 3/2 in the case of like nuclei has also been given. There are two dipolar mechanisms for line broadening: one is the local field produced at one nucleus by the z-component of the other; the second arises from the limitation of the lifetime of the spin state by simultaneous spin-exchange transitions on the part of neighbouring nuclei mutually induced by their precession. This second process is responsible for the factor 3/2. If the two nuclei in each pair are not identical, their precession frequencies differ and the spin-exchange process does not occur. Hence, in this case, the factor 3/2 is absent from Eq. (3).

If the specimen is not monocrystalline but is in powder form, it can be assumed that there are a number of crystal grains with their axes randomly distributed over all directions. The spectrum for the specimen is thus the sum of the spectra for the individual grains and hence the fine structure will be smeared out. It has been shown, by a simple

*Present address: 55 Puttanna Road, Basavangudi, Bangalore 4.

argument, taking into account the isotropic distribution of the values of θ, that the powder curve is double-humped and from the separation of the humps, the internuclear distance can be found, though the accuracy with powders is less than with a single crystal. But from powder studies, the orientation of the nuclear pairs cannot be determined.

Studies on Hydrates

The first study of proton pairs in water molecules of hydrates was made by Pake[1] on gypsum ($CaSO_4.2H_2O$). Since then a number of hydrates have been studied; the technique detailed above is also called the 'Pake method'. Studies on over 70 hydrates (both single crystal and polycrystalline) have been reported in the literature and the various results that have been derived from these studies are discussed in some detail in the following.

Molecular structure — It has been possible to get exact information regarding the stereochemistry of the compounds by these studies, a typical example for this being potassium pentaborate ($KB_5O_8.4H_2O$). The PMR study of this compound[2,3] showed that there are only two dipoles in the molecule and the molecular formula is more appropriately written as $K(H_4B_5O_{10}).2H_2O$.

Effect of some physical mechanisms — Of the many crystalline hydrates studied by PMR, the case of lithium sulphate monohydrate is interesting. PMR spectra present certain features which are as yet unexplained theoretically in a complete manner. Pake[1] first observed a certain peculiarity in the PMR study of $Li_2SO_4.H_2O$. It was found that some of the Pake curves show a shift, so that they are described by an equation of the form

$$H = 2\alpha[3\cos^2(\phi+\phi_0).\cos^2\delta-1]+\text{constant shift}$$

Here, 'shift' means a uniform deviation of H from its value expected on the basis of the equation

$$H = 2\alpha[3\cos^2(\phi+\phi_0)\cos^2\delta-1]$$

It does not refer to a displacement of the entire resonance pattern. These shifts are found to be independent of orientation.

$Li_2SO_4.H_2O$ was also studied by Soutif and Ayant[4] and Hirahara and Murakami[5]. But they did not find the shift in the Pake curves. The latest study by Silvidi *et al.*[6], however, shows the shifts as explained above. The shifts in the Pake curves are orientation-independent for each axis of crystal rotation, but do depend on the *p-p* axis and on the axis of crystal rotation.

Several attempts have been made to explain the shifts theoretically. Silvidi *et al.*[6] discuss the various mechanisms that have been suggested to explain the shift. Purcell[7] has suggested that perhaps the *p-p* axes are flipping between two orientations making angles of $\theta+\beta$ and $\theta-\beta$ with the field H_0.

Applying this concept, one obtains for the splitting

$$\langle\Delta H\rangle = 2\alpha[3\cos^2(\phi+\phi_0).\cos^2\delta.\cos^2\beta-1)+6\sin^2\beta \quad ...(4)$$

Thus, the shift in the minimum of H is $6\sin^2\beta$. Values of β were calculated from the known values of α and the shifts. But no clear suggestion regarding the origin of the flipping vibration is available.

Another motional effect may give a partial explanation. This is the modified[8] Das effect[9] that considers the vibrational-rotational motion of the *p-p* axes, in simple harmonic potential wells about their equilibrium orientations. This results in a rather complicated modification of the Pake curve. But the experimental results show that the modified Das effect is not the cause of the peculiar results obtained in the case of $Li_2SO_4.H_2O$.

Silvidi *et al.*[6] have also discussed and ruled out the possibility of the shift being due to the effects of further interaction between nuclei.

The author suggested[10] a plausible mechanism which can explain the shift. In the asymmetrical hydrogen bond, which the oxygen of the water makes with two near enough oxygens, the proton can alternate between the two possible asymmetrical states (in the double minimum potential well). If this rate of alternation becomes of the order of the precessional rate of the nearby proton, tnere can be quantum mechanical coupling between the two giving rise to an increase in the local field at the proton site. This is analogous to the mechanism that gives rise to a 3/2 factor enhancement in the local field for like nuclei, since their precessional frequencies are identical. This mechanism can be orientation-independent. The effect can also depend on the axis of rotation, since the axis of precession and the line of alternation are differently disposed. The classical models cannot explain the orientation-independence. For a satisfactory explanation, a quantum mechanical concept is very necessary as detailed above.

Internal motions and hindered rotations — It was noticed by Pake[1] that the line-width obtained with borax ($Na_2B_4O_7.10H_2O$) at room temperature is only about 4·4 gauss, whereas the usual value for the hydrates is about 10·8 gauss. This decrease in the experimental value of the line-width is attributed to the presence of internal motions inside the crystal which largely average out the line-width. In such cases, structural data cannot be obtained on the crystal. Only information concerning the presence and nature of internal motions can be inferred. If the internal motion is about an axis, the motion is called hindered rotation. Structural information on such crystals can be got only by experiments at low enough temperatures, so that the internal motions get frozen and the ideal of the rigid lattice is attained.

Antiferromagnetic and ferroelectric transitions — PMR studies on hydrates at different temperatures sometimes lead to the knowledge of the antiferromagnetic and also ferroelectric transitions if present. At the transition points, there is a definite kink in the line-width versus temperature curve, showing that the internal order has changed giving rise to the different physical states.

A few examples of the transitions that have been observed are quoted below.

Antiferromagnetic transition:

$NiBr_2.6H_2O$ (ref. 11); Neel temp. 6·5°K
$CoBr_2.6H_2O$ (ref. 12); Neel temp. 3·08°K
$CuCl_2.2H_2O$ (ref. 13); Neel temp.
$Fe_3(PO_4)_2.8H_2O$ (ref. 14); Neel temp. between 4° and 12°K

Ferroelectric transition:

Rochelle salt (ref. 15); Curie point −20°C
$K_4Fe(CN)_6.3H_2O$ (ref. 16); Curie point
$(CH_3NH_3)AlSO_4.12H_2O$ (ref. 17); Curie point 160°K

Paramagnetic hydrates — An extensive study of paramagnetic hydrates has also been made. In these cases, the line-width is enhanced not only due to the Pauli spin paramagnetism of the electrons, but also due to the hyperfine interaction term due to the interaction of the magnetic electrons with the magnetic proton.

Some crystals which are expected to be paramagnetic do not show any broadening in the PMR lines but give the same line-widths as the diamagnetic compounds (author's unpublished work). This is attributed to the fact that the demagnetizing exchange interactions largely cancel out the paramagnetism leaving the crystal diamagnetic.

Structure of the water molecule and studies of hydrogen bonding — PMR investigations of hydrates help in obtaining knowledge of the structure of the water molecule in hydrate. Usually, in hydrates the oxygen of the water molecule is hydrogen-bonded to another nearby electronegative atom (an O^- atom of the other water molecule or any group like SO_4, etc., or N or Cl as the case may be), the hydrogens of the water acting as the binding atoms. Since the positions of the hydrogen can be located, it follows that the structure of the water molecule can be obtained. A study closely associated with this is that of hydrogen bonding.

Now the water oxygen atom in a hydrate is tetrahedrally coordinated and hence the HÔH angle in a hydrate always tries to maintain its tetrahedral value of 109°. This is seen from a table compiled by Bacon[18]. But the O-H distance can vary depending on the hydrogen bond distance O-O (ref. 18). Hence, we may conclude that the H-H distance in the triangle HOH also varies. This is in contradiction to the general conclusion reached by Silvidi and McGrath[19]. They have generalized that the H-H distance is more or less constant at an optimum value of 1·595 Å in hydrates. From the graph of O-H distance versus O-O distance, we find that the O-H distance for a normal H-bond of 2·76 Å length is 1·0 Å. Using this value we find for the O-H distance an optimum value of 1·628 Å. Now the O-H distance can vary between 0·96 and 1·1 Å. Hence, we find the H-H distance can vary between 1·56 and 1·79 Å.

Again, in a typical hydrogen bond arrangement in a hydrate shown in Fig. 1, HÔH is, as explained above, about 109°. But $O_1\hat{O}O_2$ varies between the limits of 84° and 146° (ref. 18). In such a case, when $H\hat{O}H \neq O_1\hat{O}O_2$, the bond must necessarily be bent, since it is more difficult to move the heavy O_1 and O_2 atoms to the position of linearity than to allow the bond to be bent, retaining the HÔH angle at 109° as required. If, on the other hand, $O_1\hat{O}O_2$ is very nearly equal to 109°, some slight adjustments are usually made in the HÔH angle itself, so that the bond retains its linearity, since a bent H-bond requires larger energy to be formed than a linear one.

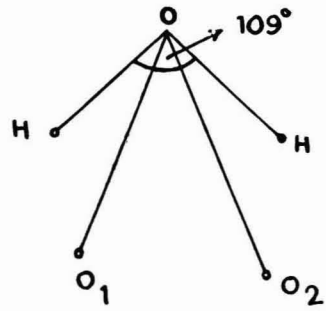

Fig. 1 — Schematic diagram of a water molecule in a hydrate

Some additional features of hydrate studies — The PMR study of zeolites does not give consistent values for the line-width (author's unpublished work). This is because the water molecule in zeolitic hydrates is constantly moving in the structure occupying the vacancies and interstices in the lattice. The fifth water molecule in $CuSO_4.5H_2O$ (ref. 20) is found to be zeolitic, while the others surround the copper ion tetrahedrally.

Again, with the increase in the number of *p-p* vectors with different orientations in the lattice, the PMR spectra become too complicated and hence are difficult to resolve. Crystals with more than four or five distinct *p-p* vectors are difficult for PMR study. Though a unit cell may contain a large number of water molecules, the space group symmetry may reduce them to a sizeable number of distinct groups of similar vectors (*p-p* vectors with the same orientation, i.e. those which are parallel to one another, are termed 'similar'). For example, in the $MgS_2O_3.6H_2O$ crystal studied by the author[21], though the unit cell contains 24 water molecules, the space group symmetry *Pnma* of the orthorhombic lattice reduces them to three distinct orientation groups of eight vectors each.

Conclusive evidence for the existence of the hydroxonium ion H_3O^+ has also been obtained by the NMR study of solid hydrates of a number of acids, e.g. $HClO_4.H_2O$ (ref. 22), $H_2SO_4.H_2O$ (ref. 23) and $H_2SO_4.2H_2O$ (ref. 24). Here, of course, the NMR spectrum is due to an isosceles triangular configuration of three identical nuclei. The structure of the H_3O ion appears to be that of a rather triangular pyramid with the HÔH angle about 115°.

Accuracy of PMR results — PMR is one of the accurate methods of proton location for reasons described earlier. The accuracy claimed in the *p-p* distance obtained is about ±0·03 Å, which is about 2%. Though the experimental accuracy in the measurements of the line-widths is only 6%, the accuracy in the value of r is 2%, since r is related to the line-width by an inverse cube law.

But it is pointed out that in PMR experiments, the distance measured is only the distance between the magnetic centres of the two nuclei and not between their centres of mass as is required. It is shown that the centre of magnetism and the centre of mass of the nucleus do not coincide. This introduces another additional error in the r values we use from PMR data.

Again it is shown[25] that the inter-pair interactions broaden the free structure lines asymmetrically. Hence, the use of peak-peak splittings is questionable. On the other hand, it is suggested that the centre of gravity of the fine structure line which is independent of inter-pair interactions should be considered and in the Pake formula the C.G.-C.G. splitting should be used. An estimate of the error involved in this, depending on the inter-pair distance, is also given[25].

Finally, the experimental values of r must be corrected for thermal vibrations. It is shown[26] that a reasonable mean value for the reduction factor is such as to lead to an equilibrium p-p distance which is 5% smaller than the value obtained directly from the Pake formula uncorrected for oscillatory motion. It is found that, after correction, the values obtained are closer to the equilibrium distance found in the isolated H_2O molecule, viz. 1·51 Å.

Regarding the motional effects, corrections must also be applied for the rocking vibrations[27] (Das effect) and waving vibrations[28] of the H_2O molecule.

Summary

A critical account of the methods of the proton magnetic resonance (PMR) study of hydrates is given. The conclusions drawn from the hydrate studies by PMR include: (1) structure of the water molecule and information concerning the hydrogen bonds, (2) other structural information like the exact structural formula of the compound, the number of molecules in the unit cell, etc., (3) study of the internal motions and hindered rotations in solids, and (4) various transitions like the ferroelectric and the anti-ferromagnetic transitions. Hydrogen bonding studies form an integral part of hydrate studies and the NMR studies coupled with the neutron diffraction data have helped to solve a number of problems connected with the H-bond. The $Li_2SO_4.H_2O$ anomaly is also discussed and a plausible mechanism to explain it is suggested. The possible sources of error and the accuracy that the PMR technique is capable of have also been briefly discussed.

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Studies on the Metabolism of Trace Elements & Seed Proteins*

TRACE ELEMENTS

Studies on Heavy Metal Toxicity

DESPITE the ubiquitous occurrence of metals in biological systems and their importance in nutrition, knowledge as regards their mode of action is incomplete. The studies on trace elements have been carried out with a view to understanding the role of essential metals like iron and magnesium in metabolism. Two approaches are generally available for such an investigation. One is the classical approach involving the withdrawal of the concerned essential element and studying the consequences thereof. The other approach is to introduce certain other metals in excess which in turn can induce the deficiency of an essential element due to ion antagonism. To begin with studies were initiated by following the latter approach, since one can discern the role of the essential element and at the same time understand the biochemical effects of trace elements at toxic levels. The toxicities of metals like cobalt, nickel, zinc and molybdenum have been studied in different systems including the molds *A. niger* and *N. crassa*, the insect *Corcyra cephalonica* St. and in a plant system *P. radiatus*. The effect of the trace elements on various parameters, viz. growth, nucleic acid, amino acid and carbohydrate metabolisms, has also been investigated. The results obtained are complex and subtler variations have been always recognized depending on the nature of the metal, the organism and the parameter studied. However, a basic similarity is seen in the case of cobalt, nickel and zinc toxicities which interfere with the iron and magnesium metabolism in all the systems. This is confirmed by the ability of iron and magnesium to counteract the metabolic derangements caused by the toxic metals. An interesting observation has been recorded in *A. niger* where the modes of counteraction by iron and magnesium are found to be different. Whereas magnesium exhibits an extracellular ion antagonism, iron does not inhibit the toxic metal uptake and hence its effect is intracellular. Molybdenum toxicity is strikingly different from that of the other three and it interferes specifically with the metabolism of the sulphur containing amino acid in *N. crassa*. Zinc toxicity also shows a singularity in the *Corcyra cephalonica* St. where it interferes with the nucleic acid metabolism at the level of purine synthesis.

*The report summarizes the work carried out under the scheme " Studies on the metabolism of trace elements and seed proteins ", sponsored by the Council of Scientific & Industrial Research at the Department of Biochemistry, Indian Institute of Science, Bangalore 12. The scheme was in operation from 9 September 1958 to 28 February 1969, with Prof. P. S. Sarma as the principal investigator. Other workers associated with the work are Drs K. Sivarama Sastry, E. Raghupathy, P. Radhakantha Adiga, G. Padmanaban, S. Lakshminarayana Rao, K. N. Subramanian, Shri N. Raman and Miss K. Malathi.

Production of Siderochromes during Cobalt Toxicity and Iron Deficiency in *Neurospora*

After surveying this gross picture a more detailed study has been carried out on cobalt toxicity and iron metabolism in *N. crassa*. In this mold, the biochemical lesions of excess cobalt are identical with iron deficiency. It has been observed for the first time that this organism secretes an iron-binding compound into the culture filtrate under conditions of cobalt toxicity or direct iron deficiency. The same compound is secreted in both the cases. The iron-binding compound (X) has been isolated from the culture filtrate as its iron complex (XFe). XFe has been found to belong to the siderochrome class of compounds, cyclic trihydroxamates, present widely in fungi. It contains 3 moles of the unique amino acid δ-N-hydroxy ornithine and it has been identified as coprogen.

Cobalt has been found to induce iron deficiency by accelerating the uptake of iron, but interfering with heme synthesis. Thus, the cell surface is in contact with a medium devoid of iron which stimulates the synthesis of the iron-binding compound.

Role of Siderochromes in Iron Transport in *Neurospora*

Coprogen has been found to be an excellent iron donor for *N. crassa*, even though the binding affinity for iron is of the order of 10^{30}. The incorporation of ^{59}Fe into coprogen of the cell was much faster as compared to $FeSO_4$. Normal mycelia of *N. crassa* contain two iron chelates of sideorochrome nature, accounting for 10% of the total iron absorbed from the medium. A precursor-product relationship has been established between the siderochrome iron and heme iron. On the basis of these results it has been concluded that the siderochrome plays a key role in iron transport in this organism and serves as the *in vivo* iron donor for heme synthesis. Under conditions of iron deficiency, iron is not available for the formation of the natural chelate and the iron-free compound accumulates in the culture filtrate.

Regulation of Heme Biosynthesis in *Neurospora*

Detailed studies have been carried out on the mode of regulation of heme synthesis in *Neurospora*. It is known that in iron deficiency, the synthesis of heme and the heme-protein, catalase, is low. It has been found that δ-aminolevulinic acid (ALA), the first intermediate in the pathway of heme biosynthesis, accumulates and that the activity of ALA dehydrase is very low, in iron deficiency conditions. A similar situation exists in cobalt toxicity and zinc deficiency. ALA synthase and heme synthase comparatively show only marginal changes under these conditions. Addition of iron and zinc to the respective metal-deficient cultures results in an induction of ALA dehydrase which is repressed

by protoporphyrin and less effectively by hemin and hemoglobin. Thus, in *Neurospora*, the second enzyme in the heme biosynthetic pathway, viz. ALA dehydrase, functions as the regulatory enzyme of the pathway in contrast with bacterial and mammalian systems in which the first enzyme, ALA synthase, has been shown to be regulatory. Iron and zinc deficiencies and cobalt toxicity have been found to interfere with the conversion of protoporphyrin into heme, thus rendering protoporphyrin available to repress ALA dehydrase. The repression can be counteracted by inorganic iron and more effectively by coprogen. Protoporphyrin is visualized as the corepressor for ALA dehydrase. Iron in the form of coprogen would convert protoporphyrin into heme, the latter having a lesser affinity for the aporepressor. Coprogen would inhibit heme binding to the aporepressor and render the repressor non-functional which would lead to a derepression of ALA dehydrase.

Regulation of Nitrate Reductase and Catalase in *Neurospora*

The mode of regulation of nitrate reductase in *Neurospora* has been investigated in detail. The effect of trace elements and amino acids on the synthesis of this enzyme has been studied. *Neurospora* develops iron deficiency when grown in sole nitrate medium provided with levels of iron normal with respect to the usual ammonium nitrate medium. The iron deficiency is caused by the depletion of the iron stores in the cells because of the increased activity of the three iron-containing enzymes, nitrate reductase, nitrite reductase and catalase. Concentration of this iron deficiency by increased level of iron in the medium leads to a much lowered nitrate reductase activity in the mycelia. This repressive action of iron on this enzyme is mediated through the iron complex, coprogen, and free amino acids like alanine. Many amino acids, especially cysteine, isoleucine, glutamine, asparagine, NH_4^+ and histidine repress nitrate reductase effectively. In the case of cysteine, isoleucine, glutamine and asparagine, this repression is also accompanied by inactivation of the enzyme present initially. Transfer of ammonium-grown mycelia to nitrate medium also causes the induction of catalase. This induction is believed to be by the H_2O_2 formed by the auto-oxidation of the flavins associated with nitrate reductase and nitrite reductase, which are induced by nitrate. Amino acids which repress nitrate reductase block the synthesis of catalase also. Cobalt toxicity in *Neurospora* has been studied as a function of the nitrogen source provided to the organism. Cobalt toxicity is maximal when nitrate is the nitrogen source and minimal in sole ammonium medium. Purified nitrate reductase is inhibited by nitrite and hydroxylamine competitively with respect to nitrate. During molybdenum deficiency, though the nitrate reductase activity is very low, the other activity exhibited by the same enzyme, viz. NADPH-cytochrome *c* reductase, is found to be normal. It is proposed that molybdenum deficiency leads to the production of an altered enzyme possessing the latter activity only.

Methods for Estimation of Magnesium, Manganese and Siderochromes

During the course of these investigations three analytical procedures have been developed. A microbiological assay procedure for the determination of magnesium at 1 μg level has been developed using *Neurospora* as the experimental organism. Sensitive chemical methods for the estimation of manganese and siderochromes have also been developed.

SEED PROTEINS

Studies on Phosphoproteins

This part of the investigation was started with studies on phosphoproteins primarily from the viewpoint of structural aspects. From the chymotryptic digests of casein a phosphopeptone was isolated and separated into three different constituents by using column chromatographic and moving boundary electrophoretic techniques. A comparative study of the amino acid composition of goat, buffalo and rabbit caseins was carried out. Parallel investigations were also initiated with the haem protein catalase, where structural modifications could be followed up by studying the biological activity.

Preliminary Studies on the Pulse *Lathyrus sativus*

In view of the emphasis laid on applied research in recent years especially in the context of increasing food production it was felt at this stage that an evaluation of the nutritive quality of the pulse *L. sativus* and a study on the disease lathyrism would be of interest. The reasons for choosing this pulse for study were: (i) It is a cheap pulse with a high protein content and cultivated in areas of low rainfall. (ii) Neurolathyrism, a crippling disease caused in the humans as a result of the consumption of *L. sativus*, is still a public health problem in India. (iii) It was claimed that excess selenium or manganese present in the seeds might be responsible for this disease and hence it was felt that investigation on *L. sativus* would fall within the purview of studies on trace element metabolism.

So studies were initiated to evaluate the nutritive quality of *L. sativus* seeds and understand the disease lathyrism, which subsequently led to several interesting findings. The title of the scheme was thus changed from "Studies on trace elements and phosphoproteins" to "Studies on trace element metabolism and seed proteins".

Isolation of Homoarginine and OX-Dapro from *Lathyrus sativus*

The proposition that the disease neurolathyrism caused by the prolonged consumption of *L. sativus* seeds is due to excess of manganese or selenium present in them could not be substantiated and the present studies have led to the discovery of two new amino acids, L. homoarginine and β-N-oxalyl-L-α-β-diaminopropionic acid (OX-Dapro). OX-Dapro has been shown to be the chief neurotoxic constituent of the seeds and could well be responsible for human neurolathyrism.

L. homoarginine has been isolated in 0·2% yields from the seeds. The properties of the compound and its monoflavianate and monopicrate derivatives have been reported. L. homoarginine is nontoxic to higher organisms. L. homoarginine is the precursor to lathyrine in *L. tingitanus*. Investigations on the metabolism of L. homoarginine by the germinating seeds of *L. sativus* have shown the presence of an enzyme (*ca* homoarginase) degrading it to lysine and urea.

OX-Dapro has been isolated from the seeds in yields varying from 0·5 to 2·0%. The structure of the compound has been established on the basis of its physico-chemical properties, degradative and synthetic studies. This has also been achieved independently by another group of Indian workers.

Neurotoxic Action of OX-Dapro and Related Compounds

OX-Dapro is growth inhibitory towards several microorganisms and the rice moth larva *Corcyra cephalonica* St. It is also severely neurotoxic to the young chick and innocuous to normal adult animals. The neurotoxic action of the compound in the chick is characteristic of its structural integrity. Several other N-oxalyl amino acids have been synthesized and shown to be neurotoxic to the chick. It has been concluded that any N-substituted oxamic acids wherein the substituent carries another free carboxyl group are generally neurotoxic to the chick. During these investigations another interesting property of N-oxalyl derivatives of diaminomonocarboxylic acid, namely the transformation in solution of ω-N-oxalyl amino acids, viz. that of Dapro, DABA and lysine to their α-oxalyl isomers has been observed.

Existence of a Blood-Brain Barrier to the Neurotoxin

The innocuous nature of OX-Dapro to normal adult animals has been explained as due to an effective blood-brain barrier (BBB) to this neurotoxin. Thus not only the chick but also other young animals, such as the new-born rat, pups, and young guinea-pigs, are also susceptible to the action when administered intraventricularly or intracranially to adult rats and mice.

Investigations on some of the experimental conditions which would effect an increased permeability of the BBB to the neurotoxin have shown that an 'acidotic' condition makes adult animals susceptible to its action. Adult animals treated with acidotic chemicals or drugs come down with severe neurological symptoms by mere intraperitoneal administration of the neurotoxin.

The Demonstration of the Onset of Neurolathyrism upon the Administration of OX-Dapro

Repeated intrathecal administration of OX-Dapro to adult monkeys has been shown to result in permanent, total areflexic, flaccid paraplegia. Histological examinations of the brains and spinal cords of these monkeys along with those from monkeys treated with other acidic amino acids have shown an obvious destruction of nerve cells of the grey matter of the spinal cord accompanied by proliferation of microglial cells, only in monkeys receiving OX-Dapro.

It is suggested that even in humans a BBB system could be operative towards this neurotoxin. Further, in view of the variety of conditions which would alter such a barrier, leading to an increased permeability, it is reasonable to conclude that gradual central effects of the compound might eventually result in the observed clinical condition of neurolathyrism in man.

Elimination of the Neurotoxin from the Seeds

Attempts have also been made to remove the neurotoxin from the seeds. Roasting has been found to destroy the neurotoxin. A preparation containing 70% protein has been obtained from the seed meal using carbonate: bicarbonate buffer extraction followed by precipitation of the protein by acidification. The protein preparation is completely free of the neurotoxin. Preliminary studies indicate that this protein preparation is very rich in the essential amino acid lysine.

Biosynthesis of OX-Dapro

The biosynthesis of OX-Dapro in germinating seeds of *L. sativus* has been studied and it is found to proceed in the following two steps:

$$\text{Oxalate} + \text{ATP} + \text{Coenzyme A} \xrightarrow[Mg^{2+}]{\text{Oxalyl CoA synthetase}} \text{Oxalyl CoA} + \text{AMP} + PP_i \quad \ldots(i)$$

$$\text{Oxalyl CoA} + \alpha,\beta\text{-diaminopropionic acid (DAP)} \xrightarrow[\text{enzyme}]{\text{Condensing}} \text{OX-Dapro} + \text{CoA} \quad \ldots(ii)$$

Germination of *L. sativus* seeds in the presence of ^{14}C-oxalate results in the incorporation of label into the oxalic acid moiety of OX-Dapro. The first step, namely oxalyl activation, catalysed by oxalyl CoA synthetase requires Mg^{2+}, ATP and coenzyme A for its activity. It is a sulphydryl enzyme and is inhibited by *p*-hydroxymercurybenzoate and iodoacetate. The second step or the condensing activity does not require any cofactors. Chemically synthesized oxalyl CoA serves effectively as a substrate along with DAP to give OX-Dapro. DAP has been shown to be the most effective substrate in comparison with other amino acids, like α,γ-diaminobutyric acid, lysine, ornithine, homoserine and serine. The two enzyme activities have been purified to 100-fold. Though the two enzyme activities could not be completely separated, certain purification procedures like alcohol and acetone precipitations completely destroy the condensing enzyme activity, while the oxalyl CoA synthetase activity is further purified. Disc electrophoresis of the 100-fold purified enzyme shows two protein bands.

Mode of Action of OX-Dapro

In order to investigate the mode of action of OX-Dapro, the changes in the levels of various

metabolites have been studied in 12 days old weanling rats, injected with OX-Dapro. A striking increase in brain glutamine level has been observed. There is also an increase in free ammonia level in brain initially, which levels off subsequently. Blood glutamine shows a small increase and free ammonia concentrations in blood and liver increase. The striking increase in brain glutamine concentration in OX-Dapro injected animals is a clear indication of ammonia toxicity, the glutamine formation being a major ammonia detoxification mechanism in the brain. Unlike the weanling rat, the adult rat does not respond to intraperitoneal administration of OX-Dapro and there is no detectable amount of OX-Dapro in the brain. These adult animals do not show any change in brain glutamine or ammonia concentration. Thus, the entry of OX-Dapro into brain appears essential for the change in glutamine level.

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World Congress on Fats & Oils

The International Society for Fat Research and the American Oil Chemists' Society (AOCS) are jointly planning a world congress to be held in Chicago, Illinois, during 27 September-1 October 1970. Besides four plenary sessions, the following symposia are being organized: (1) Metal-catalysed lipid oxidation; (2) Physical and chemical methods of testing oil and oil-based products; (3) Chemistry of cruciferous oils; (4) Modern oil processing; (5) Applications of fats and oils as foods; (6) Deep fat frying — Chemistry, technology and nutrition; (7) The role of the oilseed processor in food protein production for world consumption; (8) Biochemistry of hydrocarbon degradation; (9) Chemistry and biochemistry of polyunsaturated fatty acids; (10) Biochemistry of tocopherols; (11) Atherosclerosis and lipid metabolism; and (12) Relationship of lipids to thrombosis and blood coagulation.

In addition, there will be a number of general sessions devoted to the chemistry of fatty acids and derivatives, fat and oil industrial problems, lipid biochemistry (including biosynthesis, metabolism, nutrition, and clinical aspects), detergent testing and formulation, cosmetics and many others.

Two short courses are being offered by the AOCS in conjunction with the world congress. The first course on " Processing and quality control of fats and oils " will be given at Michigan State University, East Lansing, Michigan, in the week before the congress, 20-25 September, under the co-chairmanship of Prof. LeRoy Dugan, Michigan State University, and Dr R. J. Hlavacek, Hunt-Wesson Foods. In the week following the congress, during 4-7 October, a short course on " Behaviour of membrane lipids at surfaces " will be conducted at one of the universities in New York City, with Dr Giuseppe Colacicco, York College of the City University of New York, as chairman.

In addition, an International Conference on the Science and Technology and Marketing of Rapeseed and Rapeseed Products will be held at the Le Chanteler Hotel, Ste-Adele, Quebec, during 20-23 September 1970. It is being organized by Dr Bernd Weinberg of the Edible Oils Section, Department of Industry, 112 Kent Street, Ottawa 4, Ontario.

Further details regarding the congress can be had from the 1970 World Congress Committee, The American Oil Chemists' Society, 35 E. Wacker Drive, Chicago, Illinois 60601, USA.

Biochemistry of Nitrogen Fixation

M. S. NAIK

Biochemistry Division, Indian Agricultural Research Institute, New Delhi 12

THE nitrogen cycle in nature consists of biological transformations of nitrogen gas and its compounds, ranging from nitrate in which the nitrogen atom exists in the highest oxidized state, to ammonia in which the nitrogen atom is in its most reduced state. These transformations are of vital importance to all living organisms, since they supply the nitrogen compounds essential for life. These reactions are indeed equal in importance to the photosynthetic system which supplies carbon compounds. Biological nitrogen fixation, which means reduction of molecular nitrogen to ammonia, is an essential step in the nitrogen cycle. In addition to the great theoretical significance of this process for understanding unique biochemical mechanisms, its practical implications need hardly be stressed. It is estimated that about 10^8 tons of nitrogen are fixed annually by biological nitrogen fixation. In India, the new agricultural strategy based on intensive agriculture relies heavily on the use of massive doses of nitrogen. Nitrogen fertilizers produced by industrial processes are very expensive and are in short supply. Energy equivalent to about five tons of coal is required to reduce one ton of nitrogen. Thus, for economic reasons synthetic fertilizers can contribute only a small fraction of the total nitrogen required by crops. For a long time to come, therefore, the biological processes will play a key role in agriculture.

Excellent and exhaustive reviews on biological nitrogen fixation have appeared recently[1,2]. In this article, therefore, it is not intended to review all the facets of this problem. The main emphasis will be to describe and discuss the biochemical reactions involved in nitrogen fixation. Organisms responsible for this process are of very diverse types, ranging from obligate anaerobes to photoautotrophs, free living aerobes and symbiotic bacteria. It is the purpose of this article to bring out the essential unity of the biochemistry of nitrogen fixation in these widely different types of cells.

Chemistry of Nitrogen

As biochemical reactions are essentially chemical reactions, which occur under very special conditions obtaining in living cells, it would be of interest to recapitulate some of the important chemical properties of nitrogen gas, to serve as a background to understand the biochemical reactions. Gaseous nitrogen molecule is diatomic, with an internuclear distance of 1·095 Å. The triple-bonded structure, $N \equiv N$, is extremely stable and chemically inert, on account of the high bonding energy of the molecule. The formation of atomic nitrogen is not possible by heat, as the dissociation energy of 225·2 kcal per mole is quite high. Active nitrogen can, however, be produced in the presence of electrical discharges at pressures below 1 mm Hg. This is accompanied by a yellow afterglow effect, caused by a slow release of energy. Active nitrogen forms nitrides with sodium, arsenic, phosphorus, mercury, etc. The precise nature of active nitrogen is not clear. It is probably composed of nitrogen gas in the ground state. The afterglow effect accompanies a preassociation of atoms into molecules as follows:

$$N_2 \xrightarrow{\text{discharge}} 2N \rightarrow \text{preassociated molecules} \rightarrow \text{stable molecules}$$

Active nitrogen forms hydrogen cyanide in the presence of hydrocarbons, presumably by an attack on the carbon atoms. Ammonia or hydrazine is not formed in this reaction, thus showing that hydrogen is not attacked. Multivalent nitrogen atom can exist in different compounds in a variety of oxidation-reduction states ranging from +5 in nitrate to −3 in ammonia. Thus, under suitable conditions nitrogen can combine both with oxygen and hydrogen as follows:

$$N_2 + O_2 = 2NO \quad \text{and} \quad N_2 + 3H_2 = 2NH_3$$

By analogy, both these reactions were invoked in earlier years to explain the biological fixation of nitrogen. Thus, although convincing evidence that ammonia is the key intermediate in nitrogen fixation was presented[3,4], considerable difference of opinion existed earlier about the first step. It was not clear whether this involved the oxidation of nitrogen followed by subsequent reduction to ammonia, or it was the direct reduction of nitrogen to ammonia. Virtanen[5] and his group initially proposed the formation of hydroxylamine as an intermediate (oximes). However, now it is universally agreed that biological nitrogen fixation is a reduction process and the key intermediate, ammonia, is formed by the direct reduction of molecular nitrogen. Thus, essentially the phenomenon is chemically identical with the well-known Haber process, in which nitrogen and hydrogen combine to form ammonia. In the Haber process, molecular nitrogen is activated by using high temperatures (600°) and pressures (about 1000 atm). Combination with hydrogen takes place in the presence of a catalyst consisting of iron turnings or magnetite impregnated with molybdenum, vanadium, alumina and potassium carbonate. In the biological system, the same chemical reaction takes place at biological temperatures and atmospheric pressures. It is the purpose of this article to describe the basic biochemical reactions which accomplish this miracle of nature.

Techniques Used to Measure Nitrogen Fixation

Burris *et al.*[6] developed the use of stable isotope ^{15}N to measure nitrogen fixation. This technique

replaced the micro Kjeldahl method used earlier. Nicholas *et al.*[7] developed the technique of using the radioactive isotope ^{13}N with a very short half-life of 10·05 min. More recently, the reduction of acetylene to ethylene catalysed by nitrogenase[8-11] has been found to be a useful technique, since gas chromatography can be employed for this purpose.

Components of the Nitrogen Fixing System

As in the Haber process chemical model, the three main requirements of biochemical nitrogen fixation obviously are: (1) activation energy, usually in the form of ATP; (2) electron donor system, presumably with a low redox potential almost equal to that of hydrogen electrode (E_0' at pH 7 $=-0{\cdot}44$ V); and (3) catalysts which, of course, in the biochemical reactions, are the enzymes. Need for energy in nitrogen fixation is surprising in view of the favourable thermodynamics of the reduction of nitrogen to ammonia[12]. However, ATP requirement was unequivocally demonstrated in earlier work by the inhibition of nitrogen fixation in the presence of uncouplers of oxidative phosphorylation, such as 2,4-dinitrophenol or by trapping ATP by incorporating glucose and hexokinase[13]. The Arrhenius plot for nitrogenase has a break in the region 18-21°. Activation energies of 120-140 kcal/mole above and 31-54 kcal/mole below the break are indicated. In studies *in vitro*, the requirement of about 20 moles of ATP per mole of nitrogen fixed is no doubt excessive and represents 10-30% of sucrose available under anaerobic conditions. As regards the electron donor system, it is now known that a number of auxiliary proteins, such as ferredoxin and flavodoxin in the reduced form, are the physiological electron donors for the reduction of the nitrogen fixing enzyme complex, nitrogenase. The mechanism of supply of the above components of nitrogen fixing system is apparently different in diverse types of organisms. But in the final analysis, as will be clear from the following description, the biochemistry of nitrogen fixation is surprisingly identical, in anaerobic, photosynthetic and aerobic organisms.

Unaerobic organisms — The discovery of a non-haem iron protein, ferredoxin, by Mortenson *et al.*[14] was an important landmark in understanding the mechanism of nitrogen fixation in *Clostridium pasteurianum*. Carnahan *et al.*[15] found that in cell-free extracts of this organism, nitrogen fixation is dependent on the supply of pyruvate or α-keto-glutarate. On account of very low redox potentials of the reactions of oxidation of these substrates (E_0' at pH 7 $=-0{\cdot}6$ V), they are ideally suited as electron donors for nitrogen fixation. Phosphoroclastic cleavage of pyruvate thus supplies both ATP and reducing power by the formation of acetyl phosphate and reduced ferredoxin (Fd) as follows:

$$CH_3COCOOH + Fd(ox) + H_3PO_4 \xrightarrow{CoA} CHCOOPO_3H_2 + Fd\ (red) + CO_2$$

ATP is formed from acetyl phosphate by transphosphorylation. Reduced ferredoxin (E_0 at pH 7 $=-0{\cdot}42$ V) acts as the reductant of nitrogenase. It was also shown[16] that ferredoxin artificially reduced by potassium borohydride could also donate electrons for nitrogen fixation in the presence of ATP. Hardy *et al.*[17] proposed that the role of ATP is to activate a reduced intermediate, which then reduces nitrogen.

Photosynthetic nitrogen fixation — Tagawa and Arnon[18] reported the presence of ferredoxin in spinach chloroplasts. This was subsequently found to be identical with the photosynthetic pyridine nucleotide reductase[19]. Ferredoxin is the first reductant formed in the light reaction of photosynthesis. Thus, ferredoxin is also involved in photo-reactions of nitrogen fixing photosynthetic organisms, such as algae and green and purple sulphur bacteria. In these organisms, reduced ferredoxin formed in the light reaction provides electrons for the reduction of nitrogen. Thus, it was shown by Bennet and Fuller[20] that ferredoxin functions as an electron carrier in the light-dependent hydrogen evolution and nitrogen fixation in *Chromatium*. Since ATP is also generated by photophosphorylation of the cyclic or non-cyclic type, the light reaction provides both the reducing power and ATP for nitrogen fixation. It is thus clear that the mechanism of nitrogen fixation in *Clostridium* and in photoautotrophs is very similar. Both depend on reduced ferredoxin and ATP. The main difference is in the source of these components. In the former, phosphoroclastic reaction is responsible for the formation of ATP and reduced ferredoxin. In the latter, light reaction replaces the phosphoroclastic reaction. It is also established that the reduction of nitrite and hydroxylamine to ammonia too is dependent on reduced ferredoxin[21,22].

Aerobic organisms — Small particles obtained from *Azotobacter vinelandii* fix nitrogen gas when supplied with an ATP generating system and an artificial electron donor, sodium dithionite[23]. These particles also catalyse the ATP-dependent evolution of hydrogen gas from sodium dithionite[24]. Ferredoxin is not present in aerobic heterotrophic nitrogen fixing bacteria and hence the exact nature of the low redox potential electron donor is not clear. It is obvious that non-heme iron proteins other than ferredoxin are involved. However, the mechanism of reduction of these electron carriers is obscure, since reactions of the type of phosphoroclastic cleavage of pyruvate do not occur in *Azotobacter*. A more detailed discussion about the possible mechanism in *Azotobacter* is given in a subsequent section of this article.

As regards the other component of the nitrogen fixing system in *Azotobacter*, namely ATP, it is clear that this is formed in the respiratory electron transfer chain. Jones and Redfearn[25] have described the nature and intracellular distribution of respiratory enzymes in *Azotobacter*. They found that the respiratory chain components comprising flavin, ubiquinone and cytochromes were almost completely located in intracellular particles (P_1).

Symbiotic bacteria — Ferredoxin is not present in *Rhizobium* also. The formation of bacteroids in the nodules and the presence of leghaemoglobin are characteristic features of the symbiotic system, which distinguish it from the asymbiotic *Azotobacter*. It is of interest that haemoglobin in the nodules is almost completely deoxygenated. Low oxygen

pressure is thought to prevent the diversion of reducing power generated in the bacteroids from the reduction of nitrogen to the reduction of oxygen. This alternate electron transfer mechanism to either oxygen or nitrogen was invoked by Nicholas[26] in the case of *Azotobacter*. It is interesting to note that, while leghaemoglobin is outside the bacteroids, the nitrogenase is located inside[27-29]. This indicates that haemoglobin is not involved in nitrogen fixation. It is not an integral part of the nitrogenase. Nevertheless, it is characteristic that it is found only in nodules fixing nitrogen. It is probable that it has a role in the transport of oxygen or nitrogen. It might also prevent the inhibition of nitrogen fixation by oxygen by acting as an oxygen scavenger.

Appleby[30] has recently compared the characteristics of cytochromes and other pigments in the electron transfer reactions in nitrogen fixing and non-nitrogen fixing *Rhizobium* cells. In common with the cultured (non-nitrogen fixing) bacteria, the bacteroids contain autoxidizable cytochrome *C*(550) and cytochrome *b*. However, it is extremely interesting to note that they completely lack terminal oxidases, cytochrome a-a_3 and *o*, which are present in non-nitrogen fixing cells. They contain autoxidizable CO-reactive pigments cytochrome *C*(552) and the haem protein P-450. As the nitrogen fixing bacteroids are devoid of terminal cytochrome oxidase activity, the author has postulated that *Rhizobium* haemoglobin functions as an oxygen carrier. It is, therefore, likely that this mechanism, which is exclusively present during symbiosis, may be responsible for oxidative phosphorylation coupled to electron transfer even in the absence of cytochrome oxidase. At the same time, the nitrogen fixing system is protected from direct contact with oxygen.

Properties of Nitrogenase

Although different types of mechanisms are involved in the generation of ATP and the reducing power in the systems described above, the enzyme complex responsible for the utilization of these two components appears to be similar, if not identical. In recent years, considerable progress has been made in the direction of elucidating the mechanism of action of this enzyme complex, nitrogenase. This has been possible on account of the purification of nitrogenase from *Clostridium* and *Azotobacter*. The development of a simple assay system using sodium dithionine as an electron donor and an ATP generating system has facilitated considerably the assay of purified preparations[23]. The important properties of nitrogenase are described below.

Nitrogenase is a multienzyme complex which contains non-haem iron, labile sulphur and molybdenum. When provided with a suitable reductant and ATP it catalyses the complexation and subsequent reduction of nitrogen to ammonia. Under identical conditions it also reduces nitrous oxide, nitrides, alkynes, nitriles and isonitriles[31]. All these substrates possess a triple bond and with the exception of alkyenes have one or more non-bonding electrons. Nitrogen molecule is the natural substrate with higher affinity of 0·03-0·1 mM in cell-free extracts and 0·02-0·03 mM in whole cells. Hardy and Jackson[32] conclude that nitrogenase catalyses a step-wise two-electron reduction of different molecules. It exhibits steric and electronic specificities, which reflect the intricate topography and complexing potential of the active site. Nitrogenase is also involved in ATP-dependent hydrogen evolution and reductant-dependent ATPase. Purified nitrogenase yields two components which are called (1) molybdoferredoxin and (2) azoferredoxin in *Clostridium* or nitrogenase I and nitrogenase II in *Azotobacter*. It is a versatile redox catalyst which combines ATP hydrolysis and electron transfer in an integrated manner.

Mechanism of Action of Nitrogenase

Hardy and his coworkers have proposed the following mechanism of nitrogenase action in the light of the available evidence[8,12,33,34]. This enzyme complex consists of two distinct sites: (1) electron activation site (x), and (2) substrate binding site (y). Electrons from a donor reduce x. Reduced x then reacts with ATP in the presence of Mg^{2+} to form activated x (red)*. This activated component possesses extremely negative redox potential and is possibly more potent than any known biological reductant. Molybdenum may be involved in this step. In the absence of substrates, the electrons neutralize protons and are discharged as hydrogen gas. This is the basis of ATP-dependent hydrogenase as well as of reductant-dependent ATPase, since in this reaction ATP is hydrolysed to ADP and inorganic phosphate. These two reactions are characteristic components of the nitrogenase system. The other type of hydrogenase, which is ATP non-dependent, is not exactly typical of nitrogen fixing organisms, since it is present even in many bacteria which do not fix nitrogen. In the presence of deuterium the purified nitrogenase[35] formed HD with an endogenous electron donor. HD formation had the same requirements as nitrogen fixation and was inhibited by carbon monoxide. Site y is involved in this reaction, since HD formation is unrelated to hydrogenase. It parallels nitrogenase and the requirement of nitrogen suggests complexed intermediates of nitrogen reduction. The formation of HD from deuterium suggests the involvement of diimide and hydrazine metal complexes as intermediates. The substrate binding site y is thus supposed to be very versatile, as it can combine with a variety of molecules mentioned above. However, recently with *Azotobacter chroococcum*[36], it has been indicated that there may be some difference in the specificity for nitrogen and some other artificial substrate. Thus, it was found that the ratio of the two fractions of nitrogenase giving maximum rate of nitrogen fixation, acetylene reduction and ATP-dependent hydrogen evolution was 2:1, but for cyanide and isocyanide reduction it was 6:1. Finally, y-substrate complex is reduced by activated x reduced*. Site y is distinguished by carbon monoxide and hydrogen sensitivity. Ferrous iron is involved at y site.

Intermediates in the reduction of nitrogen to ammonia are not identified. The absence of

isotopic exchange reaction $^{15}N \equiv {}^{15}N \longrightarrow {}^{14}N \equiv {}^{15}N$ indicates that initial cleavage of nitrogen molecule to atoms may not be involved, as it presumably occurs in the chemical reduction[37]. It is likely that the intermediates diimide and hydrazine are tightly bound to the nitrogenase, since the first detectable free product is ammonia.

Physiological Reductants of Nitrogen

As stated earlier, reduced ferredoxin produced in the phosphoroclastic reaction or in the photosynthetic light reaction is the physiological electron donor of nitrogenase in *Clostridium* and photoautotrophs. Another protein flavodoxin was isolated by Knight *et al.*[38] from *Clostridium* grown in iron-deficient cultures. This is a low molecular weight flavoprotein and it can replace ferredoxin *in vivo* as the electron donor in cells grown on low levels of iron. Thus, ferredoxin (E_0' at *p*H 7 $=-0{\cdot}42$ V) and flavodoxin (E_0' at *p*H 7 $=-0{\cdot}28$ V) are the only known natural electron carriers which reduce nitrogenase[39,40]. Numerous other donors such as NAD(P)H (E_0' at *p*H 7 $=-0{\cdot}32$ V) are ineffective[17,23]. It has not yet been possible to explain the basis of this specificity.

In *Azotobacter* and other aerobes, ferredoxin is absent. The artificial reducing agent, sodium dithionite, was used to study nitrogen fixation in cell-free preparations[23]. The presence of a non-haem iron protein in nitrogen fixing particles of *Azotobacter vinelandii* was reported by Nicholas *et al.*[41], by using the electron spin resonance (ESR) technique. This protein was markedly reduced by dithionite. Shetna *et al.*[42,43] purified two non-haem iron proteins I and II from the same organism. They reported that ESR and optical spectra showed striking similarity with plant type ferredoxin. Both these proteins contained about 2 g atoms of iron and labile sulphur per mole. Molecular weights ranged from 21000 to 24000. The occurrence of these proteins of the labile sulphur iron type shows that the question whether *Azotobacter* contains ferredoxin or not is one of nomenclature only. However, the physiological function of these proteins is still obscure. It is also not known how they themselves are reduced inside the cell. In nitrogen fixing subcellular particles of *Azotobacter vinelandii*, Naik and Nicholas[44,45] reported the presence of an enzyme, which catalysed the reduction of this non-haem iron protein with NADH as the electron donor. The reduction of the protein was detected by coupling it to a nitrate reductase from *Micrococcus denitrificans*. The same enzyme (NADH-viologen reductase) also catalysed the reduction of benzyl and methyl viologen dyes with NADH as the donor. Redox potentials of these dyes (BV, E_0' at *p*H 7 $=-0{\cdot}36$ V and MV, E_0' at *p*H 7$=-0{\cdot}42$ V) are more negative than that of NADH. The redox potential of methyl viologen is almost equal to that of ferredoxin or hydrogen electrode. However, this apparent reversal of electron transfer could not be shown to be ATP dependent. The reaction was sensitive to 2,4-dinitrophenol, but this was due to the reduction of the uncoupler to 2-amino-4-nitrophenol. The enzyme was specific for NADH as the donor. NADPH and dihydrolipoate could act as indirect donors via a transhydrogenase reaction to reduce NAD. Nagai *et al.*[46] have further purified this enzyme, which was shown to contain thiol groups and at least 3 atoms of Fe per mole protein. The average moleculer weight of the purified preparation was 68000.

Effect of Combined Nitrogen Sources

Nitrogenase is inductively synthesized in cells fixing nitrogen. It is absent in cells grown on ammonia or urea. Profound metabolic changes occur in *Azotobacter* grown on combined nitrogen compounds. Knowles and Redfearn[47] reported that alternate pathways of electron transfer are functional in respiratory particles from *Azotobacter vinelandii*, depending on the type of nitrogen source. In cells actively fixing nitrogen, the major pathway involves cytochromes b_1 and a_2, the main function of which appears to be to synthesize high energy intermediates required for nitrogen fixation. These are not required in cells grown on combined nitrogen, sufficient energy being obtained via the other pathway involving cytochromes C_4+C_5 and *a*. NADH oxidase activity was more than 100% higher in cells fixing nitrogen as compared to those grown on urea. Cells grown on ammonia did not possess particles resembling nitrogen fixing particles. Lodha *et al.*[48,49] also found that cells grown on ammonia were more permeable, due to structural changes in the cell membrane. Maximum NADH oxidase and NADH-benzyl viologen reductase activities were found in extracts of nitrogen fixing cells. Glucose-6-phosphate dehydrogenase activity was, however, high in cells grown on ammonia. Mehta *et al.*[50,51] showed that urease is inductively synthesized in cells grown on urea or thiourea. Incorporation of $^{32}PO_4$ and $^{35}SO_4$ was faster in cells grown on ammonia.

Intracellular Localization of Nitrogen Fixation

In *Azotobacter vinelandii* the nitrogen fixing activity is located in small subcellular particles (P_6) obtained by differential centrifugation of cell-free extracts between 100000 and 144000 *g* for 6 hr[23]. Components of the respiratory chain are located in slightly larger particles (P_1) obtained by differential centrifugation between 35000 and 105000 *g* for 90 min[25]. Naik and Nicholas compared the activities of enzymes involved in respiration and in hydrogen transfer in these two particles (Naik, M. S. & Nicholas, D. J. D., unpublished results). The nitrogen fixing small particle (P_6) had higher NADH oxidase activity, which was partially insensitive to cyanide. ATP-dependent hydrogenase, NADH dehydrogenase and NADH-viologen reductase were mainly concentrated in P_6. An active ATPase as well as NADPH and dihydrolipoic transhydrogenases were also found in P_6. Oxidation of a variety of substrates was faster in P_1 than in P_6. It thus appears that the respiratory particle P_1 and the nitrogen fixing particle P_6 have distinct functions in the electron transfer reactions in *Azotobacter vinelandii*. In the former, energy yielding oxidation reactions take place through the cytochrome chain, while in the latter, enzymes involved

in hydrogen evolution and transfer and nitrogen fixation are concentrated, leading ultimately to the ATP-dependent reduction of molecular nitrogen.

Microelement Requirement for Nitrogen Fixation

Early observations about the requirement of iron and molybdenum have been amply confirmed by the fact that these elements are integral constituents of purified nitrogenase. Experiments with the phosphate exchange reaction suggest[52] that ATP may react with the molybdenum-non-haem iron protein complex to form an energy-rich intermediate, (Mo-Fe-protein)~ADP. Iron is also probably involved at the active site *y* in nitrogenase, where nitrogen is complexed.

It is known that cobalt is essential for nitrogen fixation[53,54] in bacteria, blue-green algae and legumes. This metal is associated with cobamide coenzymes, which function in their metabolism[55]. Cobamide coenzymes and methylmalonyl CoA isomerase have been found in root nodules. The present evidence suggests that cobalt is not directly involved in the nitrogen fixing reactions as such, but functions indirectly in the synthesis of the enzymes required for this process. Wilson and Nicholas[56] have indicated a cobalt requirement even for non-nodulated legumes and for wheat. They suggest that cobalt compounds other than cobamide materials are involved.

Requirement for sodium for nitrogen fixation in blue-green algae, *Anabaena cylindrica*, was demonstrated by Brownell and Nicholas[57]. They found that nitrogen fixation was considerably retarded in sodium-deficient cells. As in the case of cobalt, the effect of sodium may be indirect.

Summary

Nitrogenase is a multienzyme complex, which contains molybdenum, non-haem iron and labile sulphur. It consists of two components. The first is specifically reduced by suitable electron donors. The reduced component when activated by ATP is probably the most potent reducing agent known in biological systems. It is probable that a (Mo-Fe-protein)~ADP complex is formed in this reaction. The second site binds the substrate which is then reduced. Molecular nitrogen is the natural substrate, but this site is very versatile. A number of other substrates having a triple bond, such as acetylene, nitrides, cyanides, etc., are also complexed. In the absence of a substrate, the reducing power is discharged as hydrogen gas in the ATP-dependent hydrogenase reaction. Ammonia is the first detectable product of reduction of nitrogen, but the probable intermediates, diimide and hydrazine, may be tightly bound to the nitrogenase.

In *Clostridium*, the phosphoroclastic cleavage of pyruvate provides both reduced ferredoxin and ATP, while in photoautotrophs the light reaction supplies these components for nitrogen fixation. The physiological electron donor in aerobic bacteria is not known with certainty, but non-haem iron proteins are implicated. The reduction of a non-haem iron protein with NADH as the donor is possible in *Azotobacter*. In this organism, the ATP-dependent nitrogen fixing activity is located in subcellular particles, P_6, in which the enzymes involved in hydrogen transfer and evolution are also concentrated. The respiratory activity yielding ATP is located in bigger particles, P_1. Nitrogenase is inductively synthesized only in the nitrogen fixing organisms. Combined nitrogen compounds change profoundly the metabolic activities of these organisms.

In addition to iron and molybdenum, which are integral constituents of nitrogenase, cobalt and perhaps sodium may also be involved indirectly in nitrogen fixing organisms.

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Seminar on Elementary Particles & Nuclear Physics

The Institute of Mathematical Sciences, Madras, is holding a seminar on 'Elementary particles and nuclear physics' at Bangalore or Ootacamund during the third week of January 1970, with the support of the Department of Atomic Energy, Government of India. There will be invited lectures by eminent mathematicians, including Porf. Hans Bethe, N.L., Cornell University; Prof. N. P. Klepikov, Unesco Coordinator; Dr J. E. Drummond, Boeing Scientific Research Laboratory, Seattle; Prof. A. O. Morris, University of Wales, Aberystwyth; Prof. M. Gourdin, Universite de Paris, Orsay; Prof. Alladi Ramakrishnan, Director, Matscience, Madras; Dr Ramesh Chand, Matscience, Madras; Dr V. Devanathan, University of Madras; Prof. B. S. Madhava Rao, Indian Institute of Science, Bangalore; and Dr Dave Pandres (Jr), Douglas Advanced Research Laboratories, California. There will also be individual seminars by the participants on the particular subjects of their research.

Biogenesis of Mitochondria

J. JAYARAMAN

Department of Biochemistry, Indian Institute of Science, Bangalore 12

CURRENTLY we are in an exciting phase of research in the field of biochemistry; we have learnt, for example, how to make nucleic acids replicate under controlled conditions and give rise to biologically meaningful molecules[1,2]; we even know how to synthesize a gene in a test tube[3]. The next problem is understanding how an entire organelle of a cell is made. The mitochondrion serves as the power house of the cell, i.e. it converts the energy derived by the oxidation of various substrates into a more utilizable form. This conversion is brought about by several enzymes, coenzymes, etc., which form part of the mitochondrion. Some of the basic physical, chemical and biochemical properties of the mitochondria are given in Tables 1 and 2. These multifarious components have to be synthesized, brought together and kept in proper orientation structurally and spatially. The magnitude of the problem[6] is thus obvious.

An attempt is made in this review to bring together the different lines of approach towards this central problem. Instead of merely cataloguing all the papers published in this field, attention is focused on the experiments which contribute to a significant understanding of the problem. Drawing the analogy of a production plant, the subject is dealt with under four subheads: (1) Is there any machinery available in the mitochondria to make the needed component? (2) How far is this machinery utilized by the organelle? (3) What is the nature of the assembly line? (4) How are all these processes controlled?

TABLE 1 — SOME PHYSICAL AND CHEMICAL DATA CONCERNING MITOCHONDRIA*

Physical data†	
Shape	Usually ellipsoidal
Size	$3{\cdot}3 \times 1{\cdot}0\ \mu$
Average number per cell	1000
Surface area of cristae per mitochondrion	$16\ \mu^2$
Number of units of electron transfer per mitochondrion ...	64000
Chemical data‡	
Average dry weight, g ...	$1{\cdot}1 \times 10^{-12}$
Lipid, %	37
Protein, %	63
DNA (μg/mg protein) ...	3·1
RNA (μg/mg protein) ...	8·0
Cytochromes (μmoles/g protein) ...	3·18
Flavins (μmoles/g protein) ...	0·45
Ubiquinone (μmoles/g protein) ...	3·80
Copper (g atoms/g protein) ...	1·46
Iron (g atoms/g protein) ...	8·85

*Data from ref. 4 and 5.
†Rat liver.
‡Beef heart.

TABLE 2 — SOME IMPORTANT ENZYMES OF MITOCHONDRIA*

Complex I:	NADH-ubiquinone oxidoreductase
Complex II:	Succinate-ubiquinone oxidoreductase
Complex III:	Reduced ubiquinone-cytochrome *c* oxidoreductase
Complex IV:	Cytochrome oxidase
Energy transferring factors	
Tightly bound auxiliary enzymes:	D-β-hydroxy butyrate dehydrogenase
	α-Glycerophosphate dehydrogenase
	Fatty acyl CoA dehydrogenase
	Pyridine nucleotide transhydrogenase
Other proteins:	Structural protein
	Contractile protein

*Data from ref. 4 and 5.

Biosyntheses by Mitochondria

Protein synthesis — It has been known for a long time that when an intact animal or plant is given a dose of radioactive amino acid, the mitochondrial fraction shows a specific radioactivity, almost equalling that of the microsomal fraction[7,8]. Roodyn[9] showed, for the first time, that isolated mitochondria had the ability to incorporate amino acids into acid-insoluble products. This has been confirmed in several laboratories and several reviews have been published on the subject[10,11]. A comparison of some of the main properties of the mitochondrial protein-synthesizing system with those of the microsomal and bacterial systems is made in Table 3.

Identification of the product of amino acid incorporation by isolated mitochondria — The proteins of the mitochondria can be roughly grouped depending on the ease with which they are extracted. This point is illustrated in Scheme 1 (ref. 12). It was recognized early that when the mitochondria were labelled with radioactive amino acids *in vivo* and then fractionated, the insoluble proteins had more radioactivity associated with them than the soluble fractions[13]. This was much more striking in experiments with isolated mitochondria. As much as 65% of the total radioactivity incorporated by the isolated mitochondria was associated with the insoluble residue, while the soluble proteins were not labelled[14,15]. In fact, the maximum amount of radioactivity was found in the fraction containing the structural protein and this led to the general assumption that this protein is synthesized by the mitochondria. Haldar *et al.*[16], for example, isolated the structural protein fraction from the mitochondria, labelled *in vitro*, by the method of Criddle *et al.*[17] and after rigorous purification found this fraction to be highly radioactive. Analysis of this fraction on acrylamide gel electrophoresis revealed

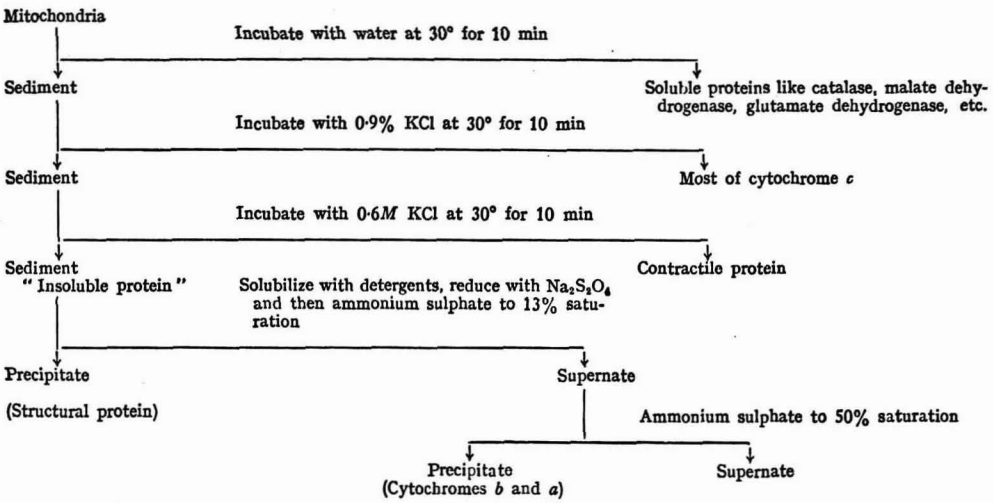
several components, many of which were radioactive, of particular interest being a protein band which corresponded to the factor 4, a component of the energy coupling system. Neupert *et al.*[18] showed that on incubating isolated mitochondria with radioactive amino acids, the outer membrane fraction had less than 5% of the specific activity of the inner membrane, while the soluble fraction was devoid of any radioactivity. Similar conclusions were arrived at by Beattie and coworkers[12,19-21], who carried out an exhaustive investigation of the kinetics of labelling of the various fractions, under *in vivo* conditions, with tissue slices and with isolated mitochondria.

Linnane and Stewart[22] have successfully exploited the fact that certain antibiotics like chloramphenicol, tetracyclines and macrolides inhibit protein synthesis carried out by mitochondria of the yeast cell, but not the protein synthesis of the cytoplasm. They grew the yeast, *Saccharomyces cerevisiae*, in the presence of the drugs and then studied the morphology and characteristics of the mitochondria in these cells[23]. Under the electron microscope, the cells grown in the presence of the drug had profiles resembling those of the normal mitochondria, but they lacked the internal cristate structure. The absorption spectra indicated that these cells lacked cytochromes $a+a_3$ and b. These results led them to suggest that the proteins of the inner membrane and the tightly bound cytochromes a and b are synthesized by the mitochondria themselves. Cycloheximide, a powerful inhibitor of protein synthesis in the cytoplasm of the yeast and mammalian tissues, has been shown not to affect mitochondrial protein synthesis[24-26]. Mahler and coworkers[27] studied the effects of both cycloheximide (actidione) and chloramphenicol added at different time intervals to a growing culture of yeast, *S. cerevisiae*, on the enzyme levels of mitochondria and concluded that the synthesis of at least some of the soluble proteins of mitochondria in the cytoplasm was regulated by entities in mitochondria, the origin of which is sensitive to chloramphenicol. But these soluble proteins require integration into particles before their activity becomes manifest. Again this integration process was sensitive to chloramphenicol. The complexity of the situation regarding the extent and nature of participation of the two systems,

TABLE 3 — COMPARISON OF THE PROTEIN-SYNTHESIZING SYSTEMS OF MITOCHONDRIA WITH THOSE OF MICROSOMES AND BACTERIA

	Mitochondrial	Microsomal	Bacterial
Rate of amino acid incorporation (μmoles/mg protein/hr)	12-72	210-1200	about 1000
Requirement for oxygen	++	(+)?	(+)?
Involvement of *p*H 5 enzymes	−	++	++
External ATP	−	++	++
External AMP	(+)?	−	−
Succinate	(+)?	−	−
Inhibition by			
Actinomycin D	(+)?	−	−
Acriflavin	+	−	−
Chloramphenicol	+	−	+
Puromycin	+	++	++
Cycloheximide	−	++	−
Ribonuclease	−	+	+
Dinitrophenol	++	−	−
Extent of incorporation	Linear up to 3 hr	Linear up to 30 min	
Contamination from other systems	Possibility of bacterial contamination	−	−
Size of ribosomes	70S ?	80S	70S
Need for formylmethionyl tRNA as initiator of protein synthesis*	++	Not demonstrated	++

*Very recently shown by Smith and Marcker[171].



Scheme 1 — Scheme for fractionation of mitochondria (adapted from Beattie[12])

mitochondrial and microsomal, in the generation of mitochondrial proteins needs no stressing.

Corroborative studies at the genetic level, particularly with yeast and *Neurospora crassa*, are discussed later.

Mitochondrial ribosomes — Wintersberger[28,29] isolated RNA from purified yeast mitochondria and studied their sedimentation characteristics and found three species with sedimentation values of 23*S*, 16*S* and 4*S*. Ribosome-like structures were detected in mitochondria and, on treatment with ribonuclease, these structures remained intact, while the cytoplasmic ribosomes disappeared[16,30]. Leon and Mahler[31] also purified RNA from isolated yeast mitochondria and studied its properties. Three components, with *S* values of 23, 16 and 4, were found, besides two minor components of 11*S* and 30*S*. The values of 23*S* and 16*S* for the two ribosomal RNA components (presumably) were lower than those for their cytoplasmic counterparts, isolated and analysed under comparable conditions. Ribosomes have also been purified from isolated mitochondria and their properties studied. O'Brien and Kalf[32,33] isolated ribosomes from rat liver mitochondria and found them to have a sedimentation value of 55*S*. Also, the ribosomal preparation consisted of spherical particles 145 Å in diameter, agreeing well with the measurements made from the electron micrographs of the whole cells. Under the same conditions, the ribosomes isolated from the cytoplasm were 190 Å in size and had a sedimentation value of 78*S*. Incubation of mitochondria with radioactive amino acids for 5 min showed the 55*S* particles to have a specific activity almost 50 times more than that of the other proteins. Elaev[34], on the other hand, had reported that rat heart mitochondria had three classes of ribosomes sedimenting at 83*S*, 63*S* and 45*S*. Rabinowitz *et al.*[35] as well as Kuntzel and Noll[36] found the ribosomes of mitochondria to have a sedimentation value of 70*S*. Rifkin and Luck[37] reported the sedimentation value of the ribosomes they isolated from the mitochondria of *N. crassa* to be 70*S*, partially reversing their previous claim that it was 80*S* (ref. 38). These ribosomes, in the presence of 2 mM of magnesium, dissociated into two 25*S* and one 15*S* subunits. Though the presence of ribosomes in mitochondria, termed 'mitosomes'[39], is fairly well established, there still exists some controversy regarding their size and very little is known about their function.

Presence of other components of the protein synthetic machinery — As mentioned earlier, the experiments of Wintersberger[28] with isolated yeast mitochondria showed the presence of a species of RNA sedimenting at 4*S*. Further studies with this RNA showed that they in fact accepted amino acids, showing that they are probably transfer RNAs. Barnett and Brown[40] have elegantly demonstrated that *N. crassa* mitochondria had transfer RNAs for as many as 18 amino acids. They also noted that at least the tRNAs of phenylalanine, aspartic acid and leucine were specific to the mitochondrial enzymes, i.e. they were not charged by the cytoplasmic amino acyl tRNA synthetases[41]. A similar specificity for the leucyl- and tyrosyl- tRNAs of the rat liver mitochondria was demonstrated by Buch and Nass[42]. These two groups of workers have also demonstrated the presence of the tRNA synthetases in isolated mitochondria and their absolute specificity for the tRNAs of the mitochondria.

Is there a messenger RNA in the mitochondria? — In experiments using pulse-labelling techniques, Wintersberger[43] noticed the presence of a species of RNA in yeast mitochondria, which had properties similar to those of messenger RNA. Comorosan *et al.*[44] isolated, from mouse liver mitochondria, a rapidly labelled RNA of molecular weight $1{\cdot}5\times10^5$ and claimed it had the properties of messenger RNA. Attardi and Attardi[45] made the interesting observation that the cytoplasm of HeLa cells had a membrane-bound RNA, which is distinct from the other known species of RNA in the cell. This was found to be synthesized on the mitochondrial DNA (MDNA). But intriguingly, part of this newly synthesized RNA seemed to be exported to the endoplasmic reticulum. The results obtained by Woodward[46] with *N. crassa* appear to provide confirmation of these results. In fact, Woodward thinks that the MDNA codes for the 'structural proteins of the cellular membranes'. "Transcription of MDNA produces a messenger RNA corresponding to structural protein, some of which is retained within the mitochondria for the synthesis of mitochondrial membranes. The remainder of the mRNA is transported to the site of protein synthesis in the cytoplasm, where the structural proteins of the other cellular membranes are synthesized." It is worth recapitulating the work of Mahler and coworkers[27] that the synthesis of some enzymes of cytoplasm is regulated by entities of mitochondrial origin, the biogenesis of which is sensitive to chloramphenicol.

Suyama and Eyer[47] studied the synthesis of RNA by isolated mitochondria of *Tetrahymena pyriformis*. They were able to show that the radioactive product sedimented in the range 14-18*S* with no radioactivity in the 4-5*S* region. The radioactive RNA hybridized with mitochondrial DNA; this hybridization was not affected by the addition of large amounts of ribosomal RNA, suggesting the probable specificity. Fukuhara[48] detected in yeast cells an RNA species different from the ribosomal RNA and the tRNAs. This RNA apparently was membrane-bound, presumably with the mitochondrial membrane and hybridized with the MDNA. The aerobic cells had a higher content of this particular RNA than the anaerobic cells. After ruling out the possibility that the hybridization with MDNA was fortuitous, Fukuhara contended that a new species of RNA, coded for by MDNA, is produced in high quantities in the yeast cells during aerobic adaptation (The formation of mitochondria in the yeast during adaptation to aerobic conditions is discussed elsewhere in this review). More recently, Leon and Mahler[31] studied the incorporation of radioactive uracil into mitochondrial RNAs by yeast cells, which had been released from glucose repression, a condition which leads to an increased mitochondrial content of the cell. Uracil was incorporated both into mitochondrial and cytoplasmic RNA, but the specific activity

of mitochondrial RNA was three times higher. The incorporation into the 11S component of the RNA was more rapid than into any other component; also the rate of incorporation was more rapid in the 'early' derepression phase than in the 'late' derepression phase. These results were interpreted to mean that the rapidly labelled mitochondrial DNA is different from the cytoplasmic RNAs. An inference as to whether this RNA is the mRNA could not be drawn, since the radioactivity was not chased by unlabelled uracil. Similar results have been obtained with isolated yeast mitochondria[49].

RNA polymerase of mitochondria — The presence of an enzyme system able to incorporate radioactive ATP into an RNA-like product was shown in rat liver mitochondria[50], lamb heart mitochondria[51] and *N. crassa* mitochondria[52]. In all these cases, it was shown that the activity was inhibited by the addition of actinomycin D, showing the involvement of DNA in the reaction.

Mitochondrial DNA — Although a number of earlier workers have reported on the presence of fibrils in mitochondria, it was Nass and Nass[53] who first demonstrated that these Feulgen-positive fibrils were sensitive to DNAse and, thus, are fibrils of DNA. These fibrils have been subsequently shown to be present in the mitochondrial matrix of a number of organisms[54]. That the mitochondria have DNA as an intrinsic component is fairly well established by isolation from a number of tissues and the different sedimentation behaviour of the nuclear DNA and the mitochondrial DNA in a cesium chloride density gradient[55] (Table 4). Also, the initial observation of Vinograd and coworkers[56,57] that the mitochondrial DNA was circular, consisting of supercoiled cyclic filaments, has been confirmed in several laboratories[58,59].

DNA polymerase of mitochondria — The ability of isolated yeast mitochondria to incorporate tritiated deoxy ATP into an acid-insoluble product, presumed to be DNA, has been demonstrated[43]. Haldar *et al.*[16] found that isolated mitochondria of rat liver and tumour cells incorporated tritiated thymidine into DNA. They also showed that the mitochondria isolated from livers of rats receiving a dose of thyroxine had a higher rate of incorporation, suggesting that the observed incorporation was physiological. Two groups of workers have recently reported on the purification of DNA polymerase enzyme from isolated mitochondria. Kalf and Chik[60] working with rat liver mitochondria purified the enzyme 22-fold, and showed that the product was a double-stranded replica of the template MDNA. Meyer and Simpson[61] purified the enzyme from both the nuclear and mitochondrial fractions of rat liver and demonstrated that the activities could be separated from one another on DEAE columns. They have also reported on the differences in the two systems regarding their requirement for magnesium and salt concentration. Preliminary results of these workers[62] also indicate that the enzyme acts more as a replicating enzyme rather than as a repair enzyme.

TABLE 4 — BUOYANT DENSITIES OF MAJOR (NUCLEAR) AND SATELLITE (MITOCHONDRIAL) DNAs*

Source	Nuclear	Mitochondrial
Yeast	1·698	1·684
Neurospora	1·713	1·701
Leishmania	1·721	1·699
Tetrahymena	1·685	1·671
Rat liver	1·703	1·701
Guinea-pig	1·700	1·700
Sheep heart	1·704	1·714
Chicken liver	1·701	1·707
Chicken embryo liver	1·698	1·707

*Data adapted from Granick and Gibor[55].

Biosynthesis of lipids — Although a completely systematic survey of all the enzymes required in the biosynthesis of all the complex lipids of mitochondria has not yet been reported, Wilgram and Kennedy[63] have found that some of these enzymes are localized outside the mitochondria. Also, it has been well established that the sterols are synthesized outside the mitochondria[64] and similar may be the case with ubiquinone[65]. The mitochondria, however, are capable of quite high rates of fatty acid synthesis and according to Hulsmann and coworkers[66], they may well be the major site of cellular fatty acid synthesis. Isolated mitochondria are also capable of carrying out the enzymatic assembly of at least portions of some of the phospholipids[4].

The Self-sufficiency or Otherwise of Mitochondria in Relation to Their Genesis

Origin of mitochondrial components — It has been discussed earlier that the soluble proteins of the mitochondria are not labelled when the isolated mitochondria are incubated with radioactive amino acids. However, only cytochrome *c* has been conclusively shown to be synthesized in the cytoplasm and transferred to the mitochondria[14,15,67]. This may be because of the lack of proper techniques to identify the enzyme proteins which may be present in inactive form. However, several circumstantial evidences listed below (and discussed elsewhere) suggest that the insoluble or tightly bound proteins of the inner and outer membranes of the mitochondria are synthesized by the organelle, while the soluble proteins and perhaps most of the lipids are synthesized in the cytoplasm and transported. (a) The physical dimensions of the MDNA speak against its capacity to code for all the proteins of the mitochondria. (b) Genetic studies with mutants of yeast and *N. crassa* have definitely shown that the synthesis of cytochrome *c* and malate dehydrogenase is controlled by the nuclear gene. (c) Amino acid incorporation studies reveal that the isolated mitochondria incorporate the amino acid exclusively in the insoluble fraction, while under *in vivo* conditions all the proteins are equally labelled. (d) Antibiotics like chloramphenicol and erythromycin, which selectively inhibit the mitochondrial protein synthesis without affecting the cytoplasmic protein synthesis, affect the formation of cristae in yeast mitochondria. Also, yeast cells grown in the presence of these drugs were deficient in cytochromes *a* and *b*. (e) Synthesis of lipids of mitochondria has been shown to be

unaffected under conditions where mitochondrial formation is inhibited, as in the presence of chloramphenicol and cycloheximide. (f) Direct transfer of proteins and lipids from microsomes to mitochondria has been demonstrated, using a system comprising radioactively labelled microsomes and unlabelled mitochondria.

The data currently available do not permit one even to speculate concerning the origin of the mitochondrial nucleic acids. The situation with regard to the lipids is somewhat less complicated. We had already seen that the mitochondria do not possess enzymes for the synthesis of complex phospholipids, sterols and probably ubiquinone. The significance of the ability of mitochondria to synthesize fatty acids and to add building blocks into lipids is at the moment far from clear.

Turnover of mitochondrial DNA — Several workers have shown[55] by autoradiographic techniques that tritiated thymidine is incorporated into MDNA. The relationship between the turnover of nuclear DNA and mitochondrial DNA has also been investigated by a number of workers. Schneider and Kuff[68] showed that the incorporation of thymidine into MDNA of rat liver cells was at least 10 times faster than into nuclear DNA, suggesting that the former turns over more frequently. Reich and Luck[69] noted that the replication of MDNA in *N. crassa* was independent of nuclear DNA. A similar situation was shown to exist in *Tetrahymena*[70,71] also. Smith *et al.*[72] carried out an extensive investigation on the content of both nuclear DNA and MDNA in a synchronously growing culture of *Saccharomyces lactis* at various time intervals. The results indicated the synthesis of the two DNAs to be out of phase, the nuclear DNA preceding the mitochondrial DNA. Neubert *et al.*[73] reported similar results with rat liver. However, Guttes *et al.*[74] found that in the slime mold, *Physarum polycephalum*, tritiated thymidine was incorporated into MDNA throughout the cell cycle, without any evidence of periodicity.

The experiments with *Tetrahymena*[70] indicated that the tritiated MDNA was carried over several generations intact; this physical continuity of MDNA has been confirmed by Stone and Miller[71]. Reich and Luck[69] carried out a series of experiments with *N. crassa*, which provided strong evidence that the MDNA replicates in the same semiconservative fashion as the nuclear DNA. They grew the organisms in a medium containing ^{15}N and then transferred them to a medium containing ^{14}N. The MDNA component was isolated at several stages, at the end of 1, 2 and 3 cell doublings, and analysed by analytical ultracentrifuge. In the first two generations, they found only ^{15}N duplex strands, apparently having been transmitted intact from the mother cell to the daughter cell. However, in the third generation cells, they were able to detect hybrid strands. A similarity in the mode of replication of the MDNA and the nuclear DNA is also supported by the observation by Kirschner *et al.*[75], of forked circular molecules of mitochondrial DNA in electron micrographs.

Can mitochondrial DNA code for all mitochondrial proteins? — Though it is not possible to make a categorical statement based on experimental evidences, theoretical considerations indicate that the MDNA can code for about half of the total mitochondrial proteins. The DNA content of the individual mitochondrion from several sources (Table 5) lies in the same range as some of the viruses and is only 1/50th of the content in an *Escherichia coli* cell. It has been calculated[76,77] that each yeast mitochondrion has 6×10^{-17} *g* of DNA, which would correspond to $2{\cdot}7\times10^{4}$ nucleotide pairs, capable of coding for 9000 amino acids forming proteins of a total molecular weight of about 900000. Sinclair and Stevens[78] determined the average length of DNA fibrils isolated from mouse liver mitochondria and found them to be 4·96 μ. This length of DNA would then contain 14600 base pairs, which will code for 5000 amino acids or proteins with a total molecular weight of about 584000. Comparing these values with the minimal molecular weight of 1·5 million, quoted by Lehninger[4], it is obvious that the MDNA can code only for part of the mitochondrial proteins.

TABLE 5 — DNA CONTENT OF SOME MITOCHONDRIA*

Origin	DNA per mitochondrion (10^{-16} *g*)
Yeast	1
Tetrahymena	3·7
Neurospora	0·2-1·8
Beef heart	0·5
Phaseolus	5
Pox virus†	2
T-even bacteriophage†	2
Polyoma virus†	0·05
Escherichia coli†	50
Yeast nucleus (haploid)†	250
Neurospora nucleus (haploid)†	900

*Data adapted from Granick and Gibor[55].
†These values for mitochondria per nucleoid are given for comparative purposes.

Expression of mitochondrial DNA — The experiments discussed up till now would indicate that mitochondria possess the heredity principle, DNA, and all the machinery needed to decode the messages and convey them into meaningful functional proteins. But the basic question, how far these are operative *in vivo*, remains largely unanswered. Indirect evidences are forthcoming, particularly from studies on yeast, that the MDNA does function. It is increasingly recognized that individual characters can be inherited not solely from the nuclear chromosomes but also through the cytoplasm[79,80]. This phenomenon is not localized to one organism, but is fairly widespread in bacteria, molds, protozoa, fungi, insects, plants and even animals. Typical examples of such type of inheritance, which has relevance to our discussion, is the occurrence of respiratory deficiency in *S. cerevisiae* and *N. crassa*.

(a) Petite mutation in yeast: Ephrussi and Slonimski[81] observed in their yeast cultures some slow-growing clones, which they designated 'petites'. Further study indicated a lack of respiratory capacity in these clones and a detailed

analysis revealed the absence of cytochromes $a+a_1$ and *b* in these cells. The conversion of normal cells to the petites was enhanced almost 1000-fold by treatment with a small concentration of acriflavine (1 in 300000). Mating haploids of these cells with one another resulted in zygotes, which by tetrad analysis were shown to be petites. However, mating with normal cells always resulted in normal colonies. A number of independently obtained cultures of petites have been screened for revertants or recombinants, but without result. Based on these results, Slonimski[82] suggested that the petite strains lacked a factor in their cytoplasm, which is necessary for the maintenance of respiratory capacity. This factor was designated as the 'rho' (ρ) factor. It is generally assumed that this rho factor is the same as MDNA or closely related to it. Support for this view comes from the following observations: (1) The action spectrum of ultraviolet induction of the petites shows the maximum efficiency at 265 mμ, the same as for nucleic acids. (2) The mutagenic action of dyes like acriflavine in producing the petites is correlatable with the well-documented capacity of these dyes to bind with DNA and inhibit the RNA polymerase activity. In fact, it has been demonstrated that acriflavine binds much more efficiently with mitochondrial DNA of the yeast than the nuclear DNA[77].

A certain amount of scepticism prevailed regarding the original suggestion that it is the absence of the rho factor that was responsible for the respiratory deficiency of the petite mutants. Recently, based on elaborate studies by several groups of workers on the presence and properties of MDNA isolated from these mutants, Slonimski and his group[83] have suggested that this thesis may not be valid any more. Instead, as mounting evidences described below show, it is the alteration in the mitochondrial DNA that confers respiratory deficiency.

Tewari *et al.*[76,77] first compared the physical properties of MDNA isolated from the normal yeast and a cytoplasmic petite. They found that the ρ^- strains had a minute amount of a satellite DNA, which was lower in density by 0·006 g/ml in cesium chloride density gradient, compared to the mitochondrial DNA of the wild type strain. Carnevali *et al.*[84] compared the bulk DNA isolated from a wild type and petite strains of yeast and found that the former had a satellite band at a density of 1·685, while in the latter it was replaced by a band at 1·670. In contrast to these observations, Moustacchi and Williamson[85] and Corneo *et al.*[86] failed to detect any satellite DNA in the petite strain. Recently, Mounoulou *et al.*[83] have pointed out critically the need to use identical growth conditions. They compared a wild type yeast and isogenic petite strain, both grown in a chemostat and found equal amounts of MDNA in both the cases. However, the buoyant density of the mutant was different from that from the normal mitochondria. Mehrotra and Mahler[87] repeated the experiments of Mounoulou *et al.*, taking precautions to maintain the same growth conditions, and confirmed that both the normal and petite strains had the same MDNA content. They further studied the physical properties of the MDNA from both the strains, in particular the buoyant density under a variety of conditions, thermal denaturation profiles and base composition. The DNA isolated from the mitochondrial particles of the mutant had an AT content of 90% as compared to 83% for the normal MDNA. This alteration, according to Mehrotra and Mahler[87], could make the MDNA of the mutant genetically incompetent.

(b) 'Poky' mutant of *Neurospora*: Mitchell and Mitchell[88] analysed several mutants of *N. crassa*, which were deficient in respiration, and showed that the deficiency is materially inherited. Three such mutants, mi-1 or poky, mi-3 and mi-4, have been studied exhaustively. The mi-1 mutant is characterized by a deficiency in cytochrome *a* and cyt *b*, while it produces an excess of cyt *c*. Both mi-3 and mi-4 are devoid of cyt *a* and have reduced amounts of cyt *c*. Reich and Luck[89] carried out some interesting experiments which resulted in a correlation between the inheritance of the mitochondrial type from the maternal parent and the transfer of the MDNA from the same parent. The MDNA of *N. crassa* has two bands at densities 1·698 and 1·702, while the MDNA of *N. sitophila* has in addition a band at 1·692. Reciprocal crosses were made between a mi-1 mutant of *N. crassa* and a wild type of *N. sitophila*. When *N. crassa* was the maternal parent, all ascopores of the progeny were mutants and none of them had a density band at 1·692. However, when *N. sitophila* was the maternal parent, the progeny was all normal and contained the band at 1·692.

Thus, these results would indicate that the respiratory competency or otherwise of the mitochondria is linked to the properties of the DNA component of the mitochondria. Still the evidence remains largely circumstantial. Two attempts have been reported which tend to provide positive evidence for the direct transfer of information from one cell to another by the mitochondria. Diacumakos *et al.*[89] injected mitochondria prepared from a cytochrome-deficient mutant of *N. crassa* into the hypha of a normal strain and found the resultant mycelium to be slow-growing and cytochrome-deficient. A similar alteration in the growth characteristics was not noted when the nucleus was injected. It is, therefore, apparent that the injected mitochondria have in some way interfered with the normal mitochondria and have transmitted their properties. Tuppy and Wildner[90] reported that when they incubated spheroplasts of petite strains of yeast with a mitochondrial suspension of normal yeast, 2·6% of the spheroplasts grew out into normal colonies. However, it may be mentioned that these results have been contradicted[91].

Mode of Assembly of Mitochondria

Origin of mitochondria de novo — Most of the support for the theory of *de novo* origin of mitochondria is derived from cytological observations and is not backed by chemical or biochemical evidences. Also, much of the earlier work did not stand the test of more rigorous experimental tools like the electron microscope. Yet, there are some

recent reports which cannot be dispensed with so easily. Berger[92] found mitochondrial structures at various stages of development, in the electron micrographs of the photo-receptor retinal cells. He concluded that the mitochondria arise *de novo* in the cytoplasm near the nuclear membrane. Adams and Hertig[93] followed the developmental changes in the mitochondria of the oocyte and observed that mitochondria appeared to develop from regions free of other mitochondria. The fact that a large percentage of these observations have been made on a particular cell, the egg cell, may be more than coincidental and should not be ignored.

Growth and division of mitochondria — Altmann proposed in 1909 that the mitochondria grow and divide independently of the nucleus[4]. But the closest direct proof to this visionary hypothesis has been only recently obtained by Luck[94,95] in *N. crassa*. A choline-requiring mutant of *N. crassa* was first grown in a medium containing radioactive choline, which extensively labelled the mitochondria. The cells were then harvested and transferred to a medium containing unlabelled choline. Samples were taken out at the end of each cell division, mitochondria prepared and the radioactivity associated with each mitochondrion determined by radioautography. Calculation of the number of grains per mitochondrion showed a random Poisson distribution, which would rule out that the mitochondria in the young cells have arisen *de novo*. The distribution data would best fit a situation where the mitochondria grow steadily during the cell cycles, by the addition of new substance to the old cells. In a subsequent work, Luck[96] demonstrated that mitochondria can accumulate more lipid material without affecting the specific activities of the various enzymes. Based on these results, he concluded that at least in *Neurospora*, mitochondria grow and divide. The inherent danger in drawing such a conclusion based only on studies with one component of mitochondria has been discussed well by Roodyn and Wilkie[97]. Other evidence that mitochondria arise by growth and division of pre-existing mitochondria has been obtained in electron microscopic studies with a small unicellular alga, *Micromonas*[98]. This organism contains one nucleus, one chloroplast and one mitochondrion. The three of them divide in synchrony at the time of cell division. Bucher and coworkers[99] have reported that the mitochondrion of the flight muscle of the locust can grow up to 60 times its size without any change in the specific activities of the enzymes. More credence to this theory is also lent by other reports[100,101] that electron micrographs of the cell show mitochondria in dumb-bell shapes and bud-like projection attached to them, which give the impression of mitochondria in the process of division.

Formation of mitochondria from other cellular membranes — Several cytologists, again purely on the basis of morphological criteria, have claimed that mitochondria are formed from other cellular membranes by a pinching-off process. The nuclear membrane, the microbodies and the endoplasmic reticulum have all, at some time or the other, been implicated in this process[4]. For example, Bell and Muhlethaler[102] followed the maturation of fern cells and claimed that mitochondria were formed from evaginations into the cytoplasm of the nuclear membrane. Reid[103] has summarized some of the evidences in favour of the role of cytomembranes in the process of mitochondriogenesis. In fact, several workers have also remarked on the apparent continuity of the mitochondrial membrane with the cytomembranes in their electron micrographs[104]. Bade[105] followed the appearance of mitochondria in regenerating mouse liver by electron microscopy. He observed a progressive transformation of the elements of the endoplasmic reticulum cisternae into double membranes and cristae of the mitochondria. At one stage in the process, an intermediate structure was found which was neither cytomembrane nor mitochondrion, and this was termed 'promitochondrion'. During regeneration, many mitochondria with incomplete outer membranes were observed and they were often found to be in close association with the endoplasmic reticulum.

Turnover of mitochondria as an entity — The classical experiments of Fletcher and Sanadi[106] deserve special mention. These workers dosed several rats with ^{35}S methionine and ^{14}C acetate and at different time intervals isolated the liver mitochondria and fractionated them into cytochrome *c*, soluble proteins, insoluble proteins and lipids. By determining the radioactivity in these fractions at all time intervals, they were able to construct decay curves for each component. Surprisingly, the curves were linear and parallel to one another, suggesting that the mitochondria turn over as an entity with a half-life of 10·3 days. Bailey *et al.*[107] performed similar experiments and obtained a value of 9 days for the half-life of the insoluble protein fraction, although the turnover rates of the lipids were much more complex than those reported by Fletcher and Sanadi. Neubert *et al.*[73] estimated the half-life of mitochondrial DNA of rat liver to be 8-9 days using pulse-labelling techniques. Since the half-life of the nuclear DNA in the same tissue was found to be 20 days, these workers suggested that the mitochondria turn over independently of the cell. Cuzner *et al.*[108] found that in brain mitochondria, cholesterol, cardiolipin and phosphatidic acid turned over slowly, if at all, whereas the remaining phosphatides had much shorter half-lives. Luck[96] demonstrated that in *Neurospora*, the ratio of protein to lipid can be varied widely without affecting the function of the mitochondria. In a similar way, the experiments of Swick *et al.*[109] demonstrate that the enzymic complement of the mitochondria can be altered without any change in the rate of formation of mitochondria as a whole. These workers labelled mitochondria *in vivo*, by continuous oral administration of radioactive calcium carbonate to the animal. The liver mitochondria were isolated at different time intervals and the decay curves constructed for 6 different fractions, which gave a half-life value of 4-6 days. When they induced the enzymes alanine and ornithine amino transferases, specifically, the enzymes had half-lives of 0·73 and 1·03 days respectively. Droz and Bergeron[110] studied the synthesis of mitochondria.

They injected tritiated leucine to rats and followed the appearance of radioactivity in the mitochondria of liver and kidney at various time intervals by electron microscope autoradiography. Sections were examined from 10 min to 6 hr after the injection. It was found that the number of grains per mitochondrion showed a Poisson distribution over the entire period. Therefore, it was concluded that there was a continuous synthesis of mitochondrial protein over the entire period of study.

Role of structural protein in mitochondriogenesis — First isolated by Criddle *et al.*[17], this protein fraction of mitochondria has been shown to contain at least 30 proteins. Richardson *et al.*[111] have shown that the various respiratory enzymes and cytochromes bind with this protein and have implicated it as the backbone material for the integrity of the mitochondrial structure. As we saw earlier, the results of amino acid incorporation studies strengthen this view. Sherman and Slonimski[112] suggested that the aberrant mitochondria present in the petite strains of yeast and *N. crassa* may be due to the alteration in the structural protein fraction, brought about probably by a mutation of the mitochondrial DNA. Indeed, Menkres and Woodward[113] found such a difference in the structural proteins of respiratory-competent and respiratory-deficient strains of *N. crassa*. They purified the structural protein fraction to such a high degree that they could find the difference in a single amino acid residue. The tryptophan residue of the normal strain was found to be replaced by a cysteine residue in the mutant strain. Also, the structural protein of the mutant showed reduced binding capacity for NADH and purified malate dehydrogenase.

Though such a difference has been predicted in the case of yeast also, there seems to be some controversy in this regard. From ultracentrifugal studies Katoh and Sanukida[114] did not find any difference between the amino acid composition and antigenic properties of the structural proteins of the normal and petite strains of yeast. But Tuppy and coworkers[115,116] claimed a difference. They purified the structural proteins from both normal and petite strains and analysed them by acrylamide gel electrophoresis. The petite strains lacked at least one protein from the corresponding normal strain. Also, these workers prepared rabbit antisera against yeast mitochondria and investigations with these showed that at least one of the antigenic determinants of the wild type structural proteins was missing from the mutant strain. Schatz[117] had shown that the F_1-ATPase associated with the mutant yeast mitochondria is cold labile, whereas the corresponding activity in the mutant is cold stable. Tuppy and Swetley[115], therefore, postulate that petite mutation causes the loss of a component in the structural protein and thereby induces cold lability of the atractyloside-sensitive nucleotide binding, catalysed by F_1 factor.

Formation of mitochondrial membranes in yeast during aerobic adaptation — The facultative anaerobe, *S. cerevisiae*, has been studied exhaustively in this connection. Marchant and Smith[118] have reviewed in detail the various membrane systems present in the yeast cell and the origin of these membranes. There is ample evidence that the yeast cells grown under anaerobic conditions do not have normal mitochondrial profiles when seen under the electron microscope. Within a very short time after exposure to air, the fully functional mitochondria are formed in the cytoplasm. These results are also supported by cytochrome analysis; the anaerobic cells are devoid of cytochromes $a+a_1$, b and c_1. Linnane and coworkers first reported that *Torulopsis utilis*, when grown anaerobically, contained a complex system of reticulate and lamellar membrane system in the cytoplasm in place of the mitochondria[119]. Though they claimed that *S. cerevisiae* also contained similar structures, they subsequently[120] commented on the essentiality of the presence of ergosterol and Tween 80 for such membrane formation. Polakis *et al.*[121] could not find any evidence for the presence of these cytoplasmic structures in the anaerobic cells even in the presence of the lipid supplements, while Morpugo *et al.*[122] and Swift *et al.*[123] supported the findings of Linnane *et al.* Osumi and Katoh[124,125] studied the stages in the mitochondria during aerobic adaptation. Within 10 min of exposure, extensive membrane systems, resembling endoplasmic reticulum, developed in the cytoplasm and after 1 hr, these gradually disappeared, giving place to mitochondrial profiles with rudimentary cristae. Only after this, fully formed mitochondria appeared. These workers also studied the processes that take place when a 'petite' strain is grown in the anaerobic conditions and is transferred to aerobic conditions. Up to about 1 hr, the changes taking place in the mutant are indistinguishable from the normal strain, but thereafter aberrant mitochondria are formed.

Linnane and his coworkers[119] reported that the membranous elements noticed in the cytoplasm of the anaerobic yeast contained some primary mitochondrial enzymes like succinic dehydrogenases, but no cytochromes. These particles were, therefore, termed 'promitochondria'. Wallace and Linnane[120] extended these observations. Polakis *et al.*[121] showed that cells adapting to aerobic conditions developed the ability to oxidize glucose, ethanol and acetate in that order. Schatz[126] obtained direct confirmation for the presence of membranous elements by isolating them from anaerobically grown cells and purifying them on a 'urografin' gradient. In a recent exhaustive study, Schatz and coworkers[127-129] clearly demonstrated the presence of these structures in the anaerobic cell by the freeze-etching technique. The particles were also isolated and found to contain succinate dehydrogenase, oligomycin-sensitive ATPase and structural protein. Study of the lipid composition of these cells also showed that they contained all the major lipids of the normal mitochondria in comparable quantities, but there was considerable variation in the fatty acid composition.

Effects of glucose repression on yeast mitochondria — It is well known that glucose represses various respiratory enzymes of yeast. Essentially, growth in the presence of glucose results in severe modification in the appearance of mitochondria and reduction in their number[104,130-133]. As glucose is

converted to ethanol, one reaches a point in the growth conditions in which the glucose concentration falls to a level which can no longer cause repression. At the same time, the cells begin to oxidize ethanol, and the number of mitochondria per cell increases. Chapman and Bartley[134] have recently reported on the kinetics of enzyme changes in yeast under conditions that cause the loss of mitochondria. The results support the thesis that it is the glucose concentration that determines whether the pathway of metabolism is anaerobic (glycolytic) or aerobic (oxygen as final hydrogen acceptor). It is to be hoped that this controlled removal of some of the enzymes, termed 'inactivation repression'[135], may reveal in more detailed studies the metabolic control mechanisms operative in the formation of mitochondria. Jayaraman *et al.*[104], from their studies, concluded that there occurs a dedifferentiation of the mitochondria under conditions of glucose repression. The morphology of the yeast cell in the repressed state is somewhat similar to that of the cell grown in the presence of chloramphenicol. The prominent features are a decrease in the number of mitochondria and an increase in large cytoplasmic membranes. Characteristically, the mitochondria in this phase possess poorly developed cristae and appear less distinct against the cell background than does standard aerobic yeast. Biochemically, the mitochondrial type particles isolated from the repressed yeast are characterized by low total and specific activities of the enzymes as well as poor respiratory control.

Development of yeast mitochondria on release from glucose repression — Utter and his coworkers[132] have shown that within a short time after the cells have been released from glucose repression, i.e. when the glucose in the medium is exhausted, most of the mitochondrial activities are restored to normal level. Jayaraman *et al.*[104] have carried out an exhaustive investigation of the appearance of the various enzymes, cytochromes, and have correlated the morphology of the yeast cells at various phases of repression and derepression. The various enzymic activities and the cytochromes appeared in the 'mitochondrial' particles at different time intervals, showing that they are not laid down concurrently *en bloc* and that there may be considerable autonomy in their synthesis. Also, as mentioned earlier, during the repression, not only the total activity of the enzymes, but also the specific activities, decreased and these increased during the derepression phase. Therefore, these workers argued " yet specific activity should certainly remain constant throughout, if increase in activity during derepression were solely to be ascribed to mitochondrial self-duplication and if there were only a quantitative difference and no qualitative difference between the mitochondria in the repressed and depressed phase ". The general model of assembly of new mitochondria which has emerged from these studies is one of stepwise interactions between hierarchies varying in autonomy.

The effects of the antibiotics, chloramphenicol and cycloheximide, on the mitochondrial formation in the derepression phase, indicated that continuous protein synthesis both by the mitochondria and the cytoplasm is necessary. Also, evidence has been obtained that the mitochondria constantly turn over and the derepression phase was characterized by a rate of synthesis much higher than the rate of degradation (Jayaraman, J., unpublished results).

The inhibitor studies also revealed that the synthesis of lipids need not be affected under a condition where the mitochondrial formation is inhibited (Jayaraman, J., unpublished results). Plagemann[136] has obtained similar results with rat liver mitochondria. He showed that cycloheximide, in concentration enough to inhibit 90% of protein synthesis, does not affect the synthesis and incorporation of phospholipids into mitochondria.

Mitochondria of respiratory-deficient mutants of yeast and Neurospora — It has been already discussed that the petite mutant of yeast, produced by acriflavin treatment, is respiratory deficient and that it has a mitochondrial DNA component different from that of the normal mitochondrial DNA. It is generally taken for granted that a mutation in the mitochondrial DNA is responsible for the aberrant mitochondria of the mutant cell. Immunochemical techniques have been used to get information regarding homologies between the mitochondria and analogous membrane components of the mutant. By the use of rabbit antiserum against respiratory subparticles from wild type *S. cerevisiae*, Mahler *et al.*[137] were able to show the presence of cross-reacting material in a similar particle preparation from a cytoplasmic mutant. Antibodies were obtained for the NADH-cyt *C* reductase portion of the respiratory chain. Kraml and Mahler[138], by immuno-diffusion experiments and by inhibition of enzyme-antiserum precipitations, showed the presence of an inactive protein in the petite mutant analogous to a protein in the cytochrome oxidase of the normal strain. The controversy regarding the identity of the structural protein fractions of the wild type yeast and the mutant yeast has already been mentioned. Great caution is, however, needed in interpreting the data obtained by immunochemical methods. Menkres and Woodward[113] have shown that antibodies against mitochondrial structural protein will precipitate the analogous protein of yeast or beef heart, in spite of significant differences in their respective amino acid composition.

Factors Controlling Mitochondriogenesis

As demonstrated by Frederics[139], mitochondria possess enormous capacity to change their shape and size in the cell. There can also be a lot of variation in the number of mitochondria per cell not only from tissue to tissue but also within the same tissue. With the evidence currently available, we can state, as a guideline, that the quality of the mitochondria is under genetic control, while the quantity of mitochondria is under the control of other factors.

Control exerted by mitochondrial DNA — The two major evidences in favour of the biological role of MDNA in the organelle formation are: (a) mutagenic agents like acriflavine, phenylethyl alcohol and dinitrophenol, which are known to bind with DNA under suitable conditions, bring about an alteration in the functional properties of mitochondria without

apparently affecting the nuclear function; and (b) the DNA components of the mitochondria isolated from wild type yeast and isogenic respiratory-deficient mutant are different. More information on the control exerted by the mitochondrial DNA has come from studies on the drug resistance by yeast cells. Linnane and coworkers[140] isolated several yeast mutants which were resistant to chloramphenicol, tetracyclines and macrolide antibiotics. By a series of ingenious experiments they showed that two mechanisms are operative by which the drug resistance is brought about. Chloramphenicol and the tetracycline antibiotics act by affecting the cell permeability and this factor is controlled by the nuclear gene. On the other hand, the macrolide antibiotics, like lincomycin, erythromycin, spiramycin and carbomycin, act apparently at the mitochondrial DNA level, since the resistance in these cases is inherited extrachromosomally. Thomas and Wilkie[141] explored this avenue further. They isolated double and triple mutants, i.e. mutants which were resistant to one or more of the above antibiotics, and thus showed that MDNA can also have mutations at more than one place, as in the case of nuclear DNA. They argued that if this be the case, then recombinants should be isolated by mating of strains which have different mitochondrial markers. In a typical experiment[142], a strain which was $E^sP^rS^r$ was mated with another strain which was $E^rP^sS^r$ and the number of clones obtained in the progeny for their drug resistance analysed. Of the total 119 clones thus analysed, 74 belonged to either of the parental group, 14 were $E^sS^sP^s$, 23 were $E^rS^rP^r$, 3 were $E^sS^rP^s$, 4 were $E^sS^rP^r$ and one was $E^rS^sP^s$. Two conclusions were derived from these results: (a) recombination between mitochondrial markers has taken place; and (b) the resistances to erythromycin (*E*) and spiramycin (*S*) are more closely linked to each other than the resistance to parmomycin (*P*). The first attempts to 'map' the mitochondrial 'gene' have been initiated.

Role of the nucleus — During their studies with the petite mutants of yeast, Slonimski and his group[82] observed some other mutants which transmitted the respiratory incompetency to the progeny in a Mendelian fashion. Crosses of these mutants with the cytoplasmic mutants gave rise to normal colonies. These mutants have been termed *p*-mutants or segregational mutants. Sherman[143] and Sherman and Slonimski[144] isolated nine different *p*-mutants and found that three of them, p_3, ly_6 and ly_8, lacked in addition the rho factor; in other words, these are double mutants in the sense both the nuclear gene and the mitochondrial gene do not allow the formation of mitochondria. The ly_6 and ly_8 mutants, as their name implies, have also a defective lysine-synthesizing system, apparently at different points. The zygotes obtained by crosses between them, however, did not require lysine as expected; but they were still rho negative. This has been interpreted to mean that the rho factor once lost cannot be regenerated even if complementation occurs at the nuclear gene level. In keeping with the recent view on the mutation of the mitochondrial DNA as responsible for the petiteness, this would mean that complementation at the nuclear gene level would not automatically bring about a complementation at the mitochondrial DNA level. More support for this conclusion is derived from the finding that genetic analysis of a revertant to lysine independence of ly_8 strain showed that the lysine requirement and respiratory deficiency are the effects of the same gene. Direct biochemical support for this thesis is also derived from the experiments of Mounoulou *et al.*[83]. On comparing the physical properties of the MDNA isolated from the wild type yeast with those of a nuclear mutant, no difference was observed.

Sherman and Slonimski[144] investigated the *p*-mutants which were rho positive, and found that there was a general lack of cytochromes, particularly cyt $a+a_3$ and *b*. They contended, based on these results, that mutation at any one of the *p* loci leads to multiple enzyme deficiencies. The three sets of evidences, namely (1) mitochondrial DNA codes for the tightly bound cytochromes like cyt *a* and *b*, (2) nuclear mutation leads to a general lack of these cytochromes, and (3) nuclear mutation does not alter the properties of the MDNA, point to the extremely complex relationship that exists between the nucleus and the cytoplasm.

Yotsuyanagi[145] made the interesting observation during electron microscopic studies that only in the rho minus strains, morphologically aberrant mitochondria were present. The nuclear mutants which are rho positive have apparently normal mitochondria. It would appear from these studies that the inner membrane of the organelle can be formed, at least to an extent, without concomitant synthesis of cyt *a* and cyt *b*.

Jakob[146] made a detailed kinetic analysis of complementation with a neutral strain, rho minus, in the case of three *p* gene mutants: p_1, p_5 and p_7. The results indicated that the complementation is not simply derepression of a previously repressed genome, but that the expression of genetic information in elaborating the respiratory system is a secondary effect of the restoration of the functional alleles of the *p* genes. She favours the theory that the primary effect of the mutation (at the nuclear level) is to alter the mitochondrial structure in such a way as to block the synthesis or function of one of the components of the electron transport. This would provide indirect support to the template model for the mitochondriogenesis discussed elsewhere in this review.

Sherman and his coworkers[147,148] isolated another type of mutants with respiratory deficiency, which were designated Cy mutants, since they were deficient in cytochrome *c*. There appear to be at least six unlinked genes controlling the production of cytochrome *c*. Carrying out elaborate analyses on the intact proteins and haem peptides formed by one of such mutants, cy 1-2 and its revertant CY 1-2, these workers showed that the cy-1 gene codes for the primary structure of cytochrome *c*. Biochemical evidence for the fact that cytochrome *c* is synthesized outside the mitochondria has already been presented. It would be pertinent to mention at this point that Munkres and Richards[149] have shown that the structural genes for malate dehydrogenase in *Neurospora* are chromosomal.

The mitochondrial malate dehydrogenases of maize[150] and man[151] have also been reported to be under the control of the nucleus. Another mitochondrial enzyme shown to be under nuclear control is β-hydroxybutyric dehydrogenase in *Paramecium*[152].

Yeast mutants have also been isolated with a view to utilizing them for understanding the mechanism of oxidative phosphorylation. Two such mutants[153,154] show altered ATPase activity, while all the other components are apparently intact. Beck *et al.*[153] described a mutant, p_9, which was unable to utilize non-fermentable substrates, but contained all the cytochromes. The mitochondrial particles from these mutants were characterized by high ATPase content and also low affinity to ADP. Interestingly, induction of mutation of MDNA by acriflavin treatment always resulted in non-viable cells. These workers conclude that the p_9 mutation alters a mitochondrial membrane structure. On the other hand, Parker *et al.*[155] studied some oligomycin-resistant mutants of yeast. These cells when grown in the presence of 10 μg/ml of oligomycin showed lower respiration and lower cytochrome content, indicating that the drug affects the biogenesis of mitochondria. In these cells, however, the frequency of mutation to the rho minus state was found to be increased 40-fold, than the normal wild type yeast. Ycas[156] as well as Reilley and Sherman[157] found mutants resistant to antimycin A and showed that the synthesis of cytochrome *a* in these cells was inhibited.

Another class of yeast mutants have been isolated by Wilkie and Negrotti[158]. These *gi* mutants grow well on non-fermentable substrates and have a normal complement of all cytochromes. Under conditions of glucose repression or anoxia, the daughter cells derived are cytoplasmic petites. The parent cells retain their respiratory competency and if put back in a non-repressing medium give rise to normal cells. Based on extensive genetic analyses, Wilkie and Negrotti contend that the continuity of the rho factor or MDNA is possible in the *gi* strain only by way of intact functional mitochondria. When the organelle is in a disintegrated or dedifferentiated state, as in the presence of glucose or in the absence of oxygen, the 'mitochondrial message' is not transmitted to the progeny. The *gi* character has been shown to be under the control of the nuclear gene. It can thus be said that the normal function of this gene is to ensure the continuity of MDNA even under conditions where the formation of mitochondria is inhibited.

Other factors affecting mitochondriogenesis — The effects of high concentrations of glucose and anaerobiosis on the formation of mitochondria in yeast have been discussed earlier. Variation in the number of mitochondria per cell is brought about in animal tissues, in response to hormonal stimulation. Tata[159] and his group suggested that thyroid hormone exerts a selective control over the synthesis of mitochondrial respiratory and phosphorylative constituents. In fact, 4 days after the administration of thyroid hormone, the amount of mitochondrial protein per gram muscle was doubled. And electron microscopic observations indicated an increase of 5-20 times in the number of cristae. Kadenbach[160], however, suggested that the increased respiration of hyperthyroid liver is not caused by an increased amount of mitochondria, but by specific changes of enzymatic equipment. Preliminary results in this laboratory also indicate that the mitochondrial protein content increases, without any change in the specific activities of respiratory enzymes, in the livers of rats administered the drug, Atromid or *p*-chlorophenoxy isobutyrate (Ramakrishna Kurup, C. K. & Aithal, H. N., unpublished results).

Scarpelli *et al.*[161] found that starved rats had $2{\cdot}26 \pm 0{\cdot}25 \times 10^{10}$ mitochondria per mg nuclear DNA, while the normal rats had almost double the amount of mitochondria, viz. $4{\cdot}48 \pm 0{\cdot}40 \times 10^{10}$. On refeeding the animal for 12 days, the mitochondrial level was restored to the normal.

Experiments conducted by Gold and Menzies[162] indicated that while the properties of mitochondria isolated from several tissues of the animal are the same, their mechanism of synthesis may vary from tissue to tissue. They injected a single dose of tritiated leucine into the rat and followed the decay of labelled mitochondria in four tissues. The mitochondria of liver and brain decayed exponentially with half-lives of 8·4 and 24·1 days respectively. On the other hand, the decay curves for lung and kidney were biphasic, the lung mitochondria showing two half-lives of 4·6 and 14·4 days, while the kidney mitochondria had half-lives of 4·3 and 11·6 days. The significance of these observations is not clear.

Several reports indicate that mitochondria within the same cell may differ from each other in certain properties. Avers *et al.*[163] found that certain clones in acriflavin-induced respiratory variants of yeast contain at least two types of mitochondria: some are cytochrome peroxidase positive and others are negative. In a study of rat heart tissue, Ogawa and Barrnett[164] found that different mitochondria had different rates of reduction of tetrazolium salts; adjacent mitochondria were frequently found, one of which contained a heavy deposit of formazan over all its cristae, while the other was free of such deposits.

Two cases of human pathology have been reported in which a muscular disorder was associated with the presence of morphologically abnormal mitochondria only in the skeletal tissue but not in the other tissues[165,166]. Even in the muscle, normal mitochondria were present side by side.

The Present Status of the Problem

Much of the work carried out in several laboratories towards understanding the basic mechanisms involved in the formation of mitochondria has necessarily centred around one organism, the yeast. Reasonable amount of evidence has accumulated to favour a 'template theory' for the formation of mitochondria. In short, it would mean that the mitochondrial DNA component acts as the information carrier, just like nuclear DNA, and is transmitted from one generation to another. This DNA also is capable of replicating, probably by a semiconservative fashion (see ref. 167 and 168 for very

recent work). Under favourable conditions, this message is transcribed and translated to give rise to components, which form the backbone of the organelle structure. The other components, which are not synthesized by the mitochondria themselves, are synthesized elsewhere in the cell and are integrated into this basic structure, seemingly in a sequential manner, and the fully functional mitochondria emerge.

Whether this is a universal mechanism of mitochondrial formation is difficult to guess with the data we have today. It is well documented that in *N. crassa*, the mitochondria arise by growth and division of the pre-existing mitochondria. Also, there are several reports on the presence, in several tissues, of mitochondria with dumb-bell shape as though they are in the process of division. Superficially, the results with rat liver seem to support the template theory. But factors like continuous regeneration of the tissues, the nutritional status of the animal and the hormonal control make the interpretation a difficult one. The recent discovery of 'mitochondrial DNA recombination' has opened up a fruitful line of research.

Concluding Remarks

Research on the subject under review is currently in an exponential phase of growth and as such it is a difficult task to keep abreast of the number of published reports, leave alone giving a critical appraisal of all of them. It can be justifiably claimed, based on the work reviewed here, that the stage has reached where definite, critical questions can be asked regarding the biogenesis of mitochondria. Some of these questions are:

(a) What is the biological role of MDNA? What is the nature of the product directed by MDNA? What is the relationship between MDNA and nuclear DNA?

(b) Two aspects of protein synthesis by mitochondria have not been resolved unequivocally: the bacterial contamination and the identity of the product. The accumulated data on the presence of mitochondria-specific tRNAs and ribosomes (see ref. 169 for recent work) as well as antibiotic inhibitors indicate the mechanism to be similar to that of the microsomal system. Confirmatory evidence is still lacking.

(c) Much work needs to be done on the protein-protein interaction and lipid-protein interaction under *in vivo* conditions, which is an essential prerequisite for an understanding of the mode of assembly of the mitochondria. The studies on the *in vitro* reconstitution of the electron transport complexes[170] should be of great help in this direction. One major handicap in extrapolating the results of the *in vitro* experiments to the *in vivo* conditions would be lack of methodology to relate the changes of enzyme activities with changes in enzyme protein concentration.

(d) A new branch of molecular biology can be said to have started with the study of mitochondrial genetics. The experiments demonstrating recombination at the mitochondrial DNA level and the isolation of several mutants of yeast with altered mitochondrial function could be cited as examples.

Summary

Mitochondria contain their own specific complements of DNA, RNA, ribosomes and tRNAs as well as the various enzymes involved in the replication of nucleic acids and the incorporation of amino acids into proteins. They are also capable of at least partial synthesis of some lipids. The mitochondriogenesis is controlled both by the nuclear genes and 'mitochondrial genes'. Environmental conditions like the lack of oxygen and the presence of glucose or antibiotics affect the process of mitochondrial assembly, providing a tool for further investigations. The bulk of the evidences is against mitochondria being completely autonomous.

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Polyribosomes: Structure & Function

JOSEPH D. PADAYATTY
Department of Biochemistry, Indian Institute of Science, Bangalore 12

STUDY of the transfer of information from genes to proteins is the most active area of contemporary research in biology. Messenger RNA carrying the information from genes associates with ribosomes and serves as template for protein synthesis. In 1962, Risebrough *et al.*[1] found that in bacterial cells, messenger RNA complexes with ribosomes forming "heavy" ribosomes. These "heavy" ribosomes are the same as the "active" ribosomes involved in protein synthesis reported by Tissieres *et al.*[2] in 1960. The existence of aggregates of ribosomes held together by strands of messenger RNA in mammalian system was reported by Gierer[3] in the beginning of 1963. Gierer's work was followed by that of Warner *et al.*[4,5]. Rich called the aggregates of ribosomes held together by strands of messenger RNA as polyribosomes or polysomes. Polysomes contain the newly synthesized polypeptides, have the highest activity in protein synthesis *in vivo* and are extremely sensitive to ribonuclease. Polysomes as functional units in protein synthesis in *in vitro* and *in vivo* systems have been supported by an imposing body of experimental evidence. It is in this polysomal structure that the activated transfer RNA molecules, the peptide bond forming enzymes, guanosine triphosphate and other factors act together to form a polypeptide chain. Considerable interest is attached to the studies on polysomes aimed at understanding the mechanism and regulation to protein synthesis.

Isolation of Polysomes

The presence of polysomes has been demonstrated in mammalian[3-8], plant[9-13], yeast[14,15], bacterial[15-21] and phage-infected cells[22,23]. Polysomal entity is extremely fragile and great care is necessary in the isolation of polysomes. Generally they are isolated after lysing the cells under mild conditions and centrifuging through a sucrose density gradient. During density gradient centrifugation, particles are separated according to their mass, shape and size. The reagents used in the isolation of polysomes should be free from ribonuclease and all manipulations should be done to minimize the degradation of messenger RNA.

Polysomes from mammalian cells — Reticulocyte polysomes have been studied extensively, because the mature red blood cells synthesize predominantly a single protein, hemoglobin. Rabbit reticulocyte polysomes are prepared as described by Rich[24]. Reticulocytosis in rabbits is induced by injecting phenylhydrazine subcutaneously[24,25]. Blood is collected either by heart puncture or from ear veins. The nascent polypeptides synthesized on polysomes of reticulocytes can be labelled by incubating whole cells according to the method of Kruh and Borsook[26]. The cells are collected by centrifugation at low speed, lysed by the addition of 8-9 volumes of "reticulocyte standard buffer" (RSB) which contains 0·01*M* Tris-HCl (*p*H 7·4), 0·01*M* KCl and 0·0015*M* Mg^{2+}. The lysate freed from the stroma by centrifugation is layered on 25 ml of 15-30% (wt/wt) sucrose in RSB and is centrifuged in a SW 25·1 rotor at 25,000 rpm for 2 hr at 4°. The centrifuge tube is punctured, the solution is pumped at a uniform rate by a Sigma motor pump and the absorbance at 260 mμ is monitored. The sucrose density gradient analysis of a clarified crude lysate of rabbit reticulocyte is shown in Fig. 1.

The number of ribosomes per polysome in each fraction is determined by electron microscopy and from sedimentation coefficient values. The number of ribosomes per polysome in the peak fraction is five. The radioactivity in the protein is maximum in the peak polysome fraction containing pentamers. Hemoglobin synthesis takes place predominantly on polysomes containing pentamers.

The reticulocyte system has been demonstrated for studies of hemoglobin synthesis[5,27], rat skeletal muscle preparation for myosin synthesis[28], rat lymph nodes for gamma globin synthesis[29] and transplanted hypophysis for the production of the hormone, prolactin[30]. The eye lens polysomes are of interest, since they are involved in the synthesis of a relatively small number of closely related proteins[31]. Bloemendal *et al.*[32] have reported the preparation of polysomes from rat liver in high yields without using detergents and this method can be used for the preparation of polysomes from

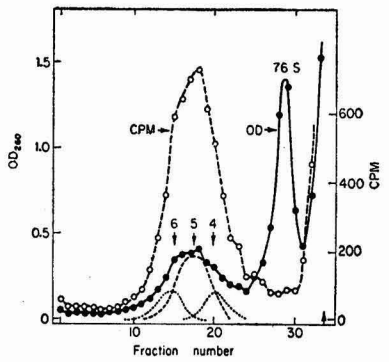


Fig. 1 — Distribution of rabbit reticulocyte polysomes and radioactivity [Reticulocytes are labelled for 45 sec with ^{14}C amino acids. One ml of the cell lysate is centrifuged through 25 ml of 15-30% sucrose in SW 25 rotor at 25,000 rpm for 2 hr at 4°. The tube is punctured and the gradient is collected in 36 fractions. Optical density at 260 mμ and radioactivity in each fraction are determined. The arrows labelled 4, 5, 6 represent sedimenting positions of tetramers, pentamers and hexamers and dotted lines are schematic representation of their distribution in the gradient (from ref. 4)]

other tissues. Polysomes from the cerebral cortex of rat were prepared by Campagnoni and Mahler[33] by a modification of the method of Bont *et al.*[34].

The human tumour HeLa cells are grown in tissue culture[35] and polysomes are prepared from these cells by simple techniques[7]. The polysomes of HeLa cells are more polydisperse than reticulocyte. A specialized polysome distribution is seen in the cells of tissues producing antibodies. Rabbits on rats are immunized, the tissues removed and polysomes prepared according to the method of Becker and Rich[36]. Polysomes thus obtained have a biphasic distribution with one cluster of polysomes containing 7 or 8 ribosomes and another cluster containing 16-20 ribosomes.

Polysomes from yeast and bacteria — Marcus and Halvorson[37] have reported a procedure for the detection and isolation of polysomes in yeast. The yields of polysomes are low from stationary phase cells or cultures allowed to cool slowly during harvest, or extracts prepared by grinding for more than 10 min. Exponential sucrose density gradient sharpens the separation of the individual species.

There is no general satisfactory method to lyse cells of *Escherichia coli* rapidly to produce undegraded polysomes. In some cases, methods involving gentle lysis have been described for specific strains of *Esch. coli*, i.e. the osmotically fragile strain of Mangiarotti and Schlessinger[38] and the pencillin sensitive strains of Kiho and Rich[17], but their methods have only limited application. The method of lysis of cells with the neutral detergent Brij-58 (polyoxyethylene-20-cetyl ether) has been successful in all K, B and C strains of *Esch. coli* and T_4 bacteriophage infected cells[22,39]. Polysomes present in whole unlysed cells degrade rapidly to monosomes but, after lysis in cold lysate, they are relatively stable. The high concentration of Brij-58 or Luberol-W used to lyse cells does not seem to have any deleterious effect on the polysomes, monosomes or DNA of the cell and does not inhibit the protein synthetic activity of the ribosome supernatant or assay of RNA polymerase. Phages MS-2, ϕX-174 and T_4 also seem to be unaffected by the concentration of neutral detergent in the lysate.

Treatment sufficient to disrupt the bacterial cell wall, yet mild enough to preserve physiological aggregates, is required to isolate polysomal aggregates from bacteria. Such a treatment has been achieved by modification of the techniques of Mahler and Fraser[40] and Repaske[41]. Their methods utilized the chelating agent ethylenediamine tetraacetate (EDTA) and the cell wall active enzyme, lysozyme, to form spheroplasts of a number of bacterial species[42]. Various procedures, such as pulse labelling, addition of inhibitors, etc., can be employed while the culture is in a physiologically steady state of growth. Different methods are used for the preparation of spheroplasts from other organisms. Techniques for the isolation of polysomes from *Esch. coli* have been described by Staehelin *et al.*[20] and by Schaechter[43]. Dresden[44] has described a modified procedure for the isolation of polysomes from *Esch. coli* K 12 and this method is used for the preparation of polysomes from *Micrococcus lysodeidticus* and *Aerobacter aerogenes*.

Phage specific polysomes — The major protein synthesized after the infection of *Esch. coli* B with T_4 phage is the phage head protein. Studies on the polysomes obtained after infection of the bacterium with the phage are of great interest from the point of view of elucidation of the relationship between the size of polysome and the protein synthesized thereon[22] and studying the effect of amber mutations on the size of polysomes[23]. Polysomes are the best source of functional messengers.

Esch. coli B is grown to 5×10^8 cells per ml in a synthetic medium[22] or in the medium of Fraser and Jerrel[45]. Cells are harvested, infected with T_4 phage at a multiplicity of three and converted to spheroplasts with EDTA-lysozyme treatment. They are suspended in a spheroplast minimal medium and incubated at 30° with constant shaking in a gyratory shaker. The growth is arrested by the addition of sodium azide solution and the cells are collected by centrifugation. The spheroplast cells are lysed with Brij-58, and the lysate almost free from cell debris is centrifuged through 27 ml of 15-30% sucrose in SW 25·1 rotor at 25,000 rpm for 2 hr at 7°. The centrifuge tube is punctured and sucrose gradient solution pumped by a Sigma motor pump at a uniform rate. The absorbance at 260 mμ is monitored and fractions of 20 drops are collected.

The T_4 phage-induced polysome profile obtained 36 min after infection at 30° is shown in Fig. 2. Phages T_7 (ref. 46), ϕX-174 (ref. 47) and monosomes are used as markers to calibrate sedimentation rates in gradients. In a number of runs, the fraction of distance moved by the marker from the top to the total distance (from top to bottom) is found to be: monosome peak (70*S*), 0·17; ϕX-174 (114*S*), 0·25; (polysome peak, 0·51); and T_7 phage (487*S*) 0·78, indicating a linear relationship between the fraction and the *S* values of markers. Assuming that monosomes of *Esch. coli* have sedimentation coefficients of 70*S* and polysomes of increasing size behave like increasingly larger members of the same polymer series, *S* values of various polysomes are determined according to the method of Martin and Ames[48]. The number of ribosomes corresponding to the *S* values of polysomes is calculated from the relationship that *S* values are directly proportional to 0·58 power of their molecular weights[49]. Polysomes at the peak of absorbance have a sedimentation coefficient of 280*S* and contain 11-12 ribosomes per polysome.

The radioactivity in each fraction of the gradient is determined. It is found that the maximum incorporation of the label is in the peak fraction. The nascently labelled proteins are synthesized on polysome in the peak fraction and are characterized as the constituents of the phage head protein. It is concluded that polysomes containing 11-12 ribosomes are involved in the synthesis of the phage head protein of molecular weight 42000 daltons.

Structure of Polysomes

Electron micrographs of thin cell sections have shown membrane-bound polysomes in such ordered

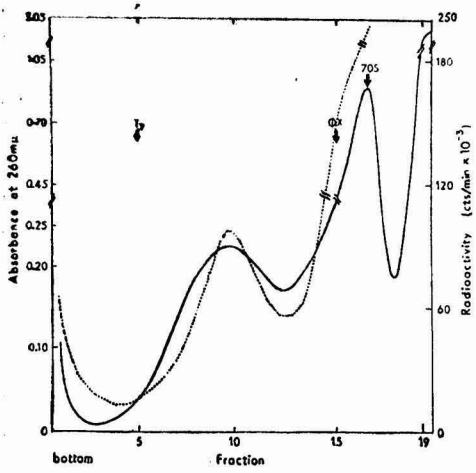


Fig. 2 — Distribution of T_4 phage specific polysomes and radioactivity [The nascent polypeptides on polysomes are labelled with ^{35}S by adding $^{35}SO_4^{2-}$ to the minimal medium in which phage particles in *Esch. coli* B spheroplasts are grown at 30° for 36 min. Cells are lysed and one ml lysate is centrifuged through 15-30% sucrose in SW 25·1 rotor at 25,000 rpm for 2 hr at 7°. The tube is punctured, the gradient is pumped at a uniform rate (20 drops per fraction) and absorbance at 260 mμ is monitored. The radioactivity in each fraction is also determined. —, absorbancy; and ....., counts per minute (from ref. 22)]

configurations as parallel double rows, loops, spirals, circles or rosettes[50]. Helical configurations have been observed[51-54] in polysomes free in cytoplasm. Cell-free preparations of polysomes often reveal a similar degree of order. Relatively small polysomes, such as those from rabbit reticulocytes, have shown both irregular linear arrays and closed-in polygonal structures[4,5,55-57]. Slayter *et al.*[57] suggested that the ordered polygonal structures might be artifacts of preparation for electron microscopy. Shelton and Kuff[58] noted that in polysomes containing 7-9 ribosomes, 6 of the ribosomes were typically arranged in a circle, with the remaining turned away like the handle of a mirror or turned inward as if to start a spiral. Ordered configurations have sometimes been observed in electron micrographs of large polysomes. Rich *et al.*[59] observed irregular clumping, presumably a drying artifact, but configurations resembling flattened helices have also been observed[58,60].

These indications of order as seen with the electron microscope suggest that ordered configurations may also exist in solution. Several attempts have been made to interpret sedimentation data in terms of model polysome configurations. Gierer[3] showed that straight chain configurations contain more than three ribosomes. Eiserling *et al.*[55] obtained fairly good agreement with the experimental results, using polygonal models for polysomes containing up to 5 ribosomes. Pfuderer *et al.*[49] fit their experimental data on the sedimentation coefficients of rat liver polysomes containing up to 12 ribosomes with helical models having 3 or 4 ribosomes per turn and a pitch of 18-20°.

Compact helices and random configurations with adjacent spheres in contact have shown that neither model can provide adequate explanation of experimental sedimentation results for rat liver polysomes[61]. The helical model is too compact, while the random configurations are too extended. The actual configuration may be a mixture of the two. The model does not seem greatly different from that of random configurations with inter-ribosomal interactions.

Polysomes and Protein Synthesis

Several types of evidence support the view that polysomes are the functional units in protein synthesis. They are (a) the sensitivity of polysomal structures to ribonuclease, (b) the progressive breakdown of polysomes with time during protein synthesis in cell-free systems, (c) the fact that polysomes are more active than monomers in general protein synthesis, (d) the lesser ability of polysomes as compared to monomers to bind polyuridylic acid and polyuridylic acid directed incorporation of labelled phenylalanine into polypeptide, and (e) the finding that puromycin causes a complete breakdown of polysomes to monosomes.

Cell-free systems — Identification of products during cell-free amino acid incorporation by polysomal preparations constitutes an active and productive area of research in protein synthesis. The classical example is that of hemoglobin, synthesized by particles from reticulocytes[62-65]. Other instances are the incorporation into serum albumin[66,67] and other serum proteins[67,68] by microsomal and ribosomal preparations from liver, into heavy and light chains of antibodies by polysomes from ascitic plasmacytoma cells[69] or rabbit lymph nodes[70], into myosin by polysomes from embryonic chick muscle[71], and the synthesis of alpha lactalbumin by particles from guinea-pig mammary gland[72]. Cell-free systems derived from brain are highly active in amino acid incorporation and well-characterized ribosomal and polysomal preparations have been described[33,73-77].

The presence of functional polysomes has been detected in cell-free systems from bovine anterior pituitary glands capable of incorporating labelled amino acids into growth hormone and prolactin fractions[78]. Messenger RNA isolated from germinating peanut cotyledons binds to monomers obtained from the dry seed and the polysomes thus formed are active in protein synthesis[79].

Monomers attach *in seriatum* to a messenger RNA molecule at the 5′ terminus, move along the strand to the opposite end, fall off concomitantly, releasing the completed polypeptide chains[80,81]. This model can account for the observed breakdown of polysomes during *in vitro* incubation, where reattachment of monomers may be slow, as has been observed with particles isolated from reticulocytes[82] and brain[74,83]. Polysomes may be formed by the attachment of ribosomal subunits, in a stepwise manner[84-87], while chain termination may lead to the release of subunits[88]. Monomeric ribosomes may not have any physiological significance and are ascribable either to polysomal breakdown or to association of the two subunits in the absence of messenger.

Quantity of polysomes and amounts of protein synthesized thereon — There is a relationship between the quantity of polysomes and the amount of protein synthesized thereon. The ratio of polysomes to monomers and dimers is increased by the fourth to seventh day after the induction of metamorphosis, thus coinciding with additional protein synthesis[89]. There is also a shift to larger polysomal aggregates during metamorphosis, which suggests a higher average size of new messenger RNA or a denser packing of ribosomes in messenger RNA during this process.

In T_4 phage-infected *Esch. coli* B, 7 min after infection, a polysome peak appears, which grows in size with time[22]. The results are shown in Fig. 3. During late times after infection, there appears to be maximum formation of the polysomes. Protein synthesis is also maximum during this period, as shown by the incorporation of radioactive amino acids into T_4 phage proteins. It seems that the amount of protein synthesized is directly related to the quantity of polysomes formed.

Sizes of polysomes, messenger RNA and protein synthesized thereon — Electron microscopic studies of the polysomes involved in the synthesis of hemoglobin of molecular weight 17500 show the presence of five ribosomes per polysome[4,57]. Polysomes containing 11-12 ribosomes are involved in the synthesis of T_4 phage head protein of molecular weight[22] 42000. The relationship between the sizes of polysomes, messenger RNAs and proteins synthesized thereon is evident from the data presented in Table 1. The determination of the number of ribosomes per polysome is not free from defects due to artifacts of preparation of the sample for electron microscopy[57]. Since polysomes can exist in a variety of shapes, sedimentation coefficient values may not reflect their actual sizes. Moreover, calculation of the number of ribosomes per polysome depends on the empirical relationship between the sedimentation coefficient values and the molecular weights[49]. The molecular weights of proteins, especially those of higher order, may not be accurate.

Constancy in spacing of ribosomes on messenger RNA — Comparing the sizes of polysomes and the corresponding messengers involved in polysome formation (Table 1), it may be inferred that there is one ribosome for an average length of 90 nucleotides of the messenger. Staehelin *et al.*[95] have reported from sedimentation analysis of polysomes from rat liver cells that the distance between two adjacent ribosomes is 90 nucleotides. Furthermore, the average length of 90 nucleotides between two ribosomes is in agreement with a distance of about 300 Å obtained by electron microscopic measurements of reticulocyte polysomes[5,96].

A messenger length of 90 nucleotides can incorporate 30 amino acids into a protein. In other words, one ribosome incorporates 30 amino acids by the time it traverses the length of 90 nucleotides of the messenger. This model involves the possibility of releasing one ribosome from the 3′ end for the movement of 90 nucleotides of messenger with concomitant attachment of another ribosome at the 5′ end of the messenger. The relationship between the sizes of polysome, messengers and the proteins synthesized thereon indicates that the spacing between ribosomes on a messenger is uniform and universal. Since the average spacing of ribosomes along the messenger, and, hence, the size distribution of polysomes is the result of a steady state, reflecting the relative rates of chain

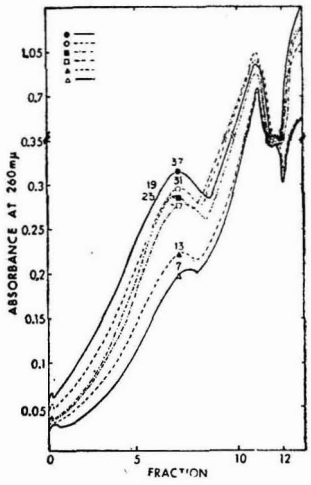


Fig. 3 — Distribution of polysomes at different times after infection of *Esch. coli* B with T_4 phage [Phage particles are grown in *Esch. coli* B spheroplasts in a minimal medium at 30°. At definite intervals of time cells are collected, lysed and 0·5 ml portions are centrifuged through 15-30% sucrose in SW 25·3 rotor at 22,000 rpm for 3 hr at 7°. The tube is punctured, the gradient pumped at a uniform rate (10 drops per fraction) and the absorbance at 260 mμ is monitored (from ref. 22)]

TABLE 1 — RELATIONSHIP BETWEEN SIZES OF POLYRIBOSOMES, MESSENGER RNA AND PROTEIN SYNTHESIZED THEREON

Protein	Mol. wt of protein	No. of ribosomes/ polysomes	No. of nucleotides/ mRNA
T_4 phage head[22]	42000	11-12	1050
T_4 phage lysozyme[90] (also Padayatty, J.D., unpublished data)	18134	4-6	480
Myosin[91]	170000-200000	50-60	4625
Actin[92]	60000-70000	15-25	1625
Tropomyosin[91]	30000-35000	5-9	800
Hemoglobin[4,57]	17500	4-6	438
Rat liver[93]	30000	8-12	750
Bovine prolactin[93]	21000	7	—
Bovine growth hormone[93]	26000	8	—
Alpha chain of collagen[94]	—	30-35	—

The number of amino acids per molecule of protein is calculated from the molecular weight (molecular weight of one amino acid is assumed to be 120) and the number of nucleotides per messenger molecule is determined on the basis that three nucleotides are involved in coding one amino acid.

initiation and chain extension, it is expected that these parameters will vary according to metabolic conditions.

Amber mutations may cause premature detachment of ribosomes from the messenger or produce smaller messengers[97], resulting in a decrease in the size of polysomes. It is found that amber mutations cause a decrease in the size of polysomes and normal sized polysomes are obtained when the amber effects are suppressed[23].

Inhibition of host protein synthesis — The inhibition of host cell protein synthesis was investigated by studying the effects of infection of Krebs ascites tumour cells by encephalomyocarditis (EMC) virus on the protein synthesizing machinery of the cell, the polysomes[98]. Microsomal preparations of uninfected cells are found to synthesize proteins *in vitro*. The addition of viral RNA as messenger to these cell extracts had no effect. By way of contrast, similar microsomal preparations from infected cells demonstrated reduced protein synthesizing activity *in vitro*, but could be stimulated by adding viral RNA. These results indicate that in normal uninfected cells, messenger RNAs are bound to the ribosomes, but in infected cells they are not bound to ribosomes. This interpretation is in agreement with the finding that polysomes in HeLa cells are degraded shortly after infection with polio virus. The process of detachment of ribosomes from messengers in polio virus-infected cells has been studied[99]. The results show that host specific messengers in infected cells are altered in such a way that the attachment of ribosomes and loading of messenger RNA cannot occur.

Inhibition of translation of early messengers — In T_4 phage-infected cells early messengers are found to be in association with polysomes during late times after infection[100]. During late times after infection, translation of early messengers is inhibited. The inhibition may be due to the appearance of modified transfer RNAs[101-103] or a new class of transfer RNA[104], resulting in amino acid substitutions or chain termination in all polypeptides coded by early cistrons. Another possibility may be that the early messenger RNA itself is altered during the latent period, rendering it still capable of attaching to ribosomes, but incapable of being translated into functional proteins.

Loading of Ribosomes on Messenger RNA

In the synthesis of alpha and beta chains of globin by both rabbit and human reticulocytes, there is apparent non-uniformity of the rate of translation over different regions of messenger RNA[105,106]. Englander and Page[107] pointed out that non-uniform rates of translation may be due to non-uniform distribution of ribosomes on messenger RNA. The possible causes of non-uniformity have been discussed by Winslow and Ingram[106] and Itano[108]. It is possible to cause non-uniform distribution of ribosomes on messengers by tryptophan starvation in reticulocyte system or by inhibiting peptide bond formation shortly after initiation by sodium fluoride. Under these conditions ribosomes are loaded at one end or the other of the message. These findings strengthen the evidence for the mechanism of action of tryptophan starvation or fluoride inhibition in causing polysome breakdown and lend support to the conclusion that under normal conditions, the ribosomes are uniformly distributed along the messenger RNA[109].

Conconi *et al.*[110] have suggested that the rate limiting steps in protein synthesis exercise their control uniformly along messenger RNA. They arrived at this conclusion on the basis of studies on the reformation of polysomes after recovery from sodium fluoride inhibition. There are two possible types of rate limiting processes which would work in such a way. The first one is a general rate limiting step in protein synthesis, such as supply of guanosine triphosphate or transfer factors. Under the second process, if the cell contains different amounts of the various transfer RNAs, then the ribosomes would wait longer at the codon corresponding to the charged transfer RNAs in short supply than at a codon for which a plentiful supply is available. And such rate limiting codons would have to be more or less uniformly distributed along the messenger RNA. This is an extention of the modulation hypothesis of Ames and Hartman[111].

It has been found that a fixed, approximately constant number of ribosomes translate the entire length of the tryptophan messenger RNA molecule[112]. The rate of ribosome movement on any region of messenger RNA is about 1350 ± 320 nucleotides per min[112]. From hybridization studies it is concluded that the first ribosome is located immediately behind the polymerase on the nascent messenger chain, and it travels in this position behind the site of RNA polymerization over the entire operon length. Morse *et al.*[112] conclude that each ribosome occupies an average RNA length of the order of 100-200 Å, comparable to the dimensions of the ribosomal subunit[113] and the ribosomes are very closely packed in bacterial polysomes.

The loading of a messenger molecule with ribosomes to give a complete polysome should require at least as much time as the synthesis of the peptide or peptides coded for by the messenger RNA, i.e. 10-20 sec at 37°. The time required to complete a polysome by attachment of ribosome to nascent messenger RNA chain might be greater than that for translation. For polycistronic messenger RNA of tryptophan operon, it is 6·5 min at 37°, for beta galactosidase messenger it is 2 min at 30°, and for phage-infected *Bacillus megaterium* it is 30-120 sec. The relationship of the time for the processes of translation, messenger RNA synthesis and loading with ribosomes is not clearly understood at present.

Bound and Free Polysomes

Ribosomes in intact cells may exist either distributed free in the cytoplasm of the cell or attached to the membrane of the endoplasmic reticulum in mammalian system[114,115] or cell membrane where endoplasmic reticulum is absent as in reticulocytes[116] or cytoplasmic membrane in bacteria[19,117,118]. The large membrane-bound particles are not broken

down by treatment with EDTA, indicating that the maintenance of the aggregates is not dependent upon the magnesium ion concentration. Exposure to pancreatic ribonuclease is also not effective in breaking down the aggregates, showing that a RNA component, which is susceptible to enzymatic attack, does not contribute to the binding[119]. Studies with puromycin or sodium fluoride treated cells indicate that binding is not dependent upon the attachment of peptidyl transfer RNA to ribosomes[119].

The rate of incorporation of amino acids into protein by membrane-bound reticulocyte ribosomes in intact cells is usually less than that of free ribosomes. In the cell-free system, membrane-bound reticulocyte ribosomes are less active than free ribosomes in incorporating amino acids into polypeptides. Thus, membrane-bound reticulocyte ribosomes making up less than 20% of the total cellular ribosome component do not account for the bulk of protein synthesis within the cell. This is in contrast to the activity of ribosomes bound to the endoplasmic reticulum[120] and the cell wall in bacteria[19,117,118].

Following pulse label, newly synthesized erythroid cell RNA appears first as free RNA and later becomes bound to the cell membrane[116]. With increasing cell age, the polysomes are less active in synthesizing protein[96,121]. This is a possible explanation for the decreased rate of protein synthesis by bound reticulocyte ribosomes.

The exact significance of membrane binding of ribosomes remains obscure. It clearly influences the stability of RNA and the activity of ribosomes in protein synthesis[122]. An increase in membrane-bound ribosome has been correlated with increase in protein synthesis which occurs during bacterial growth[123]. Changing membrane constituents may play a regulatory role in protein synthesis by alteration of the number of binding sites for polysomal attachment or by localization of coenzymes.

Palade[50], by comparing the arrangements of ribosomes in cells of different types, came to the conclusion that the incidence of rough endoplasmic reticulum is highest in cells in which the protein is synthesized for export. Liver, pancreas and mammary glands are good examples. In cells in which the protein is not exported, such as reticulocyte and tumour cells, in general, the polysomes are free.

Blobel and Potter[124] have estimated the percentage of free and membrane-bound polysomes in rat liver. The existence of two kinds of polysomes bound and free in liver cells is of interest, since liver cells are synthesizing proteins both for export and for internal use[125]. The bound polysomes are responsible for the synthesis of protein for export, such as serum albumin in liver cells[126,127] and free polysome for the synthesis of proteins for internal use.

Ribonucleoprotein Associated with Polysomes

Newly synthesized messenger RNAs are released from the polysomes as ribonucleoprotein complexes (mRNP) with relatively uniform RNA to protein ratio[128]. The mRNP complexes released from polysomes are similar in several respects to other RNP complexes in the cytoplasmic extracts which exist free of attached ribosomes. Messenger RNA in polysomes exists not as naked strand of RNA, but as an RNP complex, which has several properties in common with the polydisperse RNP that exists free of ribosome attachment. This indicates that free and polyribosomal RNP complexes may be related.

Spirin and Nemer[129] have characterized ribonucleoprotein complexes termed as inforsomes, which may represent the means of transport of messenger RNAs in developing embryos. The polydisperse RNP found in L-cells resembles inforsomes.

There is evidence that in eukaryotic cells newly synthesized molecules of messenger RNAs are transferred to the cytoplasm from their site of origin in the nucleus not as naked strands of RNA, but in association with protein, i.e. ribonucleoprotein particles[130-134]. After transfer from the nucleus, these newly synthesized messenger RNA containing particles exist in the cytoplasm for a short time as free particles until the messenger RNA is joined by ribosomes to form polysomes. The protein also enters the polysome with the messenger RNA. This is of particular interest because of the hypothesis described by Spirin[135] that protein transferred from the nucleus with the messenger RNA may be responsible for the control of protein synthesis at the translational level. Cline and Bock[136] proposed the regulation of protein synthesis at the level of translation, i.e. at polysomal level as an alternative to the classical operator regulator model of Jacob and Monod[137].

Henshaw[138] has shown that when rat liver polysomes are treated with EDTA, messenger RNAs are released from the polysomes in association with the protein. Whether this protein is the same as the one which transfers messenger RNA from the nucleus is not known. But there is similarity between the free and the polysome associated messenger RNA-containing particles. This suggests that the free particles are incorporated into polysomes and the protein could serve to modulate polysome formation.

Perry and Kelley[128] reported a similar finding in respect of L-cells. They speculated that the messenger RNA-associated protein is carried from the nucleus to the polysome. This postulation is supported by the evidence that newly synthesized messenger RNA in the nucleus is indeed associated with protein as ribonucleoprotein particles[139,140]. In cytoplasm, newly synthesized messenger RNA is associated with ribonucleoprotein particles before being incorporated into polysomes[128,130,131,133,134]. The RNA in these free particles is in all respects indistinguishable from that in the polysome-associated particles. The protein of the messenger RNA-containing particle has not been characterized. The possibility remains that the association of protein with messenger RNA is non-specific.

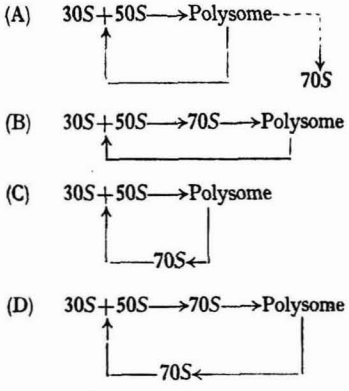
Metabolism of Polysomes

Bacterial ribosomes dissociate into 30*S*-DF complex (DF: dissociation factor), and 50*S* subunits[141,142]. The native 30*S* particle (30*S*-DF) then complexes with messenger RNA, formylmethioninyl transfer RNA[143], other initiation factors and then binds with the 50*S* particle with preceding or simultaneous

release of the dissociation factor. A polysomal ribosome is thus formed and the DF returns to form another native 30*S* particle from a free 70*S* particle[141,142].

Ron *et al.*[144] have studied the magnesium ion dependence of free and polysomal ribosome from *Esch. coli*. Polysomal ribosomes begin to dissociate into 50*S* and 30*S* subunits at about 2 mM, half dissociate at 1 mM and completely dissociate at 0·5 mM concentration of Mg^{2+}. Free ribosomes dissociate completely into subunits at 1 mM Mg^{2+} concentration. Polysomes freed from their nascent polypeptides by treatment with puromycin dissociate completely at 1 mM Mg^{2+}. At 2 mM Mg^{2+}, free ribosomes sediment as a single peak around 50*S*. They may be loosened ribosomes on the verge of dissociation and hence altered in shape, and since polysomal ribosomes do not behave in this manner, the association with peptidyl transfer RNA and messenger RNA apparently prevents such a metastable transition. Alternately at 2 mM Mg^{2+}, free ribosomes dissociate completely and then 30*S* subunits dimerize.

The fate of polysomal ribosome is not clearly understood. The following picture emerges from the studies of various investigators[145]:



When Mangiarotti and Schlessinger[38] lysed *Esch. coli* K 12 cells by a method which yielded a high proportion of polysomes, the remaining ribosomes appeared only as 50*S* and 30*S* subunits. They concluded that the 70*S* particles previously observed in other extracts were all products of polysome fragmentation[146]. In the cell, the run-off ribosomes from polysomes, following completion of their translation of a message, would yield directly only subunits, which would not reassociate until combined again with messenger RNA.

However, extracts prepared by other methods, which preserved the polysome as well, have exhibited a peak that was considered to represent largely free 70*S* ribosomes[16,38,147,148], though there is rapid exchange between subunits of 70*S* particles and free subunits in growing cell[149]. For direct participation of 30*S* subunits in initiation[150-153], an obligatory dissociation of ribosomes in the polysome cycle is by no means incompatible with the existence of a pool of free 70*S* ribosomes.

Kohler *et al.*[141] have shown the accumulation of 70*S* particles on treatment with actinomycin D or puromycin or starvation for the carbon source or for a required amino acid. The results show large increments of 70*S* peak with no detectable increase in subunits. Puromycin treatment and amino acid starvation which would eliminate nascent polypeptide (and presumably the attachment of transfer RNA) could leave messenger RNA attached to the ribosomes. However, actinomycin D treatment, and probably glucose starvation, deplete messenger RNA and thus should eliminate the basis for binding peptidyl or aminoacyl-transfer RNA as well. Thus, when released ribosomes are prevented from recycling, they accumulate in the cell as free 70*S* units. Kohler *et al.*[141] consider that the use of stoichiometric excess of deoxycholate[38] which precipitates Mg^{2+} and growth in hypertonic medium followed by osmotic shock might have caused the dissociation of 70*S* units to subunits.

Zimmermann and Levinthal[154] showed that, with actinomycin D treatment of *B. subtilis* grown in Tris-medium, 30*S* and 50*S* particles accumulate, while, with *B. subtilis* grown in phosphate medium, 70*S* particles accumulate. The run-off ribosomes from polysomes may exist as monomers (70*S*) or as their subunits (30*S* and 50*S*), depending mainly on the physiological conditions. However, there is rapid exchange between monomers and their subunits in intact cell.

Nomura and Lowry[151] reported that initiation of protein synthesis *in vitro* is a function of 30*S* subunits and not 70*S* ribosomes. They found that in the presence of messenger RNA (f_2 phage RNA) N-formylmethioninyl transfer RNA binds only to the 30*S* subunit. 70*S* particles could not bind the amino acyl transfer RNA. This implies that polysome assembly is sequential, the 30*S* subunit binding to messenger RNA before the 50*S* subunit.

Amino Acid Control of Polysome Formation

Administration of a mixture of amino acids deficient in tryptophan to rats or mice leads to loss of large polysome aggregates and accumulation of monosomes, disomes and other oligosomes[155-158]. These changes are readily reversible by feeding a complete amino acid mixture. Wunner *et al.*[157] proposed that the amino acid supply regulates the equilibrium between polysomes, ribosomes and their subunits. The effect of tryptophan deficiency on polysome formation was confirmed by Sidransky *et al.*[155], but they were unable to produce similar changes with several other essential amino acids from a mixture fed to mice. Polysomes of Chang liver tumour cells in tissue culture break down to monosomes when glutamine or arginine is removed from the medium in which they are growing.

The control of polysome formation through amino acid supply remains obscure. The difference in polysome profile between rats fed a tryptophan-deficient mixture and rats fed a complete amino acid mixture is identical in all respects to the changes in profile caused by the addition of amino acids to an *in vitro* protein synthesizing system deficient in amino acids[159]. The regulation through amino acid supply of the release of free ribosomes and

their re-utilization for polysome formation in a cell-free system demonstrate that the mechanism of control is entirely in cytoplasm. Fleck *et al.*[156] also found that the polysome pattern in liver cells could be regulated by amino acid supply even in rats treated with actinomycin D sufficient to suppress messenger RNA synthesis. It may be inferred that the amino acid supply regulates the degree of association and dissociation of ribosomes with messenger RNA. This implies that the messenger is constantly available in the cytoplasm.

During protein synthesis amino acids are required for the formation of each peptide bond from the beginning to the end of the polypeptide chain. Consequently, the continuous movement of the ribosomes along the strand of messenger RNA, from the time of association of ribosome (or ribosomal subunit) with one end of the strand until its release at the other end, is dependent on adequate supply of amino acids. In the whole cells maintained under equilibrium conditions, the rate of attachment of ribosomes to the messenger strand balances the release of ribosomes that have just completed the synthesis of the protein. In *in vitro* system, there is disaggregation of polysomes progressive with time.

When the polysome system is incubated in the absence of free amino acids, there is also breakdown of polysomes to monosomes, disomes and oligosomes. Even after prolonged incubation in the amino acid-deficient medium, the system is capable of reactivation on the addition of amino acids. This reactivation coincides with an increase in polysomes and a reduction in monosomes and disome peaks.

The idea of coupled translation and transcription proposed by Stent[160] has gained wide recognition. The model proposes that unless polysomes are formed, resulting in the translation of messenger, the messenger will not be liberated from the complex of RNA polymerase and DNA. A reduction in the rate of translation will result in a corresponding reduction in the rate of transcription of messenger RNA. Much experimental evidence now supports that ribosomes are involved in the synthesis and release of RNA from DNA template[161-164].

Contrary to the view of coupled transcription and translation processes, there are a number of reports in literature of a messenger synthesis in the absence of protein synthesis. These include the case of messenger synthesis in RC^{rel} strain[165,166] as well as RC^{str} strain of *Esch. coli*[167,168]. In these cases there must be either an uncoupling of messenger RNA synthesis from messenger RNA-ribosome interaction or uncoupling of messenger RNA-ribosome interaction from protein synthesis. Hauge[169] showed that T_2 messenger RNA synthesis takes place in a stringent amino acid starved auxotroph of *Esch. coli*; it is the coupling of polysome formation to protein synthesis which is not operative. T_2 messenger is produced in this stringent strain at a rate which is not significantly affected by 70-80% inhibition of protein synthesis and the messenger RNA so produced is associated with ribosomes, forming a normal polysome.

Sekiguchi and Cohen[170] found that 75% of messenger RNA found after T_4 phage infection is associated with ribosomes. This may indicate that messenger RNA is transferred to ribosomes whether it is translated or not. This behaviour resembles that observed with RC^{rel} and RC^{str} *Esch. coli* in the presence of chloramphenicol, where in both cases polysomes are built up in the absence of the required amino acids[171]. The need for ribosomes alone and not for translation in order to facilitate transcription is also suggested by the data of Shin and Moldave[162], Naono *et al.*[163] and Ravel and Gros[164].

The selective omission of tryptophan from an otherwise complete medium not only lowers the rate of hemoglobin synthesis, a condition observed when other amino acids are omitted, but is unique in bringing out polysome disaggregation[172]. This disaggregation may be due to the location of tryptophan codon near the 5′ ends of messenger RNAs coding for alpha and beta chains. In tryptophan deficiency, the movement of ribosomes along the messenger beyond tryptophan codon is inhibited.

Hormonal Control of Polysome Formation

Work in several laboratories has indicated that messenger RNA in nucleated cell is transported from the nucleus to the cytoplasm only when it is attached to the smaller ribosomal component[130,132,173]. In the cytoplasm, this complex appears to be united to the larger ribosomal component for the formation of polysomes. The majority of monomer ribosomes isolated from the cytoplasm are thought to be derived from polysomes. Thus, the mechanism of polysome formation must be considered in interpreting specific effects of hormone on messenger RNA synthesis and transport to the cytoplasm[174,175]. There is evidence that insulin as well as adreno-corticotropic and gonadotropic hormones may control the translational activity of cytoplasmic polysomes[176-180]. The translational effects of hormone seem to occur independently of the effects on nuclear synthesis of messenger RNA. Tata[89] emphasizes that many hormones may have multiple rather than single primary action, thereby affecting the protein synthesizing apparatus of the cells of the reproductive organ. The different effects of estrogen on the activity and the formation of uterine polysomes probably represent dual effects of the hormone on transcription which results in increased translation. Thus, the effect of estrogen on polysome formation and protein synthesis seems to be indirect.

Hamilton[181] reviewed the control by estrogen of genetic transcription and translation and concluded that β-estradiol (a) stimulates the synthesis of chromosomal and ribosomal RNAs, (b) increases the rate of precursor particles, (c) accelerates the transport of ribosomal precursor particles with attached messenger RNA to the cytoplasm, and (d) accumulates new polysomes in the cytoplasm having different amino acid incorporating properties compared to those of old ones.

Summary

Poly(ribo)somes are aggregates of ribosomes held together by strands of messenger RNA. Various methods of isolation of polysomes from different

sources are described. Polysomes may exist in a mixed (helical, random) configuration. Protein synthesis takes place on polysomes *in vitro* and *in vivo* and to a certain extent is regulated at polysomal level. A direct relationship exists between the sizes of polysomes, messenger RNAs and proteins synthesized thereon. There is a constancy in spacing of 90 nucleotides between two adjacent ribosomes on the messenger RNA and thus the loading of messengers with ribosomes is uniform under normal conditions. Free polysomes in the cytoplasm may be involved in the synthesis of proteins for internal use and membrane-bound polysomes may be responsible for the synthesis of proteins for export. Ribonucleoprotein is always associated with polysomes and may be involved in the regulation of protein synthesis at the level of translation. Polysomes are formed by the attachment of smaller subunits, initiation factors and then larger ribosomal subunits in a stepwise manner. The monomers or the subunits are detached from messenger RNA, depending on the physiological conditions. However, there is always a constant exchange between free subunits in the cell and subunits in monomers. Polysome formation and thus protein synthesis are regulated through the supply of amino acids and by the action of hormones.

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Eighth International Congress of Biochemistry

The Eighth International Congress of Biochemistry, originally proposed to be held in Rome, will now be held in Switzerland during 3-9 September 1970. The programme of the congress includes several symposia to be held in Interlaken, Luzern and Montreux.

Further details regarding the congress can be had from Mr Francis J. Griffin, 14 Belgrave Square, London SW 1.

REVIEWS

THE PHILOSOPHICAL IMPACT OF CONTEMPORARY PHYSICS by Milič Čapek (Van Nostrand-Reinhold Co., New York), 1968. Pp. xii+419. Price $ 8.00

This book is not confined, as indicated by the title, solely to contemporary physics, but is devoted to the philosophical implications of classical physics, pre-Newtonian as well as post-Newtonian. Part 1 concerning this topic deals comprehensively with the concepts of space, time, matter and motion, and presents the corpuscular-kinetic view of nature, which is the basis of classical physics. Some other collateral trends relating to time and the ultimate mechanism are also considered. This part comprising nearly one-third of the book is perhaps its best feature giving, as it does, a clear picture, specially in Chapter 6, of the main patterns of the classical mechanical picture of nature.

Part 2 deals with the revolutionary changes brought about by the theories of quantum mechanics and relativity. Each of the main physical concepts dealt with under Part 1 is systematically re-examined placing an emphasis on the historical setting and the close connection between physics and philosophy. Numerous philosophical interpretations relating to space-time (time-space as the author would have it), continuity, and determinism are carefully examined, and shown to be compatible with the corresponding physical notions. The first three chapters of this part are devoted to relativistic mechanics, of which a full and satisfactory treatment is given. The same cannot, however, be said of the next three chapters dealing with quantum theory as well as relativity. Many important aspects of the former have not been fully explained, nor sufficient emphasis laid on the fact that it was the close fusion of special relativity, and quantum mechanics, consequent on the linearity of both, that brought about the revolutionary changes in contemporary physics. The philosophical significance of this has not been carefully examined either. Some remarks on pp. 224 and 256 appear to suggest that general relativity has also been brought into close fusion with quantum mechanics, which is far from true. The introduction of terms like ' hodon ' and ' chronon ' makes all the arguments very confusing, and might well have been avoided. There are numerous repetitions of ideas, and even complete sentences throughout this part, and it is difficult to understand the main arguments, and their logical order. Chapter 17 attempts to make some positive contributions of philosophical interest to contemporary physics. This is a very ambitious programme, and it is no wonder that the contributions are too vague, and cursory, although very enlightening in some places. Chapter 18 appears to be an unnecessary repetition of ideas developed in the earlier chapters.

The last chapter containing the summary is by far the most valuable part of the book, thanks to its logical presentation. Readers who have some idea of contemporary physics and its philosophical background are well advised to read this summary first, and then delve into the several chapters containing beautiful quotations from the works of the finest philosophers of western thought of all times, the collection of which alone would make this volume charming, whatever its other defects.

The review would not be complete if some such defects were not pointed out. The author's references to mathematics in several places show the common mistake made by many laymen in confusing mathematics with theoretical physics, which, however, is not excusable in the case of a philosopher. Thus, on p. 274 the author quotes with approval the statement of someone that " the world is not a mathematics ". In fact, no mathematician claims that it is so, since mathematics is not physics, but, on the other hand, a creative art like music or painting. We also find in several places in the book a free use being made of notions like topological invariance, topology of points, topology of lumps, topology without points, mathematical continuity of space and time, and so on, without any attempt being made to clarify them.

Another defect, which is owned by the author himself in the preface, is that the book makes no reference to the striking revolutions in theoretical physics, specially in the theory of the fundamental particles, that have taken place during the last 10-15 years, since the book was already in manuscript form earlier. Just two such relevant topics of major philosophical interest might be mentioned here. The first relates to the spectacular discovery of non-conservation of parity (left or right mirror images) in weak interactions, and the consequent enunciation and proof of the *CPT*-theorem combining the notions of invariance under charge-conjugation (*C*), parity (*P*), and time reversal (*T*), and which states that symmetry could only be retained if one has a *CPT* mirror. The remarkable explanation by Feynman that the positron could be interpreted as an electron temporarily moving backward in time, and that this interpretation could be handled mathematically in a way that is entirely consistent with logic, and the laws of quantum theory, and relativity, makes it necessary that the concept of time elucidated at great length in the author's book has to be re-examined. The fact that an arrow of time can be built into some of the most elementary particle interactions has made physicists look more carefully into what the great philosophers have said about the direction of time; in other words, it appears that at the present moment, the physical implications of contemporary philosophy require a deeper study! Another topic very recently being studied, perhaps one of the most fascinating in the whole realm of physics, is the decay of the neutral K-mesons K° and $\bar{K}^{\circ}$. The violation of *CP*-symmetry in this interaction implies that it is possible to make an absolute

distinction between matter and anti-matter. This again is a problem of great philosophical significance.

In his brilliant essay on the 'Two Cultures' Lord Snow has deplored the deep schism existing between the humanities and the sciences. The present book is a welcome attempt to bridge this gulf, although one of the cultures (physics) is over-burdened by the weight of the other (philosophy)! The volume is certainly a welcome addition to the subject of the philosophy of science.

B. S. MADHAVRAO

MOLECULAR SCATTERING OF LIGHT by Immanuil L. Fabelinskii, translated from the Russian by Robert T. Beyer (Plenum Publishing Corp., New York), 1968. Pp. xxvii+622. Price $ 32.50

Of the few available treatises in the English language the translated version of the Russian edition of Dr I. L. Fabelinskii's *Molecular Scattering of Light* is the most comprehensive in its range and depth. The author, himself an active research worker at the P. N. Labedev Physics Institute of the Academy of Sciences of the USSR, has not only incorporated in the book his own contributions but has also surveyed the work of other investigators in the field, principally those of France and India. The book is chiefly devoted to the results of spectral investigation of light scattering. Aspects like Raman scattering and scattering by colloidal particles and impurities have been partly or totally omitted.

The book contains ten chapters and an appendix. The first two chapters deal with the theory of molecular light scattering in condensed isotropic media and in gases, and the spectral composition of the molecularly scattered light. The Rayleigh, Smoluchowski, Einstein and Mandelshtam, Brillouin theories have been described. A general theory of molecular light scattering in ideal crystals given by Motulevich is presented and its application to the calculation of the intensity, polarization and frequency of the Mandelshtam-Brillouin (M-B) components in cubic crystals has been discussed. In the third chapter the experimental arrangements for the study of the different aspects of light scattering have been described. These cover some of the ultrasonic devices for measuring auxiliary parameters. A review of the experimental work of molecular scattering in gases and liquids and of the fine structure in liquids has been made in the following five chapters and the results have been submitted to a critical analysis. A notable feature of these chapters is the measurements carried out by the author on the broad wing of the Rayleigh line, the determination of the absorption coefficient of hyper-sound employing the Ne-He laser and the detection of the dispersion of sound velocities in liquids. Chapter 9 is concerned with experimental determination of the M-B components and their properties in crystals. The last chapter is devoted to a description of the stimulated molecular light scattering which in recent years has assumed great importance as a result of the development of giant pulse-generating ruby lasers. The stimulated M-B scattering and the stimulated light scattering of the wing of Rayleigh line — the latter first reported by the author — have been discussed. The appendix consists of tables of scattering parameters and calculation.

The book is eminently readable and contains a stimulating account of the work, in particular of recent work, in the field. Students and research workers, who will find the book a good introduction as well as a valuable reference, owe a debt of gratitude to Mr R. T. Beyer for the service he has rendered in making accessible to them in fluent and uncramped style the English version of the Russian book. The text is free from misprints and errors and is followed by a long list of references to original papers.

K. S. IYENGAR

PREPARATIVE ORGANIC PHOTOCHEMISTRY by Alexander Schönberg, Günther Otto Schenick & Otto Albrecht Neumüller (Springer-Verlag, Berlin), Second Edition, 1968. Pp. xxiii+608. Price $ 37.00

This edition, though an enlarged version of the previous one published eleven years ago, is essentially a new book. During this period, preparative organic chemistry has grown both in size and depth. These new developments have been included in this book. This edition consists of 46 chapters. From its contents and scope of treatment, various aspects of photochemical transformations undergone by commoner types of organic molecules have been properly dealt with. Photoisomerization of dienes and trienes, ketones, esters, halides and of heterocyclic compounds containing oxygen, nitrogen and phosphorus has been discussed in a few chapters. Stereoisomerization and cyclization reactions have been dealt with in a few chapters. Photochemical cyclization, dehydrogenation, addition to carbon, carbon multiple bonds, reduction, decarboxylation, photochemical reactions of N-halogenated amines, diazoalkanes, diazoketones, diazomine salts, nitriles, epoxides and organo-metallic compounds have also been discussed in detail in this edition.

The major emphasis is on preparative aspects and on instrumentation side and a chapter on light sources and light filters has been included. Large number of interesting organic compounds like case compounds, valence isomers and cyclobutanes, which have been obtained by photochemical reaction, could not have been so easily obtained by classical methods and these have been described in detail in this book. In this respect, the utility of the book has greatly increased, as attempts have been made to complete the available data in one classified form supplemented by due references up to 1965.

In recent years, organic chemistry has made heavy ingress in the domain of photochemistry, which was formerly a chapter of physical chemistry. Recently with better understanding of physical principles and ready availability of equipments, it now constitutes a new discipline of organic chemistry and its utility in organic synthesis has been fully justified. The only criticism that can be levelled against this book is that theoretical aspects, particularly the Woodward-Hoffmann rule, have not been mentioned anywhere against the background of the wealth of information included in all the chapters of this book.

P. C. DUTTA

PHYSICAL METALLURGY OF IRON AND STEEL by Rajendra Kumar (Asia Publishing House, Bombay), 1968. Pp. xvi+456. Price Rs 35.00

The invasion of physics, chemistry and thermodynamics in the last two decades is rapidly systematizing the body of information known so far as physical metallurgy and the choice of subject matter based on one of the 80 odd metals may not nowadays meet with universal approval, but has been justified in the foreword by Prof. Honeycomb on the grounds that steel represents the most important group of engineering materials. The book has 13 chapters. In the first five chapters, Fe-C diagrams and the related topics are discussed to build up the base for the understanding of the phase transformations. Later, decomposition of austenite (VI), pearlitic transformation (VII), and bainitic transformation (VIII) are separately dealt with. Martensitic transformation and hardening of steel have been lucidly dealt with in Chapter IX. The remainder of the book deals with precipitation processes in tempering of steel, strengthening of steel, ageing and precipitating in iron and low alloy steel, and embrittlement of steel.

The book in particular is a painstaking and a good compilation of facts and figures pertaining to transformation in steel. It should have been better, however, if the author had critically examined these transformations in the light of the latest ideas and principles evolved on the thermodynamics and kinetics of structural and phase transformation. To cite an example, the underlying principles of the formation of metastable constituents, such as cementite, bainite or martensite in steel structure have not been touched upon in the discussion of the Fe-C diagram. Further, one would have expected the author to critically review the transformations in steel, in the light of the recent theories propounded regarding transformations in general. Shock hardening and irradiation of steels have been topics of great current interest, but these have not found a place in the book. Perhaps the author does not consider these topics under the general purview of physical metallurgy.

The book has comparatively few printing errors, but in general there have been a number of lapses in the citation of references. In a number of cases, details of the reference to the appropriate volume of the journal are lacking and sometimes reference, when it is due, is not cited at all. As alphabetical order has not been followed, reference numbers are necessary whenever figures or tables are cited. Though we have now adopted the metric system, the author has mixed up the inches, centimetres, centigrade and Fahrenheit — quite often on the same page and even in the same para. The reviewer was very much dismayed, however, at the fact that out of the eight hundred and odd references cited in the book only three refer to the work of Indian authors published in Indian journals! In a country where metallurgy is more or less synonymous with ferrous metallurgy and physical metallurgy synonymous with ferrous physical metallurgy, and so many metallurgists being awarded the National Metallurgists award every year, it is really disheartening that the author could not find adequate amount of good work published in India in this field for reporting in his book. The author has also not included the list of author index at the end.

Given that the above criticisms are valid, the book can be recommended as a text to the universities where physical metallurgy of iron and steel is included as a special subject. It is also a useful reference for metallurgists engaged in research and in the solving of the practical problems in the iron and steel industries. It is a commendable attempt, as there has not been any book by an Indian author so far on this subject and taking into consideration the limitations under which we work in India, it is really a praiseworthy effort. The references cited are not later than those of the year 1965 (only four of 1965!) and as neither the preface nor the foreword is dated, it is surmised that the printers have taken an unduly long time for printing the book. Our sympathies naturally go for the author, as in the meanwhile a large number of publications on phase transformations has appeared, which might have perhaps changed the author's concepts of writing such a book.

G. S. TENDOLKAR

PUBLICATIONS RECEIVED

THEORIES OF VALENCY — STRUCTURES AND PROPERTIES OF CHEMICAL COMPOUNDS by P. Ray & D. Banerjea (Indian Association for the Cultivation of Sciences, Calcutta), 1969. Pp. iv+264. Price Rs 14.00

RADIATION CHEMISTRY OF AQUEOUS SYSTEMS edited by Gabriel Stein (The Weizmann Science Press, Israel), 1969. Pp. vi+305. Price $ 13.50

CEREAL SCIENCE by Samuel A. Matz (The AVI Publishing Co. Inc., New York), 1969. Pp. vii +241. Price $ 14.00

PRINCIPLES OF ANIMAL ENVIRONMENT — ENVIRONMENTAL ENGINEERING IN AGRICULTURE AND FOOD SERIES by Merlel Esmay (The AVI Publishing Co. Inc., New York), 1969. Pp. vii+325. Price $ 16.00

CHEMICAL ANALYSIS OF INDUSTRIAL WATER by James W. McCoy (Chemical Publishing Co. Inc., New York), 1969. Pp. xi+292. Price $ 15.00

ELECTROPLATING AND RELATED PROCESSES by J. B. Mohler (Chemical Publishing Co. Inc., New York), 1969. Pp. viii+311. Price $ 10.00

THE PHYSICS OF SELENIUM AND TELLURIUM edited by W. Charles Cooper (Pergamon Press Inc., New York), 1969. Pp. ix+380. Price $ 18.50

QUANTITATIVE ANALYTICAL CHEMISTRY: Vol. I — INTRODUCTION TO PRINCIPLES by H. A. Flaschka, A. J. Barnard (Jr) & P. E. Sturrock (Barnes & Noble Inc., New York), 1969. Pp. xiii+594. Price $ 5.95

QUANTITATIVE ANALYTICAL CHEMISTRY: Vol. II — SHORT INTRODUCTION TO PRACTICE by H. A. Flaschka, A. J. Barnard (Jr) & P. E. Sturrock (Barnes & Noble Inc., New York), 1969. Pp. ix +290. Price $ 2.95

NOTES & NEWS

Picture processing by computer

New techniques of picture processing by computer developed in the recent past have become immensely valuable as powerful tools in the realms of science, engineering, education and art. By picture processing is meant either the transformation of graphical material, or the generation of pictures from data or abstract rules alone, or combinations of these operations. There are thus four categories relative to the computer's input-output terminals:

Transformation

(1) Picture→picture (some transformation is made)
(2) Picture→abstraction (something is measured or recognized)

Generation

(3) Data→picture (an invisible real-world phenomenon is made visible)
(4) Abstraction→picture (a picture is synthesized from generating rules)

The basic principles, the present state of the art and the current or potential areas of application of these different categories of picture processing are briefly reviewed by Leon D. Harman and Kenneth C. Knowlton [*Science, N.Y.*, **164** (1969), 19-29].

A typical picture reproduction system would consist of a scanner, similar to a television camera. The electrical signals from the scanner are then changed by an analogue-to-digital converter into numerical representations on magnetic tape. A general purpose, high speed digital computer is programmed to process the picture from the digital version, according to one or more algorithms. The processed picture, which is still in discrete numerical form, is then converted into the final photographic form by a microfilm printer which receives the necessary instructions from the computer. More microfilm systems yield only black-and-white output; colour pictures are possible, but the technology is not well developed yet.

Picture-to-picture transformation —Computers were used more than a decade ago for research in television bandwidth compression. Pictures were stored on an element-by-element basis, and various information-reducing manipulations were introduced experimentally. Portions of a two-dimensional picture were controllably transformed in the computer domain and then were displayed to observers for assessment of the quality of hypothetical transmission systems. There are several modifications and improvements to this sort of picture-to-picture transformation. In one version, microfilm plotters are employed rather than conventional television tube displays, for graphical rather than pictorial output. This method is usually employed for simulating a halftone process. Another process which results in apparently continuous tone pictures from binary microfilm dot-plotting uses a technique analogous to the variable dot size process of conventional halftone engraving. Another useful picture transformation procedure is that of crispening images and changing contrasts. Examples are: (i) meteorological satellite cloud pictures, where enhanced boundary processing facilitates weather analysis; and (ii) X-ray and electron microscopic photography where crispening and nonlinear density manipulations are frequently required for effective processing, either manually or by machine. Yet another kind of image transformation is that of reducing a stereoscopic pair to a single picture with the third dimension portrayed, for example, by contour lines, as in the automatic analysis of aerial stereoscopic photographs.

Picture-to-abstraction —The situation here is one which involves automatic analysis of a photographic image or a line drawing. Generally, the output is some statement of recognition or some nominal, positional, or categorical listing. For example, in particle-track detection, a single system may produce more than 10 million pictures a year. These must be screened to find and analyse the small proportion containing tracks of interest. Techniques are under development to have computers accept photographs (including stereoscopic pairs of the particle tracks), refine the traces (by thinning lines, filling gaps), and then determine line segments, directions, lengths and modes. This information can be used to reconstruct idealized line images and even to select and print out only that subset which satisfies certain predetermined criteria. The most common form of pattern recognition by computer does not yield a picture transformation. Rather, some decision-making process is initiated, or a literal or symbolic statement is produced. Machine reading of bank cheques, credit-card imprints and typescript are examples.

Data-to-picture — This category of processing by computer involves assembly and pictorial portrayal of masses of datum points permitting one to see an otherwise invisible phenomenon. A typical example of a spectral reconstruction for rapid integration by human vision could be had in volatile graphic display of air traffic in which a radar blip depicting an aircraft can be automatically accompanied by alphanumeric identification and flight information. Another kind of data-to-picture transformation is that of map making, including weather maps. A large collection of data is stored, and from it any one of a number of displays showing, for instance, population density, economic levels, and housing conditions, can be generated on request. Less than one hour of computer processing and printing can produce a set of maps which replaces weeks of skilled human effort.

Abstraction-to-picture — On this type of transformation visual images are generated via random-dot patterns by means of a microfilm printer for producing black-and-white dot structures whose individual loci and statistics are flexibly manipulated for a wide variety of studies in human perception, notably stereopsis. The technique permits rich flexibility of

stimulus generation and control, impossible by other methods.

Many visual patterns produced by this technique and related processes can represent objects not physically realizable and thus provide an entirely new domain of sensory stimulation. One notable example of this transformation is the stimulation which permits an engineer to synthesize and activate a circuit by drawing a complicated electronic schematic on the face of a cathode ray tube. "Stock parts" are evoked from the computer memory, and the engineer types in component values and enters input signals. Within seconds he has displayed to him the plotted graphic response of the system to the specified input. Other examples of explicitly ordered pictures could be generation of type fonts and the production of printed circuit designs and masks. The ability to "see" mathematical models and the results of their application can provide powerful insights. Computers have been used widely in this respect, both for pedagogy and for research. Another kind of computer picture processing concerns data which when analysed by humans are pictorial, but which need not be pictorial before machine use. Electroencephalography and electrocardiography are examples.

To conclude, image analysis with the aid of computer ranges from submicroscopic to cosmological and from natural phenomena to complete abstraction. The utility, both quantitative and qualitative, is similarly spectacular. The primary gains are in labour, time and money. Tedious human effort is saved; hours or days of delay are compressed to minutes.

Progress in laser-made plasmas

Research and development work now in progress at a few centres in the United States indicates a fair prospect for the eventual realization of controlled fusion through laser-made plasmas. A concentrated burst of laser light on small amounts of solid matter will at once vaporize and ionize them. Such ionization is capable of producing plasmas, which can be usefully employed in research on thermonuclear fusion.

Small pellets of solid matter are preferred to either a whole solid block of matter or even gas for making plasmas with laser beams. The reason for such a preference is that in both the latter cases the average energy per particle remains low on account of cluttering up of neutral particles of matter with ionized ones. In the case of pellets, however, the whole sample gets ionized and thus gives high average energies.

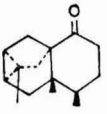
There is one drawback, however, in the case of pellets. The solid-pellet plasma expands very rapidly, thereby causing a drop in energy concentration. The heating pulse must, therefore, be very fast and the limitation is the shortness of the laser pulses currently available. Dr Alan Haught of United Aircraft has produced plasmas with average energy per particle of the order of 100 eV. Dr Moshe Lubin of the University of Rochester has obtained 1000 eV per particle by shining a laser producing $1{\cdot}5\times10^{9}$ W in bursts about 4 nsec long on a sample of lithium fluoride.

A plasma produced by laser irradiation has a number of advantages for controlled fusion work. The plasma can be produced inside a magnetic field, without physical connections to the environment outside. If one makes a plasma outside the field, one has to make a hole in the field to put the plasma inside; the subsequent closing of the hole is not always easy to accomplish. Alternatively, a neutral gas can be held and ionized inside the field by shooting a spark through the chamber. But laser light will cross a magnetic field without any external physical aid. The plasma is made inside the field without any loss of its energy by collisions with the background gas. Ionization is, therefore, complete.

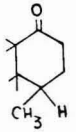
Dr Haught and Dr Lubin have found that magnetic fields stop expansion of the plasma and the energy that has been used for expansion is then reconverted to kinetic temperature. They are now engaged in observing if this excellent behaviour of plasmas continues at higher energies. If this can be achieved, then controlled fusion as in the case of hydrogen bomb is feasible. But laser technologists consider that the threshold of thermonuclear power through laser is yet far off and that if an energy of 15,000 eV is achieved that would be a factor of four, too low in energy. Moreover, a large volume is also needed to get far greater energy output than is put in. Presently Dr Haught is attempting to realize average energies of 5,000-10,000 eV and Dr Lubin is developing a laser which will create particle energies greater than 15,000 eV [*Sci. News*, **95** (1969), 384].

Ishwarone, a novel tetracyclic sesquiterpene

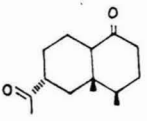
Ishwarone, a novel tetracyclic sesquiterpene isolated in 1935 from *Aristolochia indica*, and the first of its kind to occur in nature, has been assigned structure (I) on the basis of chemical and spectral evidence by a group of scientists led by Dr T. R. Govindachari at the Ciba Research Centre, Bombay.



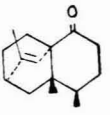
I



II



III



IV

Ishwarone, $C_{15}H_{22}O$, m.p. 57°, $[\alpha]_D^{25}+22{\cdot}9°$, λ_{EtOH} 211 mμ (ϵ 275), λ_{max} 285-290 mμ (ϵ 30); $\nu_{max}^{CCl_4}$ 1706 (6 or higher membered saturated ketone) and 1418 cm^{-1} ($-CO-CH_2$) and NMR signals at

δ 0·75 (singlet, $-C-CH_3$), 1·15

(*s*, $-C-CH_3$), 0·85 (doublet, J =

6·5 cps, $-CHCH_3$) and 0·55 (multi-

plet, cyclopropane H) is tetracyclic (resistance to hydrogenation,

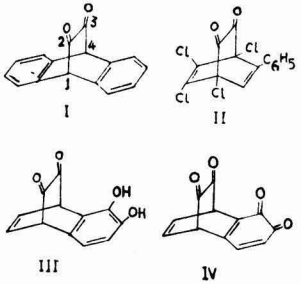
lack of $C=C$ in IR, NMR and

Raman spectra). Barton oxidation of ishwarone gave a diosphenol (methyl ether, m.p. 105-108°), which on oxidation with H_2O_2-

NaOH gives a dicarboxylic acid, ishwaric acid, $C_{15}H_{22}O_4$, m.p. 141-6°. Pyrolysis of the acid or Dieckmann cyclization of its dimethyl ester affords the cyclopentanone, norishwarone, $C_{14}H_{20}O$, b.p. 105-8°/0·5 mm, $\nu_{max}^{CH_2Cl_2}$ 1728 cm^{-1}, which in turn gives a diosphenol, $C_{14}H_{18}O_2$, m.p. 148-50°, showing NMR signals at δ 0·98 (s, $-\overset{|}{\underset{|}{C}}-CH_3$), 1·15 (s, $-\overset{|}{\underset{|}{C}}-CH_3$) and 1·87 (s, olefinic methyl). These data allow the deduction of part structure (II) for ishwarone. Further elaboration was possible using isoishwarone obtained from opening of the cyclopropane ring. Ishwarone, on treatment with dry HCl in ether at 0°, followed by brief exposure to boiling pyridine, gives an isomeric mixture of two unsaturated ketones which is homogenized by treatment with *p*-toluenesulphonic acid in benzene to isoishwarone, $C_{15}H_{22}O$, b.p. 100°/0·7 mm, $[\alpha]_D^{25°}$ −74·61°, ν_{max}^{Film} 1700 cm^{-1} (6-membered ketone) and NMR signals at δ 0·77 (s, $-\overset{|}{\underset{|}{C}}-CH_3$), 0·75 (*d*, $J = 6·5$ cps, $-\underset{|}{CH}-CH_3$), 1·78 (*d*, $J = 1·5$ cps, olefinic methyl) and 5·72 (unresolved multiplet, olefinic H). Isoishwarone with OsO_4 yields a diol, $C_{15}H_{24}O_3$, m.p. 180-82°, $[\alpha]_D^{25°}$ −80° which is smoothly cleaved by $NaIO_4$ to a diketo aldehyde, $C_{15}H_{22}O_3$, m.p. 108-10°, $\nu_{max}^{CH_2Cl_2}$ 1725 (CHO) and 1705 cm^{-1} (C=O) and NMR signals at δ 0·80 (s, $-\overset{|}{\underset{|}{C}}-CH_3$), 0·92 (*d*, $J = 7$ cps, $-\underset{|}{CHCH_3}$), 2·17 (s, $-CO-CH_3$) and 10·05 (s, −CHO). Ozonolysis of isoishwarone gave, besides the diketo aldehyde, a diketone, $C_{14}H_{22}O_2$, m.p. 87-89°, $[\alpha]_D^{25°}$ +1·97°, ν_{max}^{KBr} 1700 and 1710 cm^{-1} (carbonyls) and NMR signals at δ 0·65 (s, $-\overset{|}{\underset{|}{C}}-CH_3$), 0·92 (*d*, $J = 6·5$ cps, $-\underset{|}{CHCH_3}$) and 2·08 (s, $-COCH_3$) which is found to be identical in all respects (TLC, m.p., m.m.p., IR and ORD) with an authentic specimen of the diketone (III) prepared from valerianol. The above correlation requires that isoishwarone be formulated as (IV), assuming that the stereochemistry of the methyl ketone centre in (III) has not been affected. Ishwarone can then be represented as (I) and this was further supported by a series of reactions, e.g. treatment with ozone to oxoishwarone, with dil. HCl to a chloro compound, etc. [*Tetrahedron Lett.*, No. 3 (1969), 133].

Expulsion of bis-CO from α-diketones

Taking clue from the facile thermal decarbonylation of norbornadiene-7-ones, the expulsion of thermal bridges from bicyclo[2,2,2]octadiene-2,3-diones (I-IV) having a double bond arrangement similar to that of norbornadiene-7-one, has been attempted. Compound (I) charred on heating at 350°, giving 80% of the starting material. Compounds (II) and (III) at 180° and 220° gave respectively 32% of 2,3,4,5-tetrachlorobiphenyl and 1,2-dihydroxynaphthalene as a result of expulsion of CO.

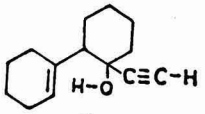


Photolysis of (I)-(IV) in benzene solution gave a smooth evolution of CO, forming respectively anthracene, tetrachlorobiphenyl (97%), 1,2-dihydroxynaphthalene (53%) and 1,2-α-naphthoquinone isolated as benzo[α]phenazine (43%).

The nature of the expelled species as C_2O_2 or bis-CO has been ascertained from mass fragmentation of (I). Support for the fact that the bridge connecting 1,2- and 3,4-bonds is cleaved (concerted or stepwise) prior to the bridge forming 2,3-bond has been found by irradiation of a bridged monoketone. Aromatization may be considered as the important driving force for the reactions described above [*Tetrahedron Lett.*, No. 3 (1969), 125].

Pyrolysis of β-hydroxy olefins

Pyrolysis of β-hydroxy olefins to give olefins and carbonyl compounds has proved to be a general and reliable transformation and an elegant synthetic method for extending carbon chains. During the pyrolysis of (I) in an evacuated

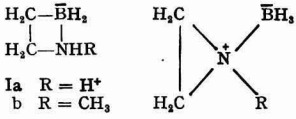


I

pyrex tube at 300° for 24 hr (or at 350° for 2 hr), the formation of 1,2,3,4,5,6,7,8-octahydrophenanthrene in almost quantitative yield has been reported, instead of the expected unsaturated ketone. Further confirmation of the absence of ketone has been obtained from the lack of olefinic protons in the NMR and absence of a carbonyl bond in the IR. The pyrolysis product has been characterized by NMR, UV, IR and mass spectral data [*Tetrahedron Lett.*, No. 3 (1969), 205].

Reaction of aziridine with sodium borohydride

Akerfeldt and Hellstorm [*Acta chem. scand.*, **20** (1966), 1418] reported that the reaction between aziridine and sodium borohydride gives the four-membered heterocyclic compound (Ia) rather than aziridineborane (IIa).



It was considered that the proposed structure was inconclusive, since it was based on one of the several possible interpretations of NMR spectrum. The reaction of aziridine with sodium borohydride was repeated in aqueous tetrahydrofuran. A product was isolated, which analysed for C_2H_8BN. The IR of this compound showed the presence of N^+-H (3300 and

2740 cm^{-1}) as would be expected from either (Ia) or (IIa).

On the other hand, in the case of (Ib) and (IIb), only (Ib) contains an N^+-H group. The reaction was, therefore, carried out under the same conditions with N-methylaziridine and a white solid was isolated which melted at 57-8° and analysed for $C_3H_{10}BN$. The IR of this compound showed no absorption characteristic of N^+-H group which eliminates (Ib) as the possible structure and suggests that the compound is (IIb). By analogy the compound obtained by Akerfeldt and Hellstorm would thus be (IIa). The ^{11}B NMR spectra of the compounds C_2H_8BN and $C_3H_{10}BN$, from aziridine and N-methylaziridine respectively, provided convincing evidence for the structures (IIa) and (IIb) [*Tetrahedron Lett.*, No. 59 (1968), 6169].

Metabolic fate of glutathione in human erythrocytes

The way in which glutathione, known to be synthesized in the red cell and having a half-life of 2-3 days, undergoes destruction in the erythrocyte remained a mystery until recently Srivastava and Beutler, using normal and glucose-6-P dehydrogenase-deficient human erythrocytes, showed that the movement of oxidized glutathione (GSSG) from the red cells is the result of an active process, unidirectional, dependent upon metabolic energy and temperature-sensitive [*J. biol. Chem.*, **244** (1969), 9]. They arrived at this conclusion on the basis of the observation that a portion of the oxidized glutathione disappears from the erythrocytes and can be recovered from the medium when the normal cells are incubated in the absence of glucose or when glucose-6-P dehydrogenase cells are incubated in the presence or absence of glucose. This movement of GSSG is unidirectional, as there is no transport of GSSG from the medium to the red cells when the normal cells are incubated in a medium containing a high concentration of added GSSG.

The continuance of outward transport of GSSG even against a concentration gradient as high as 25-fold is suggestive of the involvement of an active process in GSSG transport. Since the normal cells suspended in a glucose-free incubation medium as well as the glucose-6-P dehydrogenase-deficient red cells both in the presence or absence of glucose in the medium can transport GSSG for several hours, the source of energy for the active process appears to be 'endogenous', probably ATP. The observation that GSSG transport halts almost entirely after the endogenous compounds, such as ATP, are exhausted by preliminary incubation of red cells in a glucose-free medium for 8 hr or by the presence of 0·1*M* fluoride in the incubation medium provides evidence in support of ATP as the endogenous source of energy for the active transport. This observation has found further support from the finding of sensitiveness of GSSG transport to fluoride ion inhibition, a finding compatible with the involvement of ATP in the energy source by binding the Mg^{2+}. However, the possibility that the fluoride ions may inhibit directly a component of the transport system cannot be excluded.

Based on the hypothesis of active transport of GSSG from red cells, it is now possible to account for the low levels of total glutathione in glucose-6-P dehydrogenase-deficient erythrocytes and the fate of intraerythrocytic glutathione, which has a normal half-life of 2-3 days.

Sensitive radiochemical assay for δ-aminolevulinic acid synthetase

A new method of measuring δ-aminolevulinic acid (ALA) synthetase activity has been reported from the Department of Biochemistry, University of Adelaide, Adelaide, South Australia. The method, which is based on the incorporation of ^{14}C-succinate into ALA, is extremely sensitive, permits assay of the enzyme in normal tissues as well as in small amounts of tissue for which the existing calorimetric methods are inadequate and avoids difficulties generally encountered in the existing methods due to amino acetone synthesis. Moreover, the sensitivity of the method can be adjusted according to need by increasing the specific activity of the ^{14}C-succinate used. However, due to the high cost of the isotope used, the method is not recommended where large number of routine assays of samples with high ALA synthetase activity and small amino acetone synthetase activity are to be made.

To standard solutions of ALA.HCl in trichloroacetic acid (TCA) (or TCA supernatant of tissue incubations), 100 μmoles of labelled potassium succinate and 1 μmole of carrier ALA.HCl are added and the reaction mixture is applied to Dowex-50(H^+) columns (3×1 cm). The columns are washed with 30 ml of water and ALA is eluted with 10 ml of 2*M* pyridine-acetate buffer (*p*H 6·0). The eluates are collected in round-bottomed flasks containing 1 drop of conc. HCl and twice evaporated to dryness under reduced pressure on a Buchi rotovapor and the residues are dissolved in known volumes of water. Aliquots of the above purified eluate are mixed with a suitable volume (1 ml) of acetate buffer (*p*H 4·6) and 0·1 ml of acetylacetone. The tubes are boiled for 15 min and the ALA pyrole is extracted into 25 ml of ethyl acetate and then washed with 3 ml of water; the ethyl acetate layer is dried and the residue is dissolved in 1 ml of methanol. Aliquots (0·8 ml) of the methanolic solutions are plated into metal planchettes and counted in a Nuclear-Chicago gas flow counter [*J. biol. Chem.*, **244** (1969), 60].

Department of Atomic Energy

An outstanding achievement of the Department during the year under review (1968-69) was the going into criticality of both the reactors of the Tarapore Atomic Power Station; full commercial operation at 380 MW is expected to be achieved shortly. The other two atomic power projects, viz. the Rajasthan Atomic Power Project and the Madras Atomic Power Project, made sufficient headway during the year.

Bhabha Atomic Research Centre (BARC)—The three constituent reactors, Apsara, CIRUS and Zerlina, operated satisfactorily throughout the year. In the thermal column of Apsara, a pneumatic self-serve facility was installed for irradiation of samples. A fast neutron seed irradiation facility for irradiation of biological samples was installed in the reactor pool in collaboration

with the International Atomic Energy Agency. In the Zerlina, design modifications to the core support structure were incorporated and special operational assistance as required was extended in connection with Indo-Australian experiments on fast fission ratio measurements in UO_2 clusters under simulated boiling water conditions.

In the Physics group, the study of K X-rays emitted in the thermal neutron-induced fission of ^{235}U was carried out using CIRUS. The total yield of fragments was determined. A high resolution X-ray spectrometer employing a lithium-drifted silicon detector was fabricated for this purpose. Two units of a centrifugal molecular still were completed and various grades of high vacuum oils were produced by molecular distillation starting from crude oil available from indigenous sources. There is a recurring demand for these oils and it is estimated that their production will result in an annual saving of Rs 3·4 lakhs in foreign exchange. A notable achievement was the development of a proportional type temperature controller with an accuracy of 0·5°C.

The important development work in the Electronics group was concerned with: Mössbauer spectrometer for applications in rare earth spectral region, 2-parameter nuclear data processor (for Nuclear Physics Division), transistorized nuclear instrument modules capable of assembling nuclear counting systems, medical scanner for thyroid uptake studies, dual-channel electromyograph for diagnosis of neuromuscular paralysing disorders, transistorized liquid scintillation spectrometer for the assay of low activities and critically monitoring system for active areas for the detection of rapid changes in radiation levels. Very good epitaxial deposits of GaAs on Ge substrates have been obtained and optical systems having as many as 11 dielectric layers to improve the efficiency of ruby laser, with a view to using it as a range finder, have been completed. Work on low noise, high sensitivity oscilloscope display systems for biological measurements and frequency synthesis, handling frequencies up to 20 Mc/s with 5 kc/s spacing has reached the final stage of completion.

In the Health Physics group, a micrometeorological station with 122 m high tower was established near the Tarapore Atomic Power Project site which provides the necessary meteorological information for the control of radioactive contamination.

The computer facility built around H-400 Honeywell computer system was set up in the Modular Laboratories towards the end of August 1968. A feasibility study has been undertaken for the control systems, with particular reference to a Data Logger and Recording Annunciator System for the reactor of the Rajasthan Atomic Power Project, and a saving of Rs 4·5 lakhs in foreign exchange is expected.

In the Chemical Engineering group, air pulsers have been successfully adopted in the solvent extraction processes in place of the costly mechanical pulsating pumps. Experiments on solar evaporation, making use of extended surface technique, were continued in the Water Treatment Division. High concentration of activity in sludge was obtained and the process was found to be far cheaper than all the other alternative methods available for the volume reduction of medium level radioactive wastes.

In the Metallurgy group, using the techniques of activation analysis and radiochemical methods, methods were standardized for (i) the determination of samarium and neodymium in pure lanthanum oxide, (ii) thorium in rocks, (iii) isotopic analysis of ^{288}Ra and ^{224}Ra in the presence of ^{226}Ra, (iv) isotopic dilution analysis of samarium in rare earths, and (v) radiochemical separation of Np and Pa on $BaSO_4$. Isotopic ratios of $^{208}Pb/^{204}Pb$ have been measured on a number of galenas to determine their model ages. From the anomaly in $^{234}U/^{238}U$ ratios in a secondary formation from Naygaon, its refined age has been calculated.

In the Biomedical group, studies have been conducted for adducing evidence to show that callus cells, under appropriate conditions of exogenous nutrients, formed embryoids and later developed into plantlets. This totopotency of somatic cells observed so far only in carrot and tobacco has now been established in a medicinal plant, *Tylophura* (Asclepiadaceae).

Tata Institute of Fundamental Research (TIFR) — Research work in theoretical physics was concerned with particle physics, high energy nuclear physics, solid state physics, magnetohydrodynamics and astrophysics. In medical electronics, a device for monitoring the degree of variation of energy associated with the electrical activity of human brain has been developed and built. This equipment can be attached to a conventional electroencephalogram machine. In the area of nuclear structure studies, parameters of some low-lying electric dipole transitions in the deformed nuclei, samarium-152, gadolinium-152 and dysprosium-160, have been measured using the single-gap beta ray spectrometer fabricated at the Institute. In solid state physics, a phenomenological model has been developed to understand the factors affecting the lifetimes of positronium atoms in molecular materials. In the field of cosmic radiation, new methods developed last year for the study of cosmic ray fossil tracks in crystals in meteorites were applied to investigate the charge and energy spectra of nuclei with charges greater than 22. It was found that the time averaged intensity of iron group of nuclei, in the kinetic energy interval 700-150, MeV/nucleon, during the last 11 million years, was the same as that observed at the earth today during periods of low solar activity; however, at lower energies the flux was considerably higher. The charge spectrum over the interval of charge Z from 30 to 60 appears to differ considerably from the universal cosmic abundances. These results are important for our understanding of solar terrestrial relations and acceleration processes in massive stellar envelopes.

Physical Research Laboratory (PRL) — A superneutron monitor (18 NM 64) consisting of three independent sections, each with 6 counters, which was set up towards the end of last year, started working regularly from August 1968. A high counting rate μ-meson directional detector has been commissioned and is providing valuable information on the energy spectrum of the cosmic ray time variations related to changes

in the conditions of interplanetary space. The solar radio spectroscope was brought into regular operation for recording the dynamic spectra of solar radio bursts in the frequency range 40-240 MHz.

Thumba Equatorial Rocket Launching Station (TERLS) — As part of the Space Science Research Programme three magnetometer-Langmuir Probe payloads were launched in August 1968 from Thumba to study the parameters of the quiet day equatorial electrojet. Eight Nike-Apache, two boosted Arcas rockets and four Centaure rockets were launched during the year, with various objectives, such as measuring neutral atmospheric wind above 85 km, study of electric field in the ionosphere using $BaCl_2$ technique, obtaining information on the equatorial electron density distribution in the D-region of the ionosphere, etc. A universal launcher has been designed and manufactured by TERLS to launch 2-stage Nike-Apache or Centaure or a Menaka meteorological rocket.

Coal research in Australia

The Division of Mineral Chemistry of the Coal Research Laboratory of the CSIRO, Australia, is concerned with investigations on combustion processes in general and flames in particular. The results of three major projects are presented in report No. 36 of the laboratory. Abstracts of papers published and a list of recent publications in the field are also included. The first study covered in this report deals with the petrography of the Tomago coals, and contains the main conclusions drawn from the petrographic investigations of samples from the Tomago coal measures. From investigations on as many as 33 samples it was deduced that there were two categories of coal, depending upon the microlithotype contents of the clean coal composites of the seams, viz. (i) those with a moderate to high (more than 40%) vitrite+clarite content, and (ii) those with a very low (less than 20%) content of vitrite + clarite. Two kinds of vitrinite were identified: vitrinite A, which usually has a coherent botanical structure and occurs in layers of nearly 100% purity, and vitrinite B, which is intimately associated with other macerals and lacks coherent botanical structure. Reflectance measurements were made on polished surfaces of vitrinite from each of the six idealized Tomago seams. It was found that all the coals were of high volatile bituminous rank.

The second study on "Chromatographic gas analysis" reviews the type of apparatus used and gives examples of gas mixtures which have been separated by the technique, and are of interest to the fuel industry.

The third study entitled "Vitrinite-semifusivite transition material" deals with the work done to determine precisely where the line of demarcation between fusible and infusible components occurs. For this investigation, a small block, a few millimetres across, including the region of interest, was cut from a suitable area of coal in a large polished surface. The block was next polished and photographed and the maximum reflectance of the vitrinite and semifusivite was measured using a photomultiplier. The substance was carbonized under certain specified conditions and photographed. The carbonized layer was then polished again and a third photograph taken. A comparison of these photographs enabled measurements on expansion. The main conclusions drawn from such a study on a medium volatile coal were: (i) the line of demarcation between the fusible and infusible components of the inertinite maceral occurs in the transition zone between vitrinite and semifusivite, usually at about the point where open plant cell structures can first be recognized; (ii) an anomalous, granular type of vitrinite, producing a thick-walled coke when carbonized, is associated with the material from the vitrinite-semifusivite transition zone; and (iii) unusually high coke bireflectance values are probably the result of high pressure applied during the carbonization process.

Announcements

▪ *Bires Chandra Guha Memorial Lecturership and Sunder Lal Hora Medal* — The National Institute of Sciences of India has awarded the Guha Memorial Lecturership for distinct contributions in the field of biochemistry, nutrition, food and allied problems, and the Hora Medal for outstanding work in biological and medical sciences for the year 1969 to Dr V. Subrahmanyan and Dr K. Ramiah respectively.

Dr V. Subrahmanyan, formerly Director and now Emeritus Scientist, Central Food and Technological Research Institute, Mysore, is well known for his important contributions to indigenous food industry.

Dr Ramiah, Member, Rajya Sabha, is internationally known for his outstanding researches on genetical breeding and agronomical aspects of rice production and has a large number of publications in the field of genetics and agronomy of rice, cotton and wheat. He has been responsible for introducing several new rice varieties which are being grown in several parts of India and in some foreign countries.

▪ *Second Mastech Conference* — The CSIR-sponsored Second Mastech Conference on 'Probability and statistics and their applications to science and technology' will be held in Madras for a week in December 1969 or January 1970.

Research papers relating to probability, statistics or their applications to science and technology may be sent to Prof. Alladi Ramakrishnan, Director, Matscience, Madras. An award of Rs 1000 will be made to the author of the best paper read at the conference.

The Mastech award for the best paper at the first conference on 'Matrix analysis' held in Bangalore in September 1969 has gone to Prof. Mehdi S. Zarghamee, Aria-Mehr University, Tehran.

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