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# Journal of Scientific & Industrial Research

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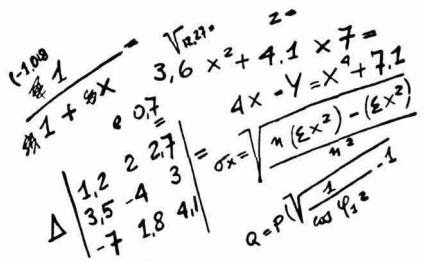

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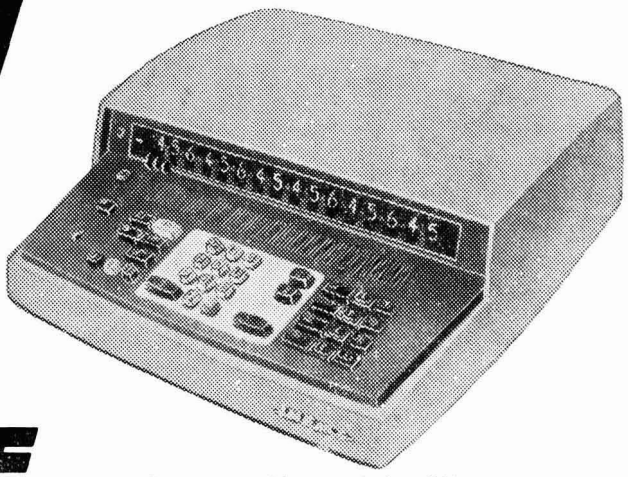

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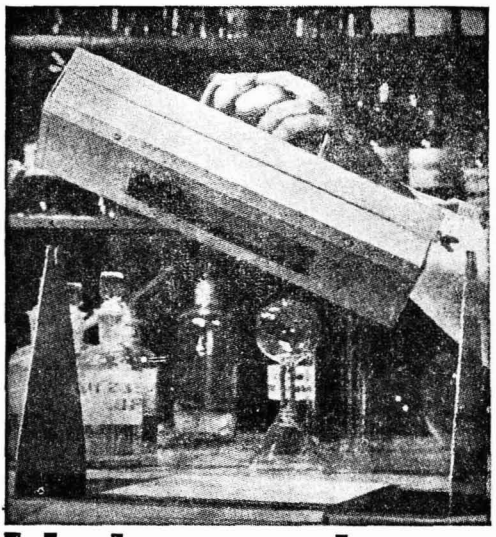

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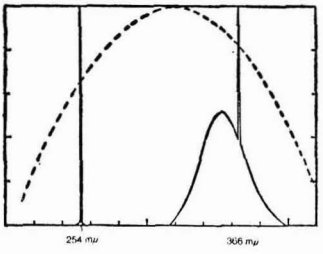


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# Current Topics

## Science & Parliament

IN India, as in most of the other developing countries, scientific and technological research is the primary responsibility of the state, which provides the bulk of the funds needed to raise the country's scientific and technological potential. This situation coupled with the fact that the state of development of science and technology in a country has considerable impact on other spheres of national activity, particularly the social and economic spheres, calls for a close link between the organization of scientific and technological research and the policy formulating system of the country, represented broadly by the parliament. The Science Policy Resolution of the Government of India was formulated and adopted by the Government in 1958, but the implementation of the policy at the institutional level has not made much headway. Positive action in this direction has much to do with the effective utilization of science and technology in the economic development of the country. In the context of the above considerations, the Seminar on 'Science & Parliament', organized in New Delhi during the last week of April 1968, by the Institute of Constitutional and Parliamentary Studies, was timely. The seminar provided a common meeting ground for leaders representing various spheres of scientific activity and the parliamentarians to have a frank discussion on various aspects of the relationship between the organization of scientific research and the decision making political system, and related problems.

Among the subjects debated at the seminar, the following were some of the major ones: (1) relationships between science policy and social, political and economic policies of the state and the respective organizational machineries; (2) creation of such environment in research establishments as may be conducive to maximum creative output; (3) augmentation of facilities for research and its diversification through allocation of more funds for science and education; (4) introduction of scientific methods in government decision making machinery; (5) international cooperation in research; (6) role of industry in encouraging research and development, and the methods used by governments to encourage innovation and development; and (7) impact of financial limits imposed by national budgets on the progress of scientific research.

Discussions during the seminar focused attention on the disparities existing in the development of various spheres of scientific and technological activity. A typical example cited was that of agricultural research which, though forming the basis of the success of industrial research programmes, has not received the priority it deserves. There was almost unanimity of opinion on the need for attaching priorities to various programmes of research in consonance with the national economic and social objectives as well as for making research organizations and government agencies responsible for them to take cognizance of the changing social and economic problems of the country. The lack of facilities for the provision of uptodate information on the status of science and technology in the country to the members of parliament was pointed out and the need for effective communication of information was stressed.

As practical means of forging a sound link between the bodies responsible for organizing scientific and technological research and the decision making political system, the seminar favoured, among other measures, the following two: (1) submission to the parliament of an annual 'Science Report' on the status of science and technology in the country; and (2) appointment of a permanent parliamentary committee for scientific research. The 'Science Report' should highlight the achievements in various fields of scientific investigation, the projects under way and the likely benefits, both economic and social, of the research effort in the country. It should also delineate the programmes in the coming year and the next five years in prespective. A statistics section of the report should give, among other things, financial, manpower and research output data. The financial data should give the breakup of expenditure between (i) government departments; (ii) government-sponsored programmes (both inter- and intra-government); (iii) research and development and promotional activities; (iv) basic, applied and developmental research; (v) different scientific fields; (vi) economic sectors; (vii) various types of government-financed agencies, e.g. government research centres, universities, cooperative research associations, etc.; (viii) distribution of expenditure on research capital costs and maintenance costs; (ix) geographical distribution of expenditure; and (x) technological balance of payment. The manpower data should give the breakup of scientific and technical manpower between (i) occupational categories; (ii) scientific disciplines, and also specialization within disciplines; and (iii) levels of educations, including supply and distribution of support personnel. The research output data should include lists of (i) patents taken and released to industry; (ii) processes under investigation and likely to be available for industrial exploitation; (iii) processes being commercially exploited with the active participation of the laboratories; and (iv) important publications of various research institutes and laboratories. It was felt that the

preparation of such a comprehensive report giving an overall picture of the status of science and technology in the country is best undertaken by a central agency to be specially organized for the purpose.

The seminar emphasized the need for the appointment of a permanent Parliamentary Committee, similar to the House Committee on Science and the Parliamentary Committee for Science in UK. Such a parliamentary committee, it was felt, could effectively fill the gap in our present science policy decision making system and integrate social, economic and political decisions with scientific and technological effort.

K. D. SHARMA

# Symposium on Particles Faster than Light

A ONE-DAY symposium on Particles Faster than Light was held at the Institute of Mathematical Sciences, Madras, on 3 March 1968, with Prof. E. C. G. Sudarshan (Syracuse University, USA) as the principal lecturer. The object of the symposium was a critical discussion of the contributions of S. Tanaka, E. C. G. Sudarshan *et al.* and G. Feinberg against the background of the well-established principles of the special theory of relativity.

Prof. Alladi Ramakrishnan, Director, Institute of Mathematical Sciences, in his introductory talk emphasized that the question of particles faster than light must be examined in the propagator formalism. In this approach it is important that the real mass be replaced by an imaginary quantity in the equation of motion. However, there seems to be other consequences that follow from the imaginary nature* of the mass, since the propagator is a function of only space, time and mass-like parameters and does not involve energy and momentum. He said that the mathematical formulation of particles travelling faster than light has a deep connection with the theory of L-matrices developed by him.

Prof. Sudarshan divided his talk into two, the first one dealing with the classical problem of tachyons (particles travelling faster than light) and the second with the problem of quantization of such entities. He referred to some experimental work carried out at the Nobel Institute in Stockholm by physicists T. Alvagov and P. Erinam and at Princeton by T. Alvagov and Krenlev. One way of detecting tachyons is to look at the decay of unstable particles. If a given particle could decay into three particles, one of which has a mass equal to that of the original decaying particles, then the other two particles would be tachyons. In his second talk, Prof. Sudarshan spoke on the quantization of tachyons. He pointed out various difficulties that one encounters in this programme.

Dr K. H. Mariwalla said that it is important to consider transformations in which the frames of reference move faster than light. The transformations referred by Prof. Sudarshan in his lecture refer only to reference frames moving with a velocity less than that of light.

Shri T. S. Santhanam spoke on imaginary mass and equation of motion, explaining the arguments of Tanaka that superphotic particles could not exist in isolation. Their existence would be felt only in their interactions with other particles.

Shri K. Srinivasa Rao discussed the Cerenkov radiation which could possibly give clues for the existence of tachyons.

*Subsequently, Prof. Ramakrishnan has studied the propagator formalism of particles faster than light in detail which indicates that there should exist particles with imaginary energy which decay very fast. (Evanescent) "L-matrices and propagators with imaginary parameters" preprint MAT-4-1968.

# Conference on Water Desalination

MOHAN LAL KHANNA
National Physical Laboratory, New Delhi 12

A CONFERENCE on Water Desalination was held at the Central Salt & Marine Chemicals Research Institute (CSMCRI), Bhavnagar, Gujarat, during 16-18 November 1967. The conference provided an occasion to scientists, representatives of engineering firms and others vitally concerned with the desalination problem to discuss various aspects of the problem, which has assumed urgency due to rapid pace of industrialization, rapid growth in population and inadequacy of the existing water resources.

Welcoming the delegates to the conference, Dr D. S. Datar, Director, CSMCRI, gave a résumé of the work on desalination being carried out at different laboratories of the Council of Scientific & Industrial Research, Defence and Atomic Energy. Inaugurating the conference, Dr Jivraj N. Mehta, Chairman of the Executive Council of CSMCRI, held that provision should be made for at least 10 gallons of water per day for human consumption and an equal amount for livestock consumption in the areas of water shortage, such as the arid zones of Rajasthan and Gujarat and the coastal region. Discussions at the eight technical sessions related to: Membrane processes; Ion-exchange techniques; Evaporation processes based on solar energy utilization; Solar energy measurement; Other desalination techniques; Physical properties of sea-water, scale formation, corrosion prevention and materials of construction; Economics of desalination processes and byproduct recovery; and Survey of water resources, evaporation control and agricultural application. The concluding session was devoted to formulating recommendations. Fifty-two papers were presented.

## Membrane Processes

N. Natarajan, V. P. Mehta and M. S. Rajawat (Defence Laboratory, Jodhpur) reported an electrodialysis method for the desalination of brackish water. The salt content of ground waters available in Rajasthan is generally within 10,000 p.p.m. of total dissolved solids (TDS). The use of permaplex A-20 and C-20 membranes in 5 as well as 45 compartment cells has been studied with different current densities, flow rates and concentrations of salt solutions and natural brackish waters. A method for producing indigenously heterogeneous types of ion-exchange membranes using PVC as a binder and nylon cloth as reinforcement has also been developed. Using these membranes, an electrodialysis unit capable of producing 20 gal. per day of desalted water from brackish water has been designed. W. P. Harkare, V. K. Indusekhar and N. Krishnaswamy (CSMCRI, Bhavnagar) have used indigenously prepared membranes of sizes $30 \times 30$ cm. and $91 \times 57$ cm. in bench scale and pilot plant units and have established the suitability of the systems for desalinating sea-water. V. K. Indusekhar, M. N. Prajapati and P. K. Narayan (CSMCRI, Bhavnagar) discussed the results of studies on the treatment of natural brackish well waters in bench scale units installed at sites. K. P. Govindan (CSMCRI, Bhavnagar) reported the results of studies on the physicochemical properties of ion-exchange membranes influencing the desalination process in relation to their ion-exchange groups, capacity, water content, ohmic resistance, diffusible electrolyte content and transport number. M. V. Bopardikar and S. R. Alagarsamy (CSMCRI, Bhavnagar) discussed a typical design and cost estimate for a brackish water conversion plant with a capacity of 3 million gal./day for water supply to 40 villages in Maharashtra. The design and cost estimate for a 5 million gal./day electrodialysis waste water effluent plant at the Worli Sewage Treatment Plant, Bombay, capable of reclaiming the water for reuse in industries were also reported. M. M. L. Khosla and R. Natarajan (Defence Laboratory, Jodhpur) discussed the reverse osmosis process, which has a bright future. The question of availability of a suitable semi-permeable membrane with sufficient mechanical strength was discussed; the investigations in progress were described.

## Ion-exchange Techniques

B. D. Dasare and U. D. Datar (CSMCRI, Bhavnagar) discussed the problem of desalination of moderately brackish waters by the mono-bed deionization technique, in which cation and anion exchangers are intimately mixed in equivalent proportions. The use of indigenously available Tulsion 14 and WB ion exchangers for this purpose has been investigated. A method for the preparation of a cation exchange resin from low temperature pitch was described by V. S. Narasimhachar, V. V. Subha Rao and R. Raidyeswaran (Regional Research Laboratory, Hyderabad). The exchange properties of the material are comparable to those of the commercially available products. The factors influencing the choice of a suitable ion-exchange technique for the treatment of brackish waters were discussed by W. P. Harkare (CSMCRI, Bhavnagar). Important among these factors are the raw water composition, quality of product water desired and its supply and demand. The economics of the treatment process decides the selection of site and technique to be adopted. Y. G. Kher (Saugar University, Sagar) reviewed the literature available on the gel filtration technique for the desalination of sea-water; the efficiency of this technique depends on the degree of crosslinking in the materials used. By manipulating the conditions of gel filtration, different kinds of viruses could be retained.

## Evaporation Processes Based on Solar Energy Utilization

M. L. Khanna (National Physical Laboratory, New Delhi) discussed the present status of the technology of desalting of saline water vis-à-vis

the enormity of the fresh water shortage problem in India. Of the various methods used presently, solar water distillation, multi-stage flash distillation, reverse osmosis and dual-purpose plants have shown great potentialities for future use. Future trends in the design, construction and operation of commercial plants in various countries, including Soviet Russia, Saudi Arabia, Greece, USA, Australia and Mexico were discussed. The need for setting up a solar water distillation pilot plant at the Central Arid Zone Research Institute, Jodhpur, was stressed. The possibility of integrating the nuclear power plants under construction in India with multi-stage flash distillation or other types of plants for desalting of brackish or sea-water of the region was suggested. S. D. Gomkale (CSMCRI, Bhavnagar) discussed the use of solar stills as a source of drinking water for isolated communities. The requirement of a comparatively large area for installation of solar still limits its use to supply of drinking water to communities in isolated places like small islands and lighthouses. As solar distilled water meets the requirements of specifications laid down for distilled water, the use of solar still for the production of distilled water is recommended, as it assures a high return on the capital investment on the still. Some civil engineering aspects of water desalination plant installations near sea shore were considered by H. D. Goghari and S. D. Gomkale (CSMCRI, Bhavnagar). Construction of underground tanks, reservoirs, foundations for light and heavy machinery involves the study of soil nature. The major difficulties encountered in solar still construction are with respect to the adjustment of basin slopes, fixing of precast items, sealing of glass joints, etc. In the humidification-dehumidification (H-D) pilot plant, the construction of solar collectors poses some difficulties due to the development of cracks in the brick-lined bottoms of the collectors. S. K. Garg, M. H. Mehta, S. D. Gomkale and R. L. Datta (CSMCRI, Bhavnagar) described the construction and operation of an H-D pilot plant wherein solar energy is used to heat sea-water for producing 1000 gal./day of fresh water. Operational data useful for scale-up of the plant capacity were presented. The possible modifications in the technique or the construction to overcome the difficulties experienced during plant operation were also discussed. S. D. Gomkale, S. K. Garg and R. L. Datta (CSMCRI, Bhavnagar) discussed the potentialities of the H-D technique in meeting the requirements of industrial water. The economics of the process can be improved if the sea-water, used in the plant for cooling purposes, is used as the feed to the plant instead of raw sea-water. P. R. Mehta and G. T. Gadre (CSMCRI, Bhavnagar) suggested the possible use of solar stills for meeting drinking water requirements of labour employed in salt works situated on the sea coast in Gujarat.

## Solar Energy Measurement

Measurement of solar and sky radiation in India formed the subject of a paper presented by A. Mani [India Meteorological Department (IMD), Poona]. The instruments used at the principal and the ordinary radiation stations since 1957 for the measurement of direct solar radiation, global solar radiation, diffuse sky radiation, net solar radiation and albedo were described. V. Desikan, O. Chacko and R. D. Agnihotri (IMD, Poona) presented maps showing the distribution of sunshine, global solar radiation and net solar radiation for different months over the Indian sub-continent. Arid and semi-arid zones in western India receive the highest amounts of energy for the major part of the year, whereas in the pluvial regions, particularly on the west coast and north-eastern part of the country, the radiation received is very small, especially during the monsoon months. These maps are important from the point of view of solar energy utilization. Solar radiation measurement programmes in coastal India were described by V. Desikan and C. G. Rahalkar (IMD, Poona). Global solar radiation is measured at 9 stations along the Indian coast from Calcutta to Bhavnagar; these stations receive on an average 440 cal./cm.$^2$/day solar radiation. During March-April, the west coast receives the maximum radiation (530 cal./cm.$^2$/day) which decreases (390 cal./cm.$^2$/day) during the monsoon months. On the east coast it is more or less uniform, except when the north-east monsoon is active. A simple method for computing the average hourly total and diffuse solar radiation intensity from the record of actual sunshine hours was described by H. P. Garg, R. Ganguli and C. L. Gupta (Central Building Research Institute, Roorkee). The computed values of direct and diffuse solar radiation on horizontal surface at Poona and Delhi have been found to be in good agreement with the measured values. Correlation of solar radiation data with surface evaporation has been attempted by M. M. Bhatt (CSMCRI, Bhavnagar); measurements of intensity of solar radiation show a maximum value of 655 cal./cm.$^2$/day in May and the minimum value of 319 cal./cm.$^2$/day in August. The following equations correlating solar radiation intensity and wind velocity with the extent of surface evaporation of water in the region for the two seasons of the year have been derived:

$$E = 0{\cdot}01G(1{\cdot}4+0{\cdot}5W)$$
$$E = 0{\cdot}01G(1{\cdot}8+0{\cdot}5W)$$

where $E$ represents surface evaporation in mm.; $G$, global solar radiation in cal./cm.$^2$/day; and $W$, wind velocity in km./hr. P. M. Oza, Y. A. Doshi, D. P. Suru, V. H. Vaidya and P. S. Rao (CSMCRI, Bhavnagar) reported a new chemical radiometer using mercuric chloride - oxalate photochemical reduction system at $p$H 5·0 for measuring solar intensity and light intensities of ultraviolet, visible and near infrared of solar radiation, which are given by the following equations:

$$U = 0{\cdot}1425+[0{\cdot}018(t_f-27)+0{\cdot}00004(ft.c-5250)]$$
$$U = (A)-0{\cdot}0184(t_f-t_i)$$

where $U$ represents moles of mercurous chloride formed/cm.$^2$/min.; $t_i$, $t_f$, initial and final temperatures respectively; and $ft.c$, light illumination. The system compares favourably in its performance with the available pyrheliometers.

## Other Desalination Techniques

S. D. Gomkale, S. K. Garg and R. L. Datta (CSMCRI, Bhavnagar) reviewed the developments in

and applications of the various evaporation techniques for water desalination. With high energy costs it is doubtful whether these techniques would produce fresh water at comparatively low costs. Ccmparative study, however, indicates that solar stills, H-D technique and low temperature flash evaporation using solar energy are more suitable for low capacity plants. The complete process design of a portable horizontal forced circulation vapour compression desalination unit of capacity 2000 gal./day of fresh water was presented by P. N. Mehta and D. K. Guha (IIT, Kharagpur). The design details are based on the use of sea/brackish water of 10 per cent concentration as raw feed and stainless steel as the material of construction. N. C. Rawal (Central Soil Mechanic Research Station, Central Water & Power Commission, New Delhi) reviewed the processes for the demineralization of saline water operated in the Soviet Union, viz. distillation with the use of fuels, solar-heat distillation, freezing, ion exchange and electrodialysis. 'Desalination and Indian Army Engineers' was the topic of a paper by Lt Col. N. C. Gupta [Research & Development Establishment (Engineers) Dighi, Poona] in which the importance of potable water for army use in a desert sector was stressed; the essential characteristics and qualitative limitations of the mobile plant required were discussed. The use of aerodynamic turbines for the combined production of electricity and fresh water using sea-water as the cooling medium was discussed by O. P. Gandhi (Gujarat Electricity Board, Power Station, Utran). It was pointed out that by utilizing waste heat available from the precooler and the intermediate cooler of the turbine, 150-300 litres of fresh water per installed kilowatt capacity of the plant can be obtained; these turbines are particularly suitable for use in arid areas. V. Krishnamurthy and K. Subbaramaiah (CSMCRI, Bhavnagar) have studied the desalination process in living systems. M. V. Bobardikar, S. R. Algarsamy and J. S. S. Lakshminarayana (Central Public Health Engineering Research Institute, Nagpur) discussed the recent technological developments in the field of desalination using nuclear energy and the possibilities of integrating it with Bombay's water supply system.

### Physical Properties of Sea-water, Scale Formation, Corrosion Prevention and Materials of Construction

R. A. Buch and B. P. Choudhari (CSMCRI, Bhavnagar) dealt with the physical properties of solar sea-water concentrates, viz. density, viscosity, surface tension, refractive index, $\not{p}$H, radiation absorption, etc. Temperatures attained by different concentrates during evaporation under identical conditions of exposure to solar radiation have been determined. B. P. Choudhari (CSMCRI, Bhavnagar) has studied vapour pressure and activity coefficients of water in sea-water concentrates. The data obtained give an indication of the evaporation equilibrium conditions for these concentrates and are useful in the study of the evaporation processes employed in the desalination of saline water. The corrosion and scale formation behaviour of copper, brass, stainless steel and aluminium has been studied by P. N. Mehta and D. K. Guha (IIT, Kharagpur) in the laboratory using synthetic saline water of composition equivalent to that of sea-water in the Rann of Kutch at $95 \pm 1°C$. Corrosion-time curve studies reveal an exponential trend. However, the curve for aluminium, after 600 hr, changes and becomes linear with high slope, which is attributed to severe deep pitting. Stainless steel has been recommended as the best material of construction for equipments handling saline waters. A. K. Lahiri and T. Banerjee (National Metallurgical Laboratory, Jamshedpur) discussed various aspects of the problem of selection of the most economical material with the requisite physical, mechanical and corrosion resistance properties and an approximate life of 20 years. The major problems encountered in the desalination processes are protection of structural materials against corrosion by saline water and marine atmosphere and handling of sea-water and brine. Cement-lined piping, rubber-lined valves, high alloy steels or iron (especially nickel- or molybdenum-bearing stainless steel) pump, etc., were recommended for handling sea-water. The suitability of different copper-based non-ferrous alloys for making heat exchangers has been studied. For evaporator units, carbon steel and alloy-clad steel have been recommended. M. J. Mehta and K. Seshadri (CSMCRI, Bhavnagar) have studied the corrosion of common metals and alloys, viz. aluminium, brass, copper, iron, monel, zinc and stainless steel, in brines and saline atmosphere with a view to assessing their relative corrosiveness. The use of inhibitors and anti-corrosive paints to prevent corrosion has also been studied. Scaling problems in plants for desalination by sea-water distillation were discussed by R. K. Sapre and R. L. Datta (CSMCRI, Bhavnagar). Recent work on the control of calcium sulphate scaling was reviewed.

### Economics of Desalination Processes and Byproduct Recovery

Carl N. Hodges (Environmental Research Laboratory, University of Arizona, Arizona, USA) described the work done at the University of Arizona and the University of Sonora Experimental Station at Puerto Penasco, Sonora, Mexico, on the combined production of power, fresh water and food. It was stressed that horticultural crops, and even agronomic crops, can be produced economically utilizing the waste blowdown sea-water from the desalination plants to modulate the temperature and humidity of a controlled-environment agricultural system. Power, water and food could be practically produced at less than half the price being paid for these products presently in the coastal desert areas of the world. A cost estimate of fresh water from the dual purpose power-cum-desalination plants was presented by P. K. Bhatnagar (Power Projects Engineering Division, Department of Atomic Energy, Bombay). Factors such as the sizes of the power plant and the desalination plant, cost of coal in relation to calorific value, interest rate, temperature of steam supplied, etc., have been taken into account while making the calculation. The results are compared graphically. The recovery of magnesia and bromine from the effluent brine of the desalination plant as byproducts was discussed by K. V. Satyanarayan, B. K. Shukla

and D. J. Mehta (CSMCRI, Bhavnagar). The economics of operating a desalination plant coupled with magnesia and bromine recovery plants of 150 and 5·8 tonne per day capacity respectively were discussed. K. M. Rao (CSMCRI, Bhavnagar) suggested forced evaporation of marine bitterns left after desalination for the production of potassium chloride. Based on data obtained from laboratory experiments with a single stage evaporator operating at atmospheric and low pressures, the estimates of operating and capital costs for single, double, triple and quadruple effect evaporators for a plant producing simultaneously 2 tonnes potassium chloride and 20,000 gal./day of fresh water were presented. In two papers, E. M. Feist and R. Matz (Negav Institute for Arid Zone Research, Beersheva, Israel) discussed the feasibility of production of fresh water and salt by multi-stage flash distillation using a solar pond. Discussing the influence of various factors on the choice of desalination techniques for India, M. H. Mehta, W. P. Harkare and D. S. Datar (CSMCRI, Bhavnagar) recommended the use of ion exchange or electrodialysis technique for brackish waters, solar still for isolated small communities, H-D technique for larger communities as well as industrial water supplies and dual purpose plant for industrially populated cities like Bombay and Madras.

### Survey of Water Resources, Evaporation Control and Agricultural Application

R. K. Shah, A. M. Trivedi and S. C. Vora (University School of Sciences, Ahmedabad) presenting a salinity map of surface and ground waters in Gujarat discussed the various parameters of importance, viz. chloride-bicarbonate ratio, electrical conductivity and the geochemical types of waters. Discussing the water resources of western Rajasthan, T. N. Bhargava, C. P. Mathur, T. C. Tak and Inder Singh (Defence Laboratory, Jodhpur) presented a map demarcating the areas containing potable and non-potable water based on detailed studies undertaken on the quality of ground water available in Barmer, Jaisalmer, Jodhpur and Bikaner districts. A. V. Rao (CSMCRI, Bhavnagar) presented the results of two years' field experiments conducted at a 470-acre reservoir near Bhavnagar using monomolecular films of a 50:50 mixture of cetyl and stearyl alcohols for controlling evaporation losses. E. R. R. Iyengar and T. Kurian (CSMCRI, Bhavnagar) reviewed the studies carried out in different countries on the application of saline water for plant growth. K. V. Raghava Rao, A. A. Rao and C. S. Doshi (Exploratory Tubewells Organization, Ministry of Food, Agriculture, Cooperation & CD) have studied the brackish and saline ground water resources as revealed through ground water exploration; the areas where sub-surface water resources need desalination treatment were highlighted. S. P. Raychoudhuri (Planning Commission, New Delhi) discussed the transformation of clay minerals in soils under irrigation with saline waters. The changes in clay mineral components shift the equilibrium, which depends on the concentration of different ionic species present in the soil solution and affects the soil structure appreciably. This slow transformation of clay minerals in saline and alkaline soils may lead to the formation of mixed layer structures of the type montmorillonite-illite, montmorillonite-vermiculite, vermiculite-chlorite, etc., whose influence on the physical properties of soil is not yet understood properly.

In the concluding session, the conference recommended the formation of a Coordination Committee on Desalination consisting of Dr D. S. Datar, Director, CSMCRI, Bhavnagar, Dr V. Ranganathan, Deputy Chief Scientist, Defence Research & Development Organization, New Delhi, and Shri V. N. Meckoni, Department of Atomic Energy, Bombay, with powers to coopt members from industry and other organizations in the country and the establishment of an information centre on desalination at CSMCRI, Bhavnagar.

# Bond Properties of Molecules

KRISHNAJI & SURESH CHANDRA
Physics Department, University of Allahabad, Allahabad

IN recent years, physicists and chemists have shown increasing interest in molecular properties and molecular structures. The nature of the chemical bond in a molecule is perhaps the most important property to be understood. Most of the information on the subject is available in terms of empirical formulae, because rigorous mathematical treatment for several bond properties of molecules is complicated and hence it is carried out only for the simple types of molecules. This article reviews the present state of information about such bond properties of molecules as group electronegativity, ionic character, partial double bond character and the *s*-character of the bonding orbitals.

## Electronegativity

Rigorous mathematical methods which attempt to express the properties of molecules in terms of the motion of individual electrons are very complicated for all except the simplest type of molecules. Attempts to define and measure the overall properties or lumped constants of the atoms, such as electronegativity, which can be used in approximate but widely applicable calculations are, therefore, of great importance.

The concept of electronegativity is very important in systematizing experimental data. Several methods have been proposed for evaluating this important qualitative property of an atom, described by Pauling[1] as " the power of an atom in a molecule to attract electrons to itself ". It is because electronegativity is concerned with atoms in molecules, rather than atoms in isolation, that its precise measurement is not easy.

Pauling set up a scale of electronegativity values and obtained relative values from the extra ionic resonance energy of a chemical bond between unlike atoms. His scale of electronegativity is defined by the expression

$$|x_A - x_B| = 0{\cdot}208\sqrt{\Delta} \qquad ...(1)$$

where $x_A$ and $x_B$ represent the electronegativities of the atoms A and B respectively, which are joined in a chemical bond, and where $\Delta$ represents the energy in excess of that expected for a purely covalent bond AB. The extra ionic resonance energy $\Delta$ (in kcal./mole) is given by

$$\Delta = D(\text{A-B}) - \tfrac{1}{2}[D(\text{A-A}) + D(\text{B-B})]$$

where $D$(A-A), $D$(B-B) and $D$(A-B) are the bond energies of the A-A, B-B and A-B bonds respectively. For convenience, $x$ for hydrogen was originally taken as zero in order to construct an electronegativity scale parallel to the electromotive series of the elements.

Using Pauling's method, Huggins[2] reassigned electronegativity values to the more common chemical elements. Huggins' values, with a few exceptions, were higher than those assigned by Pauling. If the Huggins scale is shifted downward by 0·1 and the values rounded off to two significant figures, it is found to agree well with the Pauling scale.

The best theoretical definition of electronegativity was given by Mulliken[3], based on the concept that the energy expended in going from the covalent molecule A-B to the ionic states $A^+B^-$ and $A^-B^+$ is equal if A and B have the same electronegativity. Based on qualitative theoretical considerations arising from a comparison of the results with Pauling's relative scale, Mulliken proposed that the electronegativity of an element is measured by the arithmetic mean of its first ionization energy $I_A$ and the electron affinity $E_A$.

$$x_A = \frac{I_A + E_A}{2} \qquad ...(2)$$

Since the zero point of Pauling's scale is arbitrary, there is no compelling reason to anticipate a direct proportionality between Mulliken and Pauling's scales; however, a linear relation must hold, if both the definitions represent the same property. Hinze and Jaffe[4] suggested the following correlation between the two scales:

$$0{\cdot}168(x_M - 1{\cdot}23) = x_P$$

Pauling found that by empirical manipulation, the ionization energy and the electron affinity could be made to give almost the same values of the electronegativity for monatomic elements. The sum of the ionization energy and the electron affinity expressed in kcal./mole and divided by 130 was almost identical with the original values of $x$ increased by 2·1 (making $x_H = 2{\cdot}1$ instead of zero). Though Mulliken's method has a somewhat better theoretical basis, it is not as widely applicable as that of Pauling. However, the values determined by this method have served the useful purpose of fixing the origin of the more extensive relative scale of Pauling, so that it is no longer arbitrary.

Gordy[5] defined the electronegativity of a neutral atom of a stable molecule as the potential resulting from the unscreened nuclear charge of the bonded atom effective on a bonding electron when the bonding electron is at a distance from the nucleus equal to the covalent radius $r$, where

$$x = \frac{(Z_{eff.})e}{r} \qquad ...(3)$$

However, a shift of the origin and a proportionality factor were found necessary to bring the values thus determined into agreement with the Pauling scale and the following equation has been proposed for the electronegativity of an isolated atom:

$$x = 0{\cdot}31\left(\frac{n+1}{r}\right) + 0{\cdot}50 \qquad ...(4)$$

where $n$ is the number of valence electrons of the neutral atom.

Iczkowski and Margrave[6] defined electronegativity as a derivative of energy with respect to charge. However, their definition is not satisfactory; it ignores completely the orbital dependence of electronegativity, and assumes that the energy of an atom is a continuous and a single-valued function of its charge. That the function is not single-valued is apparent from the fact that a variety of different valence states with different energies are readily obtained for a given element[4,7].

Hinze *et al.*[7] developed a new definition of electronegativity, considering it to be a property not of the atom as such, but of an individual orbital of the atom. However, electronegativity could be defined in this way only for bonding orbitals; hence, they suggested the term ' orbital electronegativity ' for the values thus calculated. Following Pauling, they defined orbital electronegativity as a measure of the power of an atom, as it may exist in a molecule, to attract an electron in a given orbital to itself. Thus, orbital electronegativity can be defined as the derivative of the energy of the atom in its valence state with respect to the charge in the orbital, i.e. the number of electrons in the orbital for which the electronegativity is defined as

$$x_j = \frac{\partial E}{\partial n_j} \qquad \ldots(5)$$

where $n_j$ is the occupation number of the $j$th orbital, the orbital electronegativity of which is $x_j$.

The above definition contains Mulliken's definition as a special case. Since Mulliken's and Pauling's definitions were shown to be substantially equivalent, it was apparent that this new definition represented a generalization of the original definitions, and left the previous work substantially unaffected. The electronegativity concept, as it was used by these authors, gives a measure of the power by which an atom, in its valence state, attracts an additional electron for bond formation. Consequently, electronegativity is a property of an atom before a bond is formed.

The advances in this field were reviewed by Pritchard and Skinner[8]. The different electronegativity values of the elements as determined by different workers employing different methods were compared by Pritchard and Skinner[8] and Gordy and Thomas[9]. However, the methods of determining electronegativity, less acceptable than those described so far, are not dealt with in this review.

## Group Electronegativity

The electronegativity of an atom in a molecule differs from that of an isolated atom, as it depends not only on the electronegativity of adjacent atoms, but on its degree of hybridization and various bond interaction effects. Of course, theoretically, the electronegativity of an isolated atom will also depend on its degree of hybridization. According to Walsh[10] and Moffitt[11], an increase in the amount of $s$-character in the hybrid orbitals of the carbon atom increases the electronegativity of the carbon atom. Many attempts were recently made to determine the group electronegativity of a radical and a wide diversity of molecular properties were found to be correlated with group electronegativities[7-9,12-29].

Of the various procedures developed, that of Hinze *et al.*[7] is important and will be discussed briefly. Using their definition of orbital electronegativity, Hinze *et al.*[7] determined group orbital electronegativity in a manner exactly analogous to the determination of atomic orbital electronegativities. Group electronegativity depends on the bond that the group will form because, by definition, it is a property of the group obtained by an adiabatic breaking of this bond. Thus, the entire electron distribution in the group is assumed to be identical to that in the final compound. This is a limitation which applies to all group electronegativities and suggests that any empirical value must depend on the compound from which they are obtained.

The procedure to obtain group orbital electronegativities outlined by Hinze *et al.*[7] requires the occupation numbers for the bonds within the group. The occupation numbers are derived from bond electronegativity, defined as the electronegativity of orbitals forming the bond, after charge has been exchanged between them. It was shown that this process of charge exchange equalizes the electronegativity of the two orbitals forming a bond to lower the energy of the molecule.

Hinze *et al.*[7] calculated the electronegativities of a series of groups. These calculations needed the degree of hybridization of the central atom which was obtained from the bond angles. This procedure of estimating hybridization from bond angles is questionable and often leads to an incorrect or impossible amount of hybridization[30]. Moreover, these calculations were made on the assumption that none of the halogens is hybridized. This is possibly an incorrect assumption, especially since recent calculations based on nuclear quadrupole coupling data have indicated considerable $s$-character in chlorine, bromine and iodine organic compounds. Also, no account of the rehybridization of the atoms was taken into consideration which might lead to erroneous values of group electronegativities. Although this procedure is fundamentally straightforward, it is very lengthy and hence not practicable.

At present, there does not seem to exist any reliable empirical expression for obtaining the group electronegativity of a radical and, hence, it appears profitable to look at some of the empirical expressions in the literature to see if these could be applied to atoms in molecules.

Pauling's relation [Eq. (1)] actually applies to atoms in molecules. For diatomic molecules it gives atomic electronegativities, while for polyatomic molecules, if dissociation energies are used, group electronegativities would be obtained. Actually, for polyatomic molecules Pauling used mean bond energies instead of bond dissociation energies and his electronegativity values obtained from polyatomic molecules were an average of the possible group electronegativities of the radicals $MX_{n-1}$, $MX_{n-2}, \ldots$ with the atomic electronegativity of $M$. Unfortunately, at present, relatively few dissociation energies of bonds in polyatomic molecules are known accurately and it, therefore, does not seem profitable to try to obtain group electronegativities from Pauling's relation.

Mulliken's expression for atomic electronegativity [Eq. (2)] can be applied to the determination of the group electronegativity of a radical by simply using the ionization potential and electron affinity of the radical. However, these quantities are not easy to obtain experimentally and, in particular, electron affinities, even for atoms, are only poorly known. This expression is, therefore, limited by the availability of experimental data and cannot be used at present.

Gordy's expression [Eq. (3)] for the electronegativity of a free atom depends only on the covalent radius ($r$) and the value of $Z_{eff.}$ as given by Eq. (4). The group electronegativity of a radical AB, derived from the molecule X-AB (the radical AB is always bonded through A) can be regarded as the electronegativity of an atom A perturbed by its chemical bonding to the atom B. Thus, if $Z_{eff.}$ and $r$ could be determined for the atom A in the radical AB, then the electronegativity of A and, hence, the group electronegativity of AB could be determined from Gordy's relation. In the radical AB, $r$ can be taken as the covalent radius of A in the bond A-B and this accounts for the effect of hybridization on the electronegativity of A, since this covalent radius depends on the degree of hybridization of A.

Gordy defined the electronegativity of an isolated atom as

$$x = \frac{(Z_{eff.})e}{r} = \frac{e[n-s(n-1)]}{r} \qquad \ldots(6)$$

where $n$ represents the number of electrons in the valence shell of the neutral atom and $s$, the screening constant of a valence electron. A simplifying assumption was made that all the electrons in the closed shells below the valence shell exert their full screening power, and that all the valence electrons exert equal screening.

Assuming $s = 0{\cdot}5$, one gets

$$x = k\left(\frac{n+1}{r}\right)$$

where $k$ is the constant $0{\cdot}50e$.

It was, however, found that a shift of the origin and a proportionality constant were necessary to bring the values thus determined in agreement with Pauling's values. The resulting equation [Eq. (4)] yields values in good agreement with those of Pauling. One drawback of Eq. (4) is that it makes an oversimplification with respect to the effective nuclear charge and, in consequence, the identity of electron affinity with atomic electrostatic surface potential is perhaps overstated. Gordy chose an unorthodox method of assessing $Z_{eff.}$ values, and surprisingly enough he did not remark upon the effect of calculating $Z_{eff.}$ by more conventional methods, e.g. those of Pauling[1] and of Slater[31].

A reinvestigation of Gordy's assumption was made by Pritchard and Skinner[8]. They calculated the effective nuclear charge from the rules given by Slater and showed that the electronegativities calculated in this way were very close to those calculated by Gordy.

However, in a radical AB, the value of $Z_{eff.}$ of the atom A will be perturbed by its chemical bonding to the atom B and this new value of $Z_{eff.}$ can be obtained as follows[32]. The electronegativity values of atoms A and B respectively in the free state may be taken as $x_A$ and $x_B$ and $m$ electrons of atom A may be assumed to be taking part in the bonding to B. For a two-electron bond, the pair of electrons could be divided between the two atoms in the ratio of their relative electronegativities so that a fraction $2x_A/(x_A+x_B)$ of electron atmosphere is on the atom A and a fraction $2x_B/(x_A+x_B)$ of electron atmosphere is on the atom B. Hence, the number of electrons on atom A due to $m$ bonds with B would be $2mx_A/(x_A+x_B)$. Since atom A lacks at least one electron from its completed outer octet (because A is the bonding part of the radical), the radical could be stabilized by the resonance contributor $^-AB^+$ if B has any nonbonded valence electrons. The amount of electron atmosphere donated to A by the resonance contributor is $x_A/(x_A+x_B)$, which may be called the relative electron attracting power of atom A for the one electron that atom B donates. If there were $p$ resonance contributors, the amount of electron atmosphere on A would be $px_A/(x_A+x_B)$. Thus by definition

$$Z_{eff.} = n-s\left[(n-1-m)+2m\frac{x_A}{(x_A+x_B)}+p\frac{x_A}{(x_A+x_B)}\right]$$

$$= n-s(n-1)-s\left[-m+2m\frac{x_A}{(x_A+x_B)}+p\frac{x_A}{(x_A+x_B)}\right]$$

Assuming the screening constant of one valence electron for another to be 0·5,

$$Z_{eff} = 0{\cdot}5\left[n+1+m-2m\frac{x_A}{x_A+x_B}-p\frac{x_A}{x_A+x_B}\right] \qquad \ldots(7)$$

$$= 0{\cdot}5(n^*+1)$$

So the net effect of the chemical bonding of atom A to atom B is to change the effective number of valence electrons. For evaluating the value of group electronegativity for a radical, $n$ in Eq. (4) should be replaced by $n^*$ and the group electronegativity of a radical AB could now be written as[32]

$$x = 0{\cdot}31\left(\frac{n^*+1}{r}\right)+0{\cdot}50 \qquad \ldots(8)$$

where

$$n^* = n+m-2m\frac{x_A}{x_A+x_B}-p\frac{x_A}{x_A+x_B} \qquad \ldots(9)$$

Eq. (8) is a more general form of Gordy's relation which can be identified with the original Gordy's equation [Eq. (4)] for a free atom ($n^*$ being equal to $n$). Thus, Eq. (8) is applicable to the calculation of both atomic and group electronegativities. This expression can also be extended to the $AB_y$ radicals. This method has been utilized[32] for evaluating the group electronegativities of a large number of radicals (Table 1).

The electronegativity values for halomethane radicals calculated by Hinze *et al.*[7] increase as hydrogen is replaced by halogens in the radical, whereas our calculations give decreasing values for a similar situation. This may be due to the fact that our calculations have taken account of the resonance contributions. The uncertainties in the method of Hinze *et al.* have been discussed earlier. If, however, the resonance contribution is neglected in our calculations, the values obtained are close to those of Hinze *et al.*, which indicates that Hinze *et al.*

TABLE 1 — GROUP ELECTRONEGATIVITIES OF DIFFERENT RADICALS

| Radical | Electronegativity value | | | | | |
|---|---|---|---|---|---|---|
| | Chandra and Chandra[32] | Hinze *et al.*[7] | Wilmshurst[15] | Kagarise[13] | Dailey and Shoolery[12] | Muller and Pritchard[29] |
| $-OH$ | 3·33 | 3·53 | 3·89 | — | 3·51 | 3·45 |
| $-SH$ | 2·56 | 2·35 | 2·61 | — | 2·45 | — |
| $-NC$ | 3·42 | — | 3·49 | — | — | — |
| $-CN$ | 2·98 | — | 3·17 | — | 2·52 | 3·20 |
| $-NH_2$ | 2·88 | 2·82 | 3·40 | — | 2·99 | 3·00 |
| $-NO_2$ | 3·19 | — | 3·45 | — | — | 3·65 |
| $-CH_3$ | 2·42 | 2·30 | 2·63 | 2·34 | — | 2·60 |
| $-CH_2F$ | 2·39 | 2·61 | — | — | — | — |
| $-CHF_2$ | 2·35 | 2·94 | — | — | — | — |
| $-CF_3$ | 2·32 | 3·29 | — | 3·20 | — | — |
| $-CH_2Cl$ | 2·31 | 2·47 | 2·74 | 2·48 | — | — |
| $-CHCl_2$ | 2·19 | 2·63 | 2·88 | 2·62 | — | — |
| $-CCl_3$ | 2·07 | 2·79 | 3·03 | 2·76 | — | 2·95 |
| $-CH_2Br$ | 2·29 | 2·40 | — | 2·44 | — | — |
| $-CHBr_2$ | 2·15 | 2·49 | — | 2·55 | — | — |
| $-CBr_3$ | 2·01 | 2·57 | — | 2·65 | — | — |
| $-CH_2I$ | 2·25 | 2·38 | — | — | — | — |
| $-CHI_2$ | 2·08 | 2·44 | — | — | — | — |
| $-CI_3$ | 1·91 | 2·50 | — | — | — | — |
| $-SiH_3$ | 1·90 | — | — | — | — | — |
| $-GeH_3$ | 1·84 | — | — | — | — | — |
| $-COOH$ | 2·65 | — | — | — | 2·57 | 2·85 |
| $-C\equiv CH$ | 2·89 | — | — | — | — | 2·90 |
| $-COCH_3$ | 2·54 | — | — | — | — | 2·65 |

probably did not take resonance contribution into account.

## Ionic Character

The description of a chemical bond usually involves specifying the amount of ionic character, the orbital hybridization, and the degree of bond multiplicity. This threefold complexity makes the interpretation of a given bond structure difficult. Since the diatomic halides are believed to involve bonds which are essentially single, complications due to multiplicity are avoided. Hence, it is possible, from a study of these comparatively simple molecules, to obtain some information about the ionic character of bonds (and to a lesser extent about hybridization) which may be used as a guide in dealing with more complex systems. To a first approximation, however, it may be assumed that each component of the multiple bond has the same ionic character.

Let us consider a molecule AB formed by the two atoms A and B, where the atom A has the same electronic configuration as in halogens, i.e. one electron less than the number required to form a closed shell. When the two atoms combine to form the molecule AB, their electron orbitals overlap, leading to a net reduction in the energy of the molecule with respect to the total energy for the two free atoms. This reduction in energy explains the stability of the molecule. The principal overlapping occurs between the outermost $p_z$-electrons directed along the internuclear axis producing a $\sigma$ bond. The two electrons forming the $\sigma$ bond move in a molecular orbital $\Psi$, which is usually chosen as a linear combination of the atomic orbitals on the free atoms.

$$\Psi = a\Psi_A + b\Psi_B \qquad ...(10)$$

where $\Psi_A$ and $\Psi_B$ are atomic orbitals for atoms A and B. If $\Psi$ is normalized, $a^2+b^2+2abS = 1$, where $S$ is the overlap integral $\int \Psi_A \Psi_B d\tau$. The ionic character for such a heteropolar bond may be defined as $(a^2-b^2)$, i.e. it is the difference between the probabilities for the electron to be found on atom A or B.

If the diatomic molecule AB of bond length $r$, which is the equilibrium internuclear distance of the two atoms forming the bond, is completely ionic $A^+B^-$, its dipole moment would be given by the product $re$, where $e$ is the electronic charge. The observed dipole moment, however, is $\mu$ and assuming this moment to be due completely to the ionic character of the bond, the ionic character ($\beta$) can be defined as

$$\beta = \frac{\mu}{e.r} \qquad ...(11)$$

Pauling[1] calculated the ionic character of chemical bonds in diatomic molecules from the observed values of the electric dipole moments using this definition. From these estimated values of ionic character he constructed a curve relating the amount of ionic character of a bond A-B to the difference in electronegativity $x_A - x_B$ of the atoms. The curve was represented by the empirical equation

$$\beta = 1 - e^{-0{\cdot}25(x_A - x_B)^2} \qquad ...(12)$$

Eq. (12) does not hold good for large $(x_A - x_B)$ values.

Hannay and Smyth[33] proposed another relation for the ionic character ($\beta$) of the single bonds between atoms A and B with electronegativities $x_A$ and $x_B$ respectively.

$$\beta = 0{\cdot}16|x_A - x_B| + 0{\cdot}035|x_A - x_B|^2 \qquad ...(13)$$

Eq. (13) is obviously not to be applied at the extreme limits of the electronegativity difference, where the approximate character of the underlying theory renders the calculation of a very small amount of covalent character or a very small amount of ionic character meaningless.

The dipole moment for a heteropolar bond is given by the expression

$$\mu_{\text{experimental}} = er\beta + \mu_{\text{overlapping}} + \mu_{\text{hybridization}} + \mu_{\text{polarization}} \quad ...(14)$$

The first term is the so-called primary moment which arises from the ionic character of the bond, and the second, third and fourth terms represent the moments due to the overlap of orbitals from atoms of unequal size, hybridization of the valence shell orbitals, and polarization of the nonbonding electrons respectively. In view of the complexity of Eq. (14), no close correlation of $\mu/er$ with electronegativity difference would be expected. A satisfactory rough correlation is actually observed. This would seem to be empirical evidence for considerable mutual cancellation of the overlap, hybridization and polarization terms contributing to the dipole moment. Pauling assumed only the first term in Eq. (14) to be important and ignored the contribution of the overlapping, hybridization and polarization terms. Although the polarization moment is considerably small, the overlap and hybridization moments are often very large[34] and could contribute greatly to the observed dipole moment. Unfortunately, it is not possible to use an equation of this form to compare the calculated and measured values of dipole moments, because the polarization term, which is always of opposite sign to the primary moment, at least for large values of the ionicity, is particularly difficult to evaluate. Since accurate estimates of these contributions are not available, the observed correlation between $\mu/er$ and electronegativity differences indicates that dipole moments may be used as some sort of guide in evaluating ionic character, but reliable values of the ionic character cannot be deduced from the dipole moment data alone.

Gordy[35-37] suggested another relation [Eq. (15)] between the electronegativity difference $(x_A - x_B)$ of the two atoms in the bond A-B and the ionic character $(\beta)$ of the bond.

$$\begin{aligned} \beta &= \tfrac{1}{2}|x_A - x_B| \quad \text{for } |x_A - x_B| < 2 \\ &= 1 \qquad\qquad\quad \text{for } |x_A - x_B| > 2 \end{aligned} \quad ...(15)$$

Gordy obtained Eq. (15) by plotting the ionic characters, as determined from nuclear quadrupole coupling constants for some diatomic molecules against the electronegativity difference of the two atoms in the molecule. To arrive at this relation, Gordy assumed no hybridization for the halogen bonds except in certain special cases and he selected a list of diatomic and polyatomic molecules.

It seems inappropriate to use a particular selection of polyatomic molecules, such as those picked by Gordy and to ignore others which appear equally reasonable, but which give conflicting results. For example, Gordy did not consider FCl and FBr because of the uncertainty in the correction for positive ionic character and the possible occurrence of the double bond character in these two molecules. Also, the assumption of no hybridization for the halogen bonds seems to be incorrect, since recent calculations on nuclear quadrupole coupling and nuclear quadrupole resonance data have indicated a considerable amount of hybridization in organic halogen compounds. The assumption that all bonds between atoms with an electronegativity difference equal to or greater than two units (on Pauling's scale) have 100 per cent ionic character is not perfectly true. For instance, it gives 100 per cent ionic character for such bonds as SiF and GeF and BF, which are normally thought to be reasonably covalent[1].

Townes and coworkers[38,39] derived an S-shaped curve for the relation between the ionic character and the electronegativity difference. A similar curve was also obtained primarily from quadrupole coupling constants in diatomic molecules. However, Dailey and Townes overlooked the double bond character and the *d*-hybridization in the analysis of the quadrupole coupling data for obtaining this curve. Moreover, the *s*-hybridization has been evaluated from their rule that the halogen bonds are hybridized with 15 per cent *s*-character whenever the halogen is more electronegative by 0·25 unit than the atom to which it is bonded, otherwise there is no hybridization. This rule is arbitrary and has been shown to be incorrect[14].

Gordy's straightline relationship was, however, a fairly satisfactory rough approximation to the S-shaped curve over most of the range of electronegativity differences. Nevertheless, these curves differ in their finer details to the extent they depend on hybridization. At the top of the curve, in the region $|\Delta x| \simeq 2$, the large number of points available for alkali halides indicate a definite deviation from a simple linear relationship. In the middle of the curve, near $|\Delta x| = 1$, points for the molecules FCl and FBr show a considerable difference between the two curves. The two curves differ most markedly for electronegativity differences lying between 1·5 and 1·0, a region in which limited data are available on which to base a decision.

The use of polyatomic molecules to establish a relation between ionicity and electronegativity differences seems inappropriate, because the bond interaction effects make it difficult to assign an accurate electronegativity value to an atom forming more than one bond and in the case of polyatomic molecules group electronegativities[32,40] must be used instead of the electronegativities of the atoms in free states.

It is not possible to decide whether Gordy's or Townes and Dailey's rule is better. Essentially, both the rules are based on plausible choices for the values of *s*-character $(s^2)$, *d*-character $(d^2)$, ionic character $(\beta)$, and double bond character $(\delta)$, in the equation[14]

$$U_p = (1 - s^2 + d^2 - \beta - \delta/2) \quad ...(16)$$

where $U_p$ represents the unbalanced *p*-electrons obtained from the quadrupole coupling constants in molecules. Since, there is only one quantity, $U_p$, known for most of the molecules from the quadrupole coupling data, one could make alternative

choices of $\beta$, $(s^2-d^2)$ and $\delta$ and still match the data fairly well with Eq. (16).

A new and important semi-empirical relation between the ionic character of a bond A-B and the electronegativities ($x_A$, $x_B$) of the two atoms forming the bond was derived by Wilmshurst[41]. For a two-electron bond the pair of electrons would divide themselves between the two atoms in the ratio of their relative electronegativities, such that a fraction $2x_A/(x_A+x_B)$ of electron atmosphere would be on the atom A and a fraction $2x_B/(x_A+x_B)$ on electron atmosphere on the atom B. The ionic character of the bond can then be defined as half the excess number of electrons on one atom relative to the other as given by the equation

$$\beta = \frac{|x_A - x_B|}{(x_A+x_B)} \qquad ...(17)$$

Eq. (17) is equally applicable to the calculation of the ionic character in diatomic molecules, as well as to the calculation of the ionic character of bonds in polyatomic molecules, but in the latter case group electronegativities must be used. In contrast to previous similar relations Eq. (17) does not allow any one single curve of ionic character against electronegativity difference to be plotted.

Barrow[42], using a one-dimensional potential well model of the chemical bond, calculated the ionic character of a number of diatomic molecules. His results, in general, agreed with the values obtained from Eq. (17), while his treatment also showed that no one single ionic character versus electronegativity relation would be expected.

## Double Bond Character

If a bond has properties intermediate between those of an ideal single and an ideal double bond (between the same atoms), it is described as a single bond with double bond character. When $p_x$- and $p_y$-electrons on both atoms forming the bond also take part in bond formation between the two atoms, portions of the electron cloud from the outermost $p_x$- or $p_y$-states of the atoms move into the region between the atoms in order to overlap with each other and produce $\pi$ bonds perpendicular to the $\sigma$ bond. The double bond character due either to the $p_x$- or $p_y$-electrons is defined by the fraction $\delta$ as

$$\delta = \begin{bmatrix}\text{Electron density in the}\\ p_x \text{ (or } p_y\text{) state of the}\\ \text{free atom}\end{bmatrix} - \begin{bmatrix}\text{Electron density in the}\\ p_x \text{ (or } p_y\text{) state of the}\\ \text{bound atom}\end{bmatrix}$$

For the case of planar molecules, the double bond character may be ascribed entirely to the conjugation of $p_x$- or $p_y$-electrons, and a relation may be established between the asymmetry parameter for the halogen nuclei and the double bond character of the bond in which the halogen is taking part[43-46]. Goldstein[47] developed the following expression for evaluating $\delta$ with the help of the nuclear quadrupole coupling data:

$$\delta = \frac{\chi_{xx}-\chi_{yy}}{\frac{-3}{2}eQq_{\text{atomic}}} = \frac{\eta\chi_{zz}}{\frac{-3}{2}eQq_{\text{atomic}}} \qquad ...(18)$$

where the notations have their usual meaning. In the molecules having cylindrical symmetry about the line joining an atom to its neighbour, no distinction is possible between the $p_x$- and $p_y$-electrons. Hence, no measure of the double bond character is available from $\eta$, which has a value of zero. Moreover, this method is limited only to those molecules whose microwave spectra are well studied and which show nuclear quadrupole hyperfine structure. Thus, this method, though accurate, is somewhat complicated in analysis and applicable only to a limited number of molecules.

The length of a single bond between the two atoms in a molecule is generally less than the sum of the covalent radii of the two atoms. This shortening is primarily attributed to the partial ionic character of the bonds. However, in most of the cases the observed shortening of the bonds is much larger than that theoretically expected on the basis of the partial ionic character. This additional shortening is explained by assigning a double bond character to the bonds[1,48].

Based on these considerations, Pauling proposed a semi-empirical rule to evaluate the double bond character of the bonds[1]. According to this rule, if $R_1$ and $R_2$ represent the bond distances for single and double bonds between the two atoms and $R$, the observed distance, then

$$R = \frac{a_1R_1+3a_2R_2}{a_1+3a_2} \qquad ...(19)$$

where $a_1$ and $a_2$ are the fractional importance of the single and double bond structures. Thus $a_2$ corresponds to $\delta$ and $a_1 = 1-\delta$, so that

$$\delta = \frac{R_1-R}{R_1+2R-3R_2} \qquad ...(20)$$

The distances $R_1$ and $R_2$ might be obtained from the singly- and doubly-bonded covalent radii of the atoms tabulated by Pauling. Thus, if $R_{1A}$ and $R_{1B}$ refer to the single bond covalent radii of atoms A and B, then

$$R_1 = R_{1A}+R_{1B}-0{\cdot}09|x_A-x_B| \qquad ...(21)$$

The last term is a correction due to Stevenson and Schomaker[49] with $|x_A-x_B|$ representing the electronegativity difference between the two atoms. A similar relation holds for the double bond radii of the two atoms. Most of the calculations on the double bond character were made following this approach[1,48]. These calculations use the values of the partial ionic character which are very likely to be incorrect, as discussed earlier. Also, it was seen that Eq. (21) did neither hold good nor did it reproduce fairly the observed single bond lengths[50,51]. The values of the partial double bond character from this equation would, therefore, be erroneous.

A method for evaluating the partial double bond character based on the principle of electroneutrality[52] was suggested by one of the present authors. As pointed out earlier, the amount of the negative charge that would be placed on the atom A (if $x_A > x_B$) in the bond A-B due to the partial ionic character is given by $(x_A-x_B)/(x_A-x_B)$. The same amount of charge would be placed on the atom B, but with an opposite sign. Exceptionally large charges may result from the partial ionic character of the bonds between atoms having widely different electronegativities, if there is no way in which the charges can be reduced. Electron donors such as the halogens and oxygen atoms are able, under

these circumstances, to swing another pair of electrons into position for bond formation and electron acceptors, e.g. cyanide and nitro groups, can provide an orbital for a pair of electrons from the rest of the molecule, thus giving some double bond character to the bond. This double bond character can be estimated from the concept of the electroneutrality according to which, in general, the electronic structures of substances are such as to cause each atom to have essentially zero resultant electric charge. The charge $|x_A-x_B|/(x_A-x_B)$ would be reduced to zero, if this bond has $[\{|x_A-x_B|/(x_A-x_B)\}100]$ per cent double bond character. Hence, partial double bond character is given by[52]

$$\delta = \frac{|x_A-x_B|}{(x_A+x_B)} \qquad ...(22)$$

For polyatomic molecules, the value of group electronegativity is to be used in Eq. (22). The double bond character in a bond is equal to the ionic character of the bond which gives rise to the partial double bond character. The advantage of this method of evaluating $\delta$ is that it involves only the knowledge of the values of $x_A$ and $x_B$ which can be obtained easily.

## The *s*-Hybrid Character of the Bonding Orbitals

A chemical bond is generally formed by using strongly directed atomic orbitals as a result of mixing *s* and *p* atomic states. Actually, it is well known from valence theories that a stronger bond is produced if the bonding electrons are in orbitals made up of *s*- and *p*-states of the atoms instead of pure *p*-states. This mixing of *s* and *p* atomic states, leading to strongly directed atomic orbitals, is known as hybridization[53]. The *s*-hybrid character of the atomic orbitals of any atom bonded with other atoms depends upon the angles between the various bonds. For a molecule consisting of a central atom bonded by two other atoms, let the two bonds be described by the following two *s*-*p* hybrids

$$(1-s_1^2)^{1/2}\Psi_{p_1}+s_1\Psi_s \qquad ...(23)$$

$$(1-s_2^2)^{1/2}\Psi_{p_2}+s_2\Psi_s \qquad ...(24)$$

The wave functions $\Psi_{p_1}$ and $\Psi_{p_2}$ refer to *p*-states of the atom, symmetric about the two bond directions, and $\Psi_s$ refers to the *s*-state. Since the two bonds are independent of each other, the two states shown in Eqs. (23) and (24) must be orthogonal. If $\theta$ is the angle between the two bonds, this condition requires that

$$s_1s_2+[(1-s_1^2)(1-s_2^2)]^{1/2}\cos\theta = 0 \qquad ...(25)$$

if only *s*- and *p*-orbitals are involved and the two bonds are similar (i.e. $s_1 = s_2 = s$), then Eq. (25) yields[1,39,46]

$$s^2 = \frac{\cos\theta}{\cos\theta-1} \qquad ...(26)$$

which gives the amount of *s*-character on each bond. However, Eq. (26) is not always an accurate indication of the hybridization and sometimes gives a misleading or impossible estimate[30]. There are two possible sources of error in the value of $s^2$ deduced from Eq. (26) (ref. 46). Firstly, there may be some *d*-hybridization present due to which the wave functions [Eqs. (23) and (24)] do not give accurate descriptions of the atomic orbitals on the central atom[54] and, secondly, the repulsion between the atoms bonded to the atom of interest[53] causes error. While the first source of error would be more important for heavier atoms, the second would depend on the sizes of the attached atoms as well as on the net charges on them in the molecule. Moreover, this method does not give any information about the *s*-character of the bonds when the atom in question is chemically bonded to only one other atom. Such a situation occurs in most of the halogen compounds. Hence, recourse to other more general and accurate methods is taken in determining the *s*-character of the bonding orbitals.

### *s-Character from Nuclear Quadrupole Coupling Constants*

The measurement of nuclear quadrupole couplings in molecules provides a new means of investigating the electronic structures of molecules. This coupling constant depends on the value of nuclear spin $\vec{I}$, on the nuclear quadrupole moment $Q$, and on the tensor quantity $\vec{\nabla}\vec{\nabla}V$, where $V$ is the electrostatic potential due to all charges external to the nucleus having a quadrupole moment. A set of coordinate axes is usually chosen, so that the symmetry tensor may be described in terms of the three components, $q_{zz} = \partial^2V/\partial z^2$, $q_{yy} = \partial^2V/\partial y^2$, and $q_{xx} = \partial^2V/\partial x^2$, with $|q_{zz}|$ the largest. In the analysis of the experimental data for cases where the field is symmetric about the *z*-axis, $q_{xx} = q_{yy} = -\frac{1}{2}q_{zz}$ and the experimental results are given as values of $eQq_{zz}$, which is the quadrupole coupling constant. If the field does not have symmetry about the *z*-axis, the data are usually reported in terms of $eQq_{zz}$ and an asymmetry parameter

$$\eta = \frac{q_{xx}-q_{yy}}{q_{zz}} \qquad ...(27)$$

The fundamental quantities $eQq_{zz}$ and $\eta$ may be determined by quadrupole resonance, microwave, molecular and beam spectroscopy measurements. The asymmetry parameter $\eta$ does not involve any properties of the nucleus and can be interpreted in terms of the electron distribution in molecules. The quantity $eQq_{zz}$ involves a nuclear property $Q$ and a molecular property $q_{zz}$. The nuclear quadrupole moment $Q$ is a measure of the departure of the nuclear charge distribution from a spherical shape. It depends on the state of the nucleus and may be considered fixed, since nuclei will almost always be encountered in their ground state. The sign and magnitude of $Q$ can be very roughly estimated from nuclear theory and in some cases it may also be approximately estimated from radio frequency atomic beam techniques[55]. The quantity $q_{zz}$ can be estimated if the charge distribution over the molecule is known. This involves a precise and rigorous knowledge of the wave functions for the electrons in the molecule. A direct theoretical determination of these quantities is usually very complex and since accurate calculations of $q_{zz}$ for the molecules cannot be performed, many workers[12,36,37,39,56,57] relied upon simpler semi-empirical considerations to evaluate $q_{zz}$ and to derive

relations between the charge distribution and the quadrupole coupling constants.

Townes and Dailey[56,57] developed a simple relation between $eQq$ and the approximate wave functions for the bonding electrons in a molecule. For the special case of a diatomic chlorine compound they gave the approximate equation:

$$eQq \simeq (1+S^2-s^2+d^2)(1-\beta)eQq_{\text{atomic}} \quad ...(28)$$

In deriving Eq. (28) it was assumed that the wave function for the bonding electrons in the neighbourhood of the chlorine atom may be represented by

$$\Psi = \sqrt{1-s^2-d^2}\,\Psi_p \pm \sqrt{s^2}\,\Psi_s \pm \sqrt{d^2}\,\Psi_d \quad ...(29)$$

where $s^2$ is the amount of the $s$-character in the bonding orbital; $d^2$, the amount of the $d$-character; $\beta$, the ionicity; and $S^2$, the value of the square of the overlap integral. The general form of this relation has not been challenged, but methods of using it have differed widely.

From Eq. (28) it is evident that the quadrupole coupling for an atom in a molecule is also affected by the overlap of its valence wave functions with that of the atom to which it is bonded. The role of overlap effects in determining quadrupole coupling constants is a puzzling one and, unfortunately, the precise effect of overlap on the quadrupole coupling constants is very difficult to evaluate theoretically. Townes and Dailey in their analysis of the experimental values of quadrupole coupling constants rejected the overlap effects. The rejection of the overlap contribution implies that neither the valence bond nor the molecular orbital theories provide an adequate link between atomic and molecular wave functions for this purpose. The overlap integrals are not negligible and in $Cl_2$ and $Br_2$ they amount, according to Mulliken[58], to 0·34 and 0·31 respectively. Qualitatively their omission suggests that no bond has been formed and quantities such as ionic character and hybridization, defined on the basis of atomic wave functions neglecting overlap, cannot be carried over to molecules and consequently are of doubtful significance.

Schatz[59] attempted to overcome the above difficulty by including the overlap term explicitly in calculations of the field gradient in $Cl_2$, HCl and $CH_3Cl$, using Slater orbitals. He reported the presence of about 17 per cent $s$-character in the $Cl_2$ bonding orbital. This value, however, appears to be improbably high. Gordy[37] argued that it was incorrect to apply molecular orbital theory in this way, since the choice of the wave functions implies that the electronic charge is displaced from the region near the nucleus, which is important in determining quadrupole coupling, when a bond is formed. Such a displacement is unlikely, since the required overlap may be obtained more readily by a choice of orbital which redistributes the charge on the periphery of the atom. In this case, the molecular wave function would be changed relatively little near the nucleus and so the field gradient in the homopolar diatomic molecule and the atom would be nearly the same, as is found experimentally for chlorine. An analysis by Das[60], of the coupling constants of a number of boron compounds, supported this view. The molecular orbital method including overlap gives too low a value for the field gradient, and better results are obtained by omitting the overlap term. The resolution of the difficulty must lie in the use of more accurate wave functions to describe molecules. There is evidence that molecule formation is accompanied by a contraction of the orbital compared with its distribution in the free atom; this may be accounted for by simply attributing to the nucleus a larger formal charge than is implied in the use of Slater orbitals[61]. Craig and Magnuson[62] discussed the behaviour of sulphur from this point of view, and Bersohn[63] obtained better agreement between the calculated and experimental field gradients at the hydrogen nucleus in some C-H and O-H bonds in this way.

Richardson[64] suggested that the neglect of overlap is partly compensated for by using the very approximate Slater wave functions rather than the more accurate functions. Thus, while dealing with nuclear quadrupole coupling constants it appears permissible to ignore the overlap integral and normalize the wave functions by setting $S = 0$ (refs. 37, 65). However, Townes and Dailey[56] overlooked the effects of the partial double bond character of the bond and also neglected the possible occurrence of $d$-hybridization. Further, they stated quite arbitrarily that the halogen bonds are hybridized with 15 per cent $s$-character whenever the halogen is more electronegative by 0·25 units than the atom to which it is bonded but otherwise there is no hybridization.

Gordy[37,65] considered a diatomic molecule A-B bonded by a pair of electrons in the bonding orbital $\Psi$. Taking the A-orbital in question to be represented by the wave function $\Psi_A$, and the B-orbital with which it gets involved by $\Psi_B$, the linear combination of the atomic orbitals[66], i.e. the new molecular orbital $\Psi$ may be expressed as

$$\Psi = a\Psi_A + b\Psi_B \quad ...(30)$$

with the yet undetermined mixing coefficients $a$ and $b$. This is itself an approximation, since it neglects the hybridization terms from other atomic orbitals. The coefficients $a$ and $b$ are chosen to have values which associate $\Psi$ with minimum energy. The charge density due to the bonding electrons (proportional to $\Psi^2$) is given by

$$\Psi^2 = a^2\Psi^2 + 2ab\Psi_A\Psi_B + b^2\Psi_B^2 \quad ...(31)$$

if $\Psi_A$ and $\Psi_B$ are real. Thus, the approximate picture of the bond is that of the electronic charge $2ea^2$ centred on A, $2eb^2$ centred on B and $4eab\int\Psi_A\Psi_B d\tau$ associated with the overlap. The two electrons are assumed to be in the bonding orbital represented by $\Psi$ and their combined contribution to $q_{zz}$, the associated field gradient on the atom A along the internuclear axis, is given by

$$(q_{zz})_{\text{bond}} = 2a^2\int\Psi_A q\Psi_A^* d\tau + 4ab\int\Psi_A q\Psi_B^* d\tau + 2b^2\int\Psi_B q\Psi_B^* d\tau \quad ...(32)$$

where $q$ is equal to $e(3\cos^2\theta-1)/r^3$, $r$ is the distance from a point to nucleus A, and $\theta$, the angle between the direction of $r$ and the internuclear ($z$) axis. Since $q$ depends on the inverse cube of $r$, only that part of $\Psi$ which is centred on A will contribute significantly towards it. The last term of Eq. (32) represents

the contribution to $q_{zz}$ of electronic charge density in the atomic orbital of B, which, because of the inverse cube variation of $q$ and $r$, is negligible. The middle term, larger than the last, is less than 1 per cent of the first term for halogens. Thus, the last two terms in Eq. (32) may be dropped, giving

$$(q_{zz})_{\text{bond}} = 2a^2\int\Psi_A q\Psi_A^* d\tau = 2a^2 q_{\text{atomic}} \quad ...(33)$$

which, except for the factor $2a^2$, is simply the contribution of a single electron in the atomic orbital $\Psi_A$, where $q_{\text{atomic}}$ is $q_{zz}$ for the type of electron cloud concerned in $\Psi_A$.

The case when $\Psi_A$ is the wave function of a $p_z$-orbital is very important as then the other $p$-orbitals would be filled with unshared pairs and the $d$-orbitals would be empty. The total $q_{zz}$ can then be obtained by treating it as arising from a $p_z$-electron deficit of $(2-2a^2)$ electrons in an otherwise spherical cloud. The atomic coupling, on the other hand, arises from a deficit of exactly one $p$-electron in an otherwise closed shell. Therefore,

$$(q_{zz})_{\text{total}} = 2(1-a^2)q_{\text{atomic}} \quad ...(34)$$

Normalization of $\Psi$ in Eq. (31) yields

$$a^2 + b^2 + 2ab\int\Psi_A\Psi_B d\tau = 1 \quad ...(35)$$

and, if the overlap term is neglected, $a^2+b^2 = 1$. The ionic character $\beta$ of a bond is defined as

$$\beta = a^2 - b^2 \quad ...(36)$$

whereupon Eq. (34) becomes

$$(q_{zz})_{\text{total}} = (1-\beta)q_{\text{atomic}} \quad ...(37)$$

The observed quantity is $eQq_{zz}$ and this is related to the atomic measurements by

$$eQq_{zz} = (1-\beta)eQq_{\text{atomic}} \quad ...(38)$$

A useful concept is that of $U_p$, the number of unbalanced $p$-electrons oriented along the bond. It is given by

$$U_p = \frac{eQq_{zz}}{eQq_{\text{atomic}}} \quad ...(39)$$

The preceding discussion is based on the assumption that ionic character alone determines the electron distribution in a bond; the presence of hybridization will now be considered. A pure covalent bond, through hybridization of the atomic orbitals, can alter the angular distribution of the electronic charge cloud near the nucleus and can thus influence the coupling without lifting charge density appreciably away from the nucleus, although some lifting accompanies the hybridization. To examine the effect of bond orbital hybridization and ionic character, Eq. (33) has to be considered again. It may be assumed that the bonding orbital $\Psi_A$, while remaining primarily $p_z$, hybridizes with an $s$-orbital, and a $d$-orbital of A so as to give a better bonding (orbital) with $\Psi_B$. $\Psi_A$ can then be written as

$$\Psi_A = \alpha_s\Psi_s + \alpha_p\Psi_p + \alpha_d\Psi_d \quad ...(40)$$

where $\alpha_p$ is expected to be greater than $\alpha_s$ or $\alpha_d$, and normalization gives

$$\alpha_s^2 + \alpha_p^2 + \alpha_d^2 = 1$$

The contribution of electrons in the orbital $\Psi_A$ to $q_{zz}$ is given by

$$(q_{zz})_{\text{bond}} = 2a^2[\alpha_s^2\int\Psi_s q\Psi_s^* d\tau + \alpha_p^2\int\Psi_p q\Psi_p^* d\tau + \alpha_d^2\int\Psi_d q\Psi_d^* d\tau] \quad ...(41)$$

This is a modified version of Eq. (33), if the cross terms are neglected. The first integral in Eq. (41) is zero, because the $s$-electronic cloud (represented by $\Psi_s$) is spherically symmetric. The second integral represents the contribution of the $p$-electron discussed earlier. The third integral is small, because the $d$-orbital is non-penetrating and is negligible on account of the small penetration of $\Psi_d$ towards the nucleus. It is further reduced by the factor $\alpha_d^2$, which should ensure that its contribution to $q_{zz}$ in the halogen is less than 1 per cent. Both the first and the last terms are, therefore, dropped to give

$$(q_{zz})_{\text{bond}} = 2a^2\alpha_p^2\int\Psi_p q\Psi_p^* d\tau \quad ...(42)$$

Eq. (42) gives only the contribution of the hybridized bonding orbital. To get the total $q_{zz}$ other orbitals must be considered, some of which will be counter-hybridized. The $p$-orbital involved to be the $p_z$ is chosen. The counter-hybridized $sp_z$-orbital will have $\alpha_s^2$ amount of $p$-character and will contain an unshared pair of electrons, while the counter-hybridized $p_zd$-orbital will have $\alpha_d^2$ amount of $p_z$-character but will be empty. As before, the $p_x$- and $p_y$-orbitals contain unshared pairs. There will be resultant $p_z$ population of $2a^2\alpha_p^2 + 2\alpha_s^2$. The total $q_{zz}$ will arise from a $p_z$-electron deficit of $(2-2a^2\alpha_p^2-2\alpha_s^2)$ in an otherwise spherical cloud. With the normalizations, $\alpha_s^2+\alpha_p^2+\alpha_d^2 = 1$, $a^2+b^2 = 1$, and $\beta = a^2-b^2$, the expression for $(q_{zz})_{\text{total}}$ is obtained as

$$(q_{zz})_{\text{total}} = [1-\alpha_s^2+\alpha_d^2-\beta(1-\alpha_s^2-\alpha_d^2)]q_{\text{atomic}}$$

Taking $\alpha_s^2 = s^2$ and $\alpha_d^2 = d^2$,

$$(q_{zz})_{\text{total}} = [1-s^2+d^2-\beta(1-s^2-d^2)]q_{\text{atomic}} \quad ...(43)$$

where $s^2$ and $d^2$ are the amounts of $s$- and $d$-characters in the bonding orbital $\Psi_A$ of the atom A. Assuming that $s^2$ can be neglected, and that hybridization of the $d$-orbital is negligible, both Eqs. (43) and (28) reduce to

$$eQq = (1-s^2)(1-\beta)eQq_{\text{atomic}} \quad ...(44)$$

If the view is taken that $\beta$ is always a positive quantity, e.g. $|a^2-b^2|$, then $eQq_{zz}$ or $eQq_A$ and $U_p$ can be obtained by considering the bond as an appropriate hybrid of covalent and ionic forms.

Since in Eqs. (28) and (43) only $U_p$ is known for most of the molecules and three quantities are unknown, these equations cannot be used as such for the interpretation of the nuclear quadrupole coupling data. Gordy[37] assumed no hybridization ($s^2 = 0$, $d^2 = 0$) for the halogen bonds to obtain a relation between the electronegativity difference of the two atoms in the bond A-B and the ionic character of the bond as determined from nuclear quadrupole coupling data. Further, no allowance was made for the double bond character of the bond in his analysis.

A relation can be developed for estimating the $s$-character of the atomic orbitals forming the bond in terms of the ionic character, double bond character and the $p$-electron defect on some simple physical arguments[14]. In a molecule the nucleus

of an atom is subject not only to the influence of its own electrons, but also to those of all the other atoms composing the molecule and the effect of all these electronic charges should be considered. The field gradient $q$ along the $z$-axis at the nucleus of the atom A (of a molecule A-B) is due to: (i) the two electrons in the molecular orbital $\Psi$, which is a linear combination of the atomic orbitals on the free atoms, i.e. $\Psi = a\Psi_A + b\Psi_B$; (ii) the two pairs of $p_x$- and $p_y$-electrons on the atom A; (iii) the electrons in the inner shells; and (iv) the nucleus of the atom B and all its electrons except the one that takes part in the molecular orbital $\Psi$ (this may be regarded approximately as a unit positive charge at the position of the nucleus of the atom B).

However, as pointed out earlier, there are certain general facts which simplify the calculation of the resultant $q_{zz}$. An $s$-orbital or an undisturbed closed shell is spherically symmetrical. It makes no contribution to $q_{zz}$ except for small polarization or distortion effects. Calculations and experimental evidences indicate that such distortion effects may increase the contribution of a neighbouring ion to $q$. However, this is less than 2 per cent of the value due to valence electrons[39]. Hence, quadrupole effects due to neighbouring ions (a neighbouring ion distorts the electron distribution about the nucleus including that of the closed shells) or to distortion of surrounding spherical shells of electrons can be neglected. The electrons on the other atom B are also too far away to make significant contribution to $q_{zz}$ because of the inverse cube variation of $q$ with $r$.

The polarization of a closed shell was shown to be unimportant by Townes and Dailey[56]. They estimated that the contributions to the field gradient which arise in this way are only 1 per cent of those produced by an electron in that shell. The polarization effects on the valence electrons outside the atomic closed shell, however, must also be considered. Calculation shows that they can contribute up to about 10 per cent of the field gradient given by a valence electron in the lowest $p$-state, and so may also, in general, be ignored[56]. Contributions to $q$ from electrons in other states are usually negligible when there are unbalanced $p$-electrons in the valence shell. This represents a sweeping simplification of the problem, and yet it is not hard to justify. Therefore, all electrons, except those in the valence shell of the coupled atom, can be ignored and the problem resolves to one of guessing what happens to the electronic cloud of its valence shell when the atom forms a chemical bond. Furthermore, in the absence of any formal charge on the atom or bond orbital hybridization, the contribution of the nonbonding electrons in the valence shell will be the same as in the free atom except for small orientation and distortion effects.

If a bond is not entirely covalent but has some ionic character, the charge cloud of the bonding electron pair can no longer be regarded as divided equally between the two atoms. As shown earlier, if $x_A$ and $x_B$ are the values of electronegativity of the free atoms A and B forming the bond A-B and $x_A > x_B$, the negative charge that would be placed on the atom A due to the partial ionic character of the bond A-B is given by $(x_A - x_B)/(x_A + x_B)$. This leads to a net population of $2x_A/(x_A + x_B)$ or $(1+\beta)$ in the $p_z$-state of atom A. However, if $x_A < x_B$, the net charge placed on atom A due to the ionic character would be positive and the net population of the hybrid orbital pointing towards bond of atom A will be $(1-\beta)$.

Since electrons in different atomic orbitals have widely different couplings, hybridization of the bonding orbitals markedly affects the coupling. The most pronounced hybridization effects are obtained by mixing $s$-orbitals, which have zero coupling, with $p$-orbitals, which have the largest coupling of an orbital. Since there is, on an average, only one electron per atom in the bonding orbital, the $s$-electron cloud is decreased by the hybridization and the $p$-electron cloud is increased by the complementary hybridization. In general, other states with appropriate symmetry can also mix with the $p_z$-state. Thus, $d$-hybridization, which involves some mixing of the $d$-state with the $p_z$-state, should also be considered. If there is some $s$-hybridization, say $s^2$, it is obvious that the electron originally in the $p_z$-state loses an amount $s^2$ of $p$-character, while the two electrons in the $s$-state each gain an amount $s^2$ of $p$-character. The net population in the $p_z$-state of the atom A is, therefore, increased by an amount $s^2$. On the other hand, since the $d$-state is originally empty, $d$-hybridization leads to a loss of $p$-character by an amount $d^2$.

When, as is most often the case, the $\pi$ bond component in the double bond character is formed through donation of an unshared $p$-pair by the coupling atom A, the unbalanced $p$-charge is reduced and the coupling is lowered. An amount $\delta$ of the double bond character causes a reduction of $\delta/2$ $eQq_{\text{atomic}}$ in the quadrupole coupling. If $N_x$, $N_y$ and $N_z$ represent the electron population or the time averaged fractional number in the $p_x$, $p_y$ and $p_z$ states respectively of the atom A, from the above discussion it is evident that

$$\left.\begin{aligned} N_x &= 2 \\ N_y &= (2-\delta) \\ \text{and} \quad N_z &= (1+s^2-d^2+\beta) \end{aligned}\right\} \qquad \ldots(45)$$

The quantity

$$\left[\frac{N_x+N_y}{2} - N_z\right] = U_p \qquad \ldots(46)$$

is called the amount of unbalanced $p$-electrons oriented along the bond and is termed the $p$-electron defect.

For any type of bond, the net effect of the valence $p$-electrons may be expressed as the number of unbalanced $p$-electrons oriented along the bond, $U_p$. The quadrupole coupling constant is $U_p$ times the coupling per $p$-electron, or $U_p.eQq_{\text{atomic}}$. The sign of $U_p$ is positive for a deficit of $p_z$-electrons and the quadrupole coupling constant $eQq_{zz}$ has the same sign as $eQq_{\text{atomic}}$ for a $p_z$-electron. If $N_z$ happens to be greater than $(N_x+N_y)/2$, the sign of $U_p$ is negative for an excess of $p$-electrons, and the quadrupole coupling constant $eQq_{zz}$ has the opposite sign as $eQq_{\text{atomic}}$ for a $p_z$-electron. Thus

$$\frac{eQq_{zz}}{eQq_{\text{atomic}}} = U_p \qquad \ldots(47)$$

Eq. (45), therefore, yields[14]

$$U_p = (1-s^2+d^2-\beta-\delta/2) \qquad ...(48)$$

Eq. (48) can be used to evaluate the s-hybrid character, $s^2$, provided the values of $d^2$, $\beta$ and $\delta$ are known. There is evidence that s-hybridization in the halogens would occur at the negative and not at the positive pole[37]. Where there is a positive (not a negative) charge on the halogen, the halogen bonds are hybridized with d-character. The evidence is against the d-hybridization on the negative ion. Using the calculated values of $\beta$ and $\delta$ (as discussed earlier) and the experimentally determined values of $U_p$, the values of $s^2$ may be evaluated from Eq. (48). The values of s-hybrid character of chlorine, bromine and iodine orbitals obtained are given in Table 2.

### *s-Character from Nuclear Spin-Spin Coupling Constants*

This method of evaluating the s-character is limited only to those molecules whose microwave spectra show nuclear quadrupole hyperfine structure. Evaluation of the s-hybrid character of the bonding orbital of an atom, not having any nuclear quadrupole moment, is based on the measurement of indirect nuclear spin-spin coupling constants in nuclear magnetic resonance spectra. The nuclear spin-spin coupling between $^{13}C$ and proton directly bonded to each other, $J_{^{13}C\text{-}H}$, has been widely studied and discussed[29,67-85].

The possible utility of $^{13}C$-H coupling constants in determining the nature of the carbon orbital bonded to hydrogen was first pointed out by Muller and Pritchard[29]. Shoolery[67] established the following relationship between $J_{^{13}C\text{-}H}$ and the fractional s-character, $s^2_H$, of the carbon orbital bonding to hydrogen

$$J_{^{13}C\text{-}H} = 500 s^2_H \qquad ...(49)$$

Knowing $s^2_H$ from Eq. (49), $s^2_X$, the fractional s-character of the carbon atomic orbital of a C-X bond, can be computed if the carbon atomic orbitals are required to be orthogonal. The calculated values of the s-character of the carbon orbital in the C-F, C-Cl, C-Br and C-I bonds[14] are given in Table 3.

It was subsequently shown by Malinowski[80] that the effect of the substituents on $J_{^{13}C\text{-}H}$ values in substituted methanes is additive to a good approximation. This observation was rationalized by Gutowsky and Juan[77,78] who used both a potential box model and a valence bond formulation to derive the additivity effect. This treatment developed for methanes has been extended by them to $J_{^{13}C\text{-}H}$ in substituted ethylenes and to the $^{29}Si$-H couplings in silanes. Karabatsos *et al.*[86-88] showed that the correlation between the magnitude of $J_{^{13}C\text{-}H}$ and the extent of $sp$-hybridization is a general one and can be applied to interactions between $^{13}C$ and protons separated by two bonds with some modification.

However, the deviations from the additivity relations for highly electronegative substituents such as fluorine and methoxy were observed and empirical corrections suggested[74,79,83,84,89]. The theoretical justification for the quantitative character Eq. (49) is far from impregnable[82,90-92], but it is empirically very satisfactory in a series, such as $CH_4$, $C_2H_4$ and $C_2H_2$. It is presumably also satisfactory for a series of $CH_3Y$ compounds[93].

TABLE 2 — PERCENTAGE s-CHARACTER IN THE HALOGEN BOND ORBITALS DETERMINED FROM NUCLEAR QUADRUPOLE COUPLING CONSTANTS

| Molecule | $U_p$ (exp.) | $\beta$ | $\delta/2$ | $s^2-d^2$ | s-character % |
|---|---|---|---|---|---|
| | | NUCLEUS: $^{35}Cl$ | | | |
| FCl | 1·33 | 1·143 | 0·071 | −0·258 | 25·8* |
| $Cl_2$ | 0·99 | 0·000 | 0·000 | 0·010 | 1·0 |
| BrCl | 0·94 | 0·034 | 0·017 | 0·009 | 1·0 |
| ICl | 0·75 | 0·090 | 0·045 | 0·115 | 11·5 |
| HCl | 0·61 | 0·177 | 0·088 | 0·125 | 12·5 |
| TlCl | 0·14 | 0·250 | 0·125 | 0·485 | 48·5 |
| NaCl | 0·01 | 0·540 | 0·270 | 0·180 | 18·0 |
| ClCN | 0·76 | 0·003 | 0·0015 | 0·242 | 24·2 |
| $CH_3Cl$ | 0·69 | 0·107 | 0·053 | 0·150 | 15·0 |
| $SiH_3Cl$ | 0·36 | 0·224 | 0·112 | 0·304 | 30·4 |
| $GeH_3Cl$ | 0·42 | 0·240 | 0·120 | 0·220 | 22·0 |
| $CF_3Cl$ | 0·71 | 0·128 | 0·064 | 0·098 | 9·8 |
| $SiF_3Cl$ | 0·39 | 0·232 | 0·116 | 0·262 | 26·2 |
| | | NUCLEUS: $^{79}Br$ | | | |
| FBr | 1·42 | 0·177 | 0·088 | −0·331 | 33·1* |
| ClBr | 1·14 | 0·034 | 0·017 | −0·123 | 12·3* |
| $Br_2$ | 0·99 | 0·000 | 0·000 | 0·010 | 1·0 |
| NaBr | 0·08 | 0·510 | 0·255 | 0·155 | 15·5 |
| BrCN | 0·89 | 0·031 | 0·015 | 0·126 | 12·6 |
| $CH_3Br$ | 0·75 | 0·073 | 0·036 | 0·141 | 14·1 |
| $SiH_3Br$ | 0·44 | 0·191 | 0·095 | 0·274 | 27·4 |
| $GeH_3Br$ | 0·49 | 0·207 | 0·103 | 0·200 | 20·0 |
| $CF_3Br$ | 0·80 | 0·094 | 0·047 | 0·059 | 5·9 |
| $SiF_3Br$ | 0·57 | 0·199 | 0·099 | 0·132 | 13·2 |
| | | NUCLEUS: $^{127}I$ | | | |
| ICl | 1·28 | 0·090 | 0·045 | −0·235 | 23·5* |
| $I_2$ | 0·94 | 0·000 | 0·000 | 0·060 | 6·0 |
| NaI | 0·11 | 0·470 | 0·235 | 0·185 | 18·5 |
| ICN | 1·06 | 0·087 | 0·043 | −0·038 | 3·8* |
| $CH_3I$ | 0·84 | 0·016 | 0·008 | 0·136 | 13·6 |
| $SiH_3I$ | 0·54 | 0·136 | 0·068 | 0·256 | 25·6 |
| $CF_3I$ | 0·94 | 0·037 | 0·018 | 0·005 | 0·5 |

*Percentage d-character in the halogen bond orbital.

The nuclear coupling constants used in calculating $U_p$ values were determined from the hyperfine structure of microwave spectra[35].

$eQq_{atomic}$ values (Mc/s.): chlorine[94], −109·74; bromine[95], 769·62; and iodine[96], 2292·44.

TABLE 3 — PERCENTAGE s-CHARACTER IN THE CARBON BOND ORBITAL DETERMINED FROM NUCLEAR SPIN-SPIN COUPLING CONSTANTS

| Compound | $J_{^{13}C\text{-}H}$ | s-character % ($s^2_X$) |
|---|---|---|
| $CH_3F$ | 149·1* | 10·6 |
| $CH_2F_2$ | 184·5* | 13·1 |
| $CHF_3$ | 239·1* | 17·4 |
| $CH_3{}^{35}Cl$ | 150† | 10·0 |
| $CH_2Cl_2$ | 178† | 14·4 |
| $CHCl_3$ | 209† | 19·4 |
| $CH_3{}^{79}Br$ | 152† | 8·8 |
| $CH_2Br_2$ | 179* | 14·2 |
| $CHBr_3$ | 206† | 19·6 |
| $CH_3{}^{127}I$ | 151† | 9·4 |
| $CH_2I_2$ | 173† | 15·4 |

*Values reported by Frankiss[79].
†Values reported by Muller and Pritchard[29].

## Summary

A proper understanding of the concept of electronegativity of an isolated atom is necessary for obtaining the group electronegativity of a radical. The definitions given by different workers for electronegativity are critically examined in this review. The methods for evaluating the group electronegativities of radicals are also reviewed. The description of a chemical bond necessarily involves an assessment of the ionic and double bond characters. A critical survey is made of the methods for evaluating these quantities in a molecule. Another important quantity pertinent to a chemical bond is the *s*-hybrid character of the bonding orbitals. Methods for evaluating the orbital hybridization from bond angles, nuclear quadrupole coupling constants, nuclear spin-spin coupling constants are discussed. The bond properties of a number of radicals and molecules are tabulated.


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# Studies on Ubiquinone

T. RAMASARMA
Department of Biochemistry, Indian Institute of Science, Bangalore 12

A DECADE ago, in 1957, a new compound ubiquinone (coenzyme Q) was added to the list of naturally occurring compounds of potential significance in biochemistry. The author had the good fortune to witness and participate in the initial phases of its discovery and characterization during 1957-58 in the laboratory of Prof. D. E. Green at the Institute for Enzyme Research, University of Wisconsin (USA). The historical aspects of its simultaneous and independent discovery in the laboratories of Prof. R. A. Morton at Liverpool and at Wisconsin, and the chemical characterization by the two famous pharmaceutical laboratories of Hoffmann-La Roche, Switzerland, and Merck, Sharpe & Dohme, USA, were described earlier[1].

In importance and impact on biochemistry, ubiquinone can be compared with such other compounds as ATP, NAD, folate and cytochromes. The enormous biological interest was in part due to its ubiquitous occurrence and the structural features with resemblance to fat-soluble vitamins — to vitamin K in having a quinone nucleus, to vitamin A in its isoprene side chain, and to vitamin E by its ability to cyclize to chromenol, ubichromenol. Ubiquinone with its 59 carbons and molecular weight of 863 is the largest single molecule in animal tissues, excluding polymers (Fig. 1). The long polyprenyl side chain confers its lipid character, understandably necessary since ubiquinone is a common constituent of membrane structures. There are no extraordinary molecular properties other than the oxidation-reduction of the quinone. Yet the molecule exhibits wide biological responses with remarkable specificity. A good deal of information on the compound and its functions is made available by the efforts of many laboratories. Several good reviews were published periodically summarizing the advances made in the field[1-9]. In this article an attempt will be made to present the contributions from this laboratory in the progress of ubiquinone research.

## Methodology

Late in 1957, when ubiquinone class of compounds were being identified in the Enzyme Institute in a variety of aerobic organisms, it was recognized that all of them possessed the same ultraviolet absorption spectrum and elution characteristics in adsorbent chromatography but differed in their melting points. The difference was recognized to be due to the differing chain length from 10 to 6 isoprene units[10]. To characterize the homologue it was necessary to obtain enough quantity to crystallize and determine

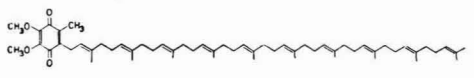

Fig. 1 — Structural formula of ubiquinone-10 [This homologue with 10 isoprene units in the side chain is the most common homologue in the animals including the man]

the melting point and molecular extinction. Such procedures would be too demanding on time for a survey on a large scale. Therefore, a reverse phase paper chromatographic system was developed using papers impregnated with Dow Corning Silicone Fluid No. 550 and 80 per cent *n*-propanol/water as the solvent which separated the homologues satisfactorily (Fig. 2)[11]. This system was useful in rapid identification of the homologues and even their separation on quantitative scale[12]. This method with modifications using vaseline[13] or stopcock grease[14] had remained a valuable tool in the quick identification of the homologues.

The isolation procedure essentially consists of saponification in ethanol or methanol under reflux in the presence of the antioxidant, pyrogallol, and subsequent separation by adsorbent chromatography on columns followed by crystallization. The recoveries are better than 90 per cent under these conditions. Recently, linear gradient concentration of ethyl ether had been employed instead of the batch elution (Fig. 3). After the column, the fractions were

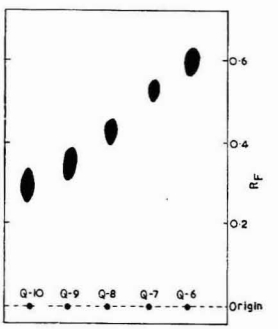


Fig. 2 — Mobility of five ubiquinone homologues in ascending reverse phase paper chromatogram [Solvent, *n*-propanol-water (4: 1); paper, Whatman No. 3MM impregnated with Dow Corning Silicone Fluid No. 550 (ref. 11)]

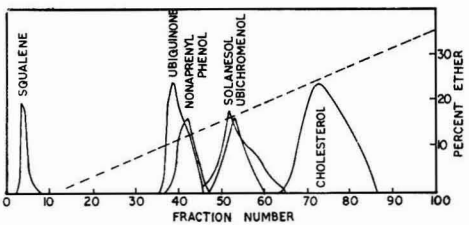


Fig. 3 — Elution pattern of standard mixture of squalene, ubiquinone, nonaprenyl phenol, solanesol, ubichromenol and cholesterol on a deactivated alumina (20 g.) column [Linear ethyl ether gradient elution using petroleum ether (250 ml.) and ethyl ether/petroleum ether (250 ml.) for the gradient were employed, and 5 ml. fractions were collected and analysed]

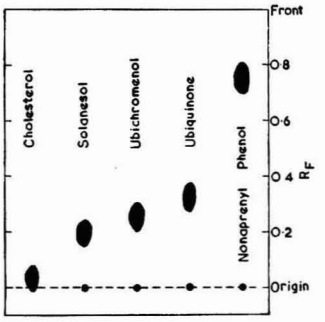


Fig. 4 — Separation of ubiquinone and related compounds on thin layer plates of silica gel G [Solvent, benzene]

further purified on thin layer plates, either silica gel G or alumina G by which ubiquinone was obtained free from other contaminants. The separation on silica gel G is usually used for the separation of nonaprenyl phenol (Fig. 4).

Ubiquinone content was determined by the decrease in the absorption at the peak (275 mμ) on reduction with borohydride. This method has some drawbacks when impurities are present. Turbidity developed when other lipids were not removed. Egg yolk, one of the earliest materials tested in this laboratory[14], offered many difficulties because of its high content of triglycerides, phospholipids, sterols, carotenes, etc. While extensive purification on column chromatography removed these impurities, losses of ubiquinone were high. When small quantities of ubiquinone were present, the problem was greater. So reverse phase paper chromatography had been adapted to separate ubiquinone from the interfering material. It was then extracted from the paper, reduced with borohydride, ubiquinol re-extracted with petroleum ether and reacted with α,α-dipyridyl-ferric chloride reagents. The Emmerie-Engel method for measuring quinols and chromanols had been adapted to work in aqueous solutions which helped to stabilize the otherwise unstable colour, and to increase the colour intensity[15]. The sensitivity of the method is high and can measure as low as 0·01 μmole quantities and had been used in routine simultaneous analysis of ubiquinone and tocopherol in large number of tissues in the rat. This method was used for reporting for the first time the presence of small quantities of ubiquinone (about 6 μg./ml.) in the human blood[14].

## Intracellular Distribution of Ubiquinone

The general discussion following the Ciba Symposium on *Quinones in electron transport*[1] held in May 1960 was initiated by the comment by Chance that while ubiquinone (coenzyme Q) was discovered in mitochondria, there was lack of information on its possible localization in other cell components. By that time we already had such data in our laboratory showing that ubiquinone has extra-mitochondrial occurrence. Vitamin A deficiency in the rat was known to increase liver ubiquinone content[16]. With a good deal of interest on vitamin A in this laboratory, we wanted to find out where the extra ubiquinone resided in the liver cell of vitamin A-deficient rats and whether or not this could all be accommodated in the mitochondrion itself. When the normal animals were first tested, to our horror, we found that only about 40-60 per cent of the total ubiquinone was in the mitochondrial fraction. We repeated the experiment several times to convince ourselves that our methods were correct and this pattern was true. By testing the marker enzyme activity, it was established that mitochondrial contamination could not account for ubiquinone's presence in other fractions[17]. We also found that such distribution is true in other tissues and other animals[18]. We later realized that there was a brief report from the Liverpool Laboratory that ubiquinone was present in other cell fractions, but it was concluded that it was predominantly in the mitochondria[19]. We extended our studies by testing the cell components prepared by different methods to confirm the natural occurrence and have established it with respect to microsomes and soluble supernatant[20]. It was not conclusive with respect to nucleus where oxidative activities and ubiquinone content always paralleled. Indirect evidence, however, was obtained to show that nuclear ubiquinone was also partly constitutive, based on the differences in specific radioactivities of mevalonate-2-$^{14}$C incorporation into ubiquinone in the cell components[21]. The pattern of intracellular distribution was amply confirmed in other laboratories[22,23]. But Moury and Crane[24] raised a doubt on the natural occurrence in microsomes. We reconfirmed[25] our work and showed that microsomes prepared by the conventional method and characterized by the enzyme activities indeed possessed significant amounts of ubiquinone. The presence of ubiquinone in liver microsomes may partly be of dietary origin representing the absorption of the dietary compound through the endoplasmic reticulum. Liver microsomes obtained from rats fed on ubiquinone-free diet for long periods also possessed considerable ubiquinone, confirming its endogenous nature.

The intracellular distribution pattern remains the same despite large variations in the total ubiquinone content of the livers of normal rats or in rats fed diets of low protein content[26]. Under other nutritional and stress conditions, this pattern was altered (Table 1).

The concentration of ubiquinone in the particulate systems seems significant in view of the ratio of ubiquinone to protein being about 1 : 2 for microsomes and 1:1 for mitochondria taking one million as the average molecular weight of the protein which exists in highly polymerized and organized state in the membranes. In view of the known occurrence of ubiquinone in all the cellular particulate material it appears that it is a general constituent of membranes and may have significant role in membrane function.

## Biosynthesis of Ubiquinone

*Independent synthesis of ubiquinone in rat tissues*— Ubiquinone is known to occur in every tissue of the rat. It was of interest to investigate whether individual tissues have the capacity to synthesize the compound or whether partial requirement will arise

TABLE 1 — INTRACELLULAR DISTRIBUTION OF UBIQUINONE IN THE LIVERS OF RATS UNDER DIFFERENT NUTRITIONAL AND OTHER CONDITIONS

| Status of the rats | mμmole/g. liver | % in each cell fraction | | | |
|---|---|---|---|---|---|
| | | Nucleus | Mitochondria | Microsomes | Supernatant |
| Normal (ref. 17) | 96 | 37 | 41 | 15 | 7 |
| Protein deficiency (ref. 26) | 13 | 23 | 46 | 16 | 15 |
| Vitamin A deficiency (ref. 27) | 260 | 20 | 35 | 17 | 28 |
| Thyrotoxicosis* | 276 | 17 | 45 | 12 | 26 |
| Cold exposure (ref. 28) | 242 | 21 | 39 | 29 | 11 |
| CPIB-fed* | 220 | 29 | 60 | 3 | 8 |
| Ubiquinone-9-fed* | 230 | 27 | 50 | 13 | 10 |

*Unpublished data from this laboratory.

in any particular tissue. Gloor and Wiss[29] showed earlier that mevalonate-2-$^{14}$C was incorporated into the side chain of ubiquinone in the rat liver. Using acetate-2-$^{14}$C, Lawson *et al.*[30] found incorporation of radioactivity into ubiquinone of liver, skin and intestine. Joshi *et al.*[31] extended these studies with mevalonate-2-$^{14}$C and found incorporation into ubiquinone in heart, intestines and kidney and spleen. In all these cases the specific activities were different from tissue to tissue and in the case of kidney and spleen the turnover rate was surprisingly higher than in the liver (Table 2). This led to the conclusion that the tissues are capable of independent synthesis of ubiquinone, with regard to the side chain, especially in view of the apparent lack of exchange of ubiquinone between tissues which will be discussed later. This view is confirmed by Gold and Olson[32] using a tissue-slice system.

*Endogenous nature of the homologue, ubiquinone*-10— Rat liver contains homologue ubiquinone-9 (nine isoprene units in the side chain) to the extent of 85 per cent and about 10 per cent ubiquinone-10, the rest being lower homologues. Since ubiquinone-10 was found in the diet and can be absorbed, this homologue was considered to be exogenous[33]. While this may be partly true in the case of liver, the presence of this homologue in other tissues of the rat must be endogenous, since dietary compound did not appear in other tissues. It was established even in the liver that ubiquinone-10 was biosynthesized and, in fact, with turnover possibly greater than ubiquinone-9[31,34]. At the moment we do not know why the rat has the peculiar feature of having a majority of ubiquinone-9, in contrast to ubiquinone-10 in other animals, how the constant proportion of the two homologues were synthesized and their functional significance. There is absolute specificity of the major homologue at least in one system, which will be described later.

*Intermediates in the synthesis of ubiquinone* — Incorporation of mevalonate-2-$^{14}$C into the side chain is indicative of *de novo* formation of ubiquinone and is used as a measure of its synthesis. The complete sequence of reactions and the enzymatic mechanisms involved are not yet understood. So far no cell-free homogenate system synthesizing ubiquinone could be perfected. Some crucial step may be irreversibly damaged during homogenization. Most of the experiments on biosynthesis are largely in intact animals or tissue slices, utilizing incorporation of radioactive tracers. Fig. 5 summarizes the

TABLE 2 — INCORPORATION OF MEVALONATE-2-$^{14}$C INTO UBIQUINONE AND UBICHROMENOL IN RAT TISSUES*

| Tissue | Counts/min./μmole | |
|---|---|---|
| | Ubiquinone | Ubichromenol |
| Intestines | 3350 | 10000 |
| Kidneys | 2700 | 5000 |
| Spleen | 1000 | — |
| Liver | 250 | 530 |
| Heart | 120 | — |

*Data from ref. 31.

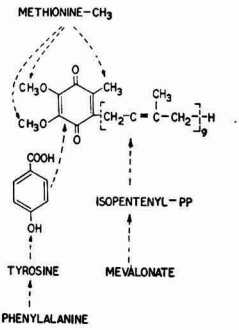


Fig. 5 — The origin of various parts of ubiquinone molecule from the studies of incorporation of radioactive tracers

knowledge on the incorporation of tracers into the various parts of the molecule.

The reactions from *p*-hydroxybenzoate to the fully substituted quinone and from mevalonate to the polyprenyl side chain are yet to be deciphered. There are eight reactions involved in the formation of the ring: three C-hydroxylations, two O-methylation, one C-methylation, one decarboxylation and one C-alkylation by polyprenyl group. The possibility of similar reaction types being accomplished in a single step and the occurrence of concerted reactions cannot be ruled out. The possibilities are too many to make a guess at the probable sequence of the reactions.

It is not yet certain whether alkylation of side chain occurs early in the sequence as in the case of photosynthetic anaerobe, *Rhodospirullum rubrum*, where

the product was nonaprenyl phenol[35], which then undergoes substitution in the ring. During the author's brief stay in 1966 in the laboratory of Prof. R. E. Olson in St Louis (USA), attempts to detect nonaprenyl phenol or its labelling by a variety of radioactive tracers were not successful. We have now confirmed, in this laboratory[36], the absence of nonaprenyl phenol in a variety of aerobic organisms and, therefore, the chances of this compound being an intermediate in aerobic synthesis seem to be vanishingly small (Sharma, B. V. S., unpublished data). A new compound, called compound S, having twin absorption peaks at 272 and 310 mμ, was found in several sources in this laboratory[36]. We considered that this may be the precursor in aerobic synthesis of ubiquinone, but our recent experiments showed that this compound is an isolation artifact.

Information on the first intermediate of ubiquinone having both the ring and the side chain in animal tissues will be of paramount importance, because lipid intermediates will be found only when alkylation occurs early in the synthetic sequence. The only one such intermediate so far reported was the ' compound X ' of Olson and Aiyar[37]. Several benzoate-derived lipids were detected (Fig. 6), but these did not possess the isoprene side chain (Ramasarma, T., unpublished work). In fact, Lynen *et al.*[38] proposed that the quinone nucleus may condense with polyprenyl pyrophosphate with the elimination of pyrophosphate, yielding ubiquinone. Evidence for this mechanism was claimed by Stoffel and Martius[39], who showed the formation of ubiquinone from appropriate substrates by a mitochondrial enzyme system. This implies that the last step in the biosynthesis is the attachment of side chain and that it occurs in mitochondria. These experiments could not be reproduced in other laboratories and also are inconsistent with the finding that the fully substituted ring (ubiquinone-O) did not participate in ubiquinone synthesis. The site for the last step in the formation of ubiquinone appears to be the soluble cytoplasm, because our data on the specific radioactivities of ubiquinone in cell components at various time intervals after a single dose of mevalonate-2-$^{14}$C showed that soluble supernatant had the highest value at the early time interval which decreased rapidly accompanied by increase in other fractions[40].

*Pathway of prenyl polymerization* — The assumption that the isoprene pathway of sterol synthesis beyond mevalonate will be shared by ubiquinone has never been verified. Where the branching takes place is still not clear. Experiments in this laboratory showed that the two pathways are common at least up to isopentenyl pyrophosphate, since SKF 525A[41], a powerful inhibitor of some step between isopentenyl pyrophosphate and squalene, inhibited the incorporation of mevalonate-2-$^{14}$C into both cholesterol and

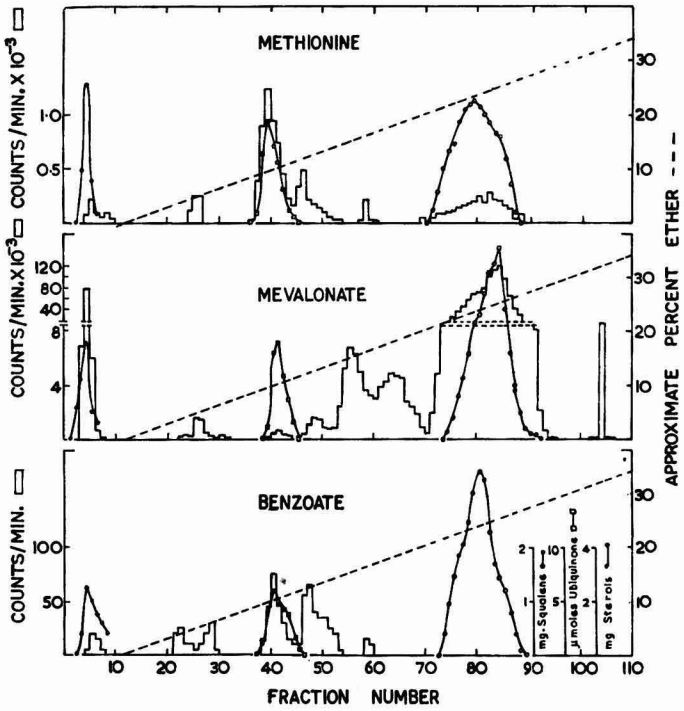


Fig. 6 — Incorporation of methionine, mevalonate and benzoate into the unsaponifiable lipids of rat liver [Two rats per group were dosed intraperitoneally with the tracers (benzoate-U-$^{14}$C, 100 μC./rat; mevalonate-2-$^{14}$C, 5 μC./rat; or methionine-methyl-$^{14}$C, 50 μC./rat). After 30 min., the animals were killed, the livers were processed for unsaponifiable lipids. The lipids were chromatographed on deactivated alumina columns as described in the legend for Fig. 3]

ubiquinone and also reduced their concentrations in the liver (Inamdar, A. R., unpublished data).

Polyprenyl pyrophosphates of long chains had not been detected and their participation in ubiquinone synthesis is still a conjecture. Despite the occurrence of solanesol in the rat liver, its participation as an intermediate had been ruled out by isotopic experiments[42,43]. Prenyl polymerization thus remains an unsolved problem, while most other biological polymerizations are reasonably understood.

## Absorption and Internal Transport of Ubiquinone

*Fate of administered ubiquinone in the rat* — Diets deficient in ubiquinone have not produced any change in the concentration of ubiquinone in the rat tissues[44,45] — understandable in view of its biosynthesis. The absorption of exogenous ubiquinone from the diet was demonstrated by Lawson *et al.*[45] and Rudney[46], and we have studied this in detail in this laboratory using radioactive ubiquinone.

Given intracardially, the compound was removed from the blood within a few minutes and could be detected only in the liver and to a small extent in the spleen. However, once the compound was deposited in the liver, it was not mobilized by any other tissue even after long periods[47].

On administration by oral route, ubiquinone-10-$^{14}$C was absorbed by the liver exclusively and by no other tissue. After long time intervals, ubiquinone in the liver decreased, but did not appear in other tissues. These observations support the total lack of internal transport of ubiquinone in the rat tissues[21].

The fate of administered ubiquinone is in marked contrast with that of another lipid-quinone, vitamin K (methyl-$^{14}$C)[48], which was distributed in all tissues, irrespective of the mode of administration. This pattern of uniform distribution was also found in the case of α-tocopherol[49] and ubichromenol[31].

*Experiments on saturated ubiquinone* — The absorption and metabolism of ubiquinone by the liver was altered when the double bonds in the side chain were saturated[21]. Firstly, the animal was incapable of absorbing the saturated compound through the intestines. This inability was not due to any impairment of the reduction of the ring in the intestines. Similar failure of absorption was also noticed for vitamin $K_1$ and ubichromenol when their side chains were saturated. Secondly, once bound to the liver by intracardial administration the saturated compound was metabolized only slowly (Fig. 7). Therefore, both for absorption and catabolism of ubiquinone the double bonds in the side chain are necessary. Plastoquinone, a related quinone having isoprene side chain, occurring in plants was never detected in the tissues of the rat, although fed plant material and apparently was not absorbed, indicating that certain specificity of the ring structure also seems to be involved in the absorption.

*Catabolism of ubiquinone under stress conditions* — The absorbed compound in the liver was largely present as unchanged ubiquinone with a small quantity of degraded lipid products. Under stress conditions[50], the catabolism of the absorbed compound decreased. Therefore, the absorbed compound was retained in the liver to a greater extent and for a longer period under these experimental conditions compared to the normal animals (Fig. 8).

*Reactions of exogenous ubiquinone* — Will the exogenous ubiquinone equilibrate with the endogenous form and participate in its reactions? Intravenously or orally administered ubiquinone-10-$^{14}$C was deposited in the liver cell and was distributed in all the cell fractions with a majority being in the mitochondrial and the microsomal fractions. Since simple addition of ubiquinone to liver homogenate before centrifugation recovered it in the supernatant fraction, the above results were considered to represent the actual penetration of the compound into the cell[20]. The specific radioactivities at different time intervals indicated that the compound had probably entered the cell through the endoplasmic reticulum and rapidly redistributed in other parts of the cell.

Ubiquinone added to mitochondria interacted with the electron transport, possibly at the site of endogenous form or with the component before it, since its oxidation, but not its reduction, was inhibited by antimycin A[51]. It was possible to obtain rates of a magnitude close to overall oxygen uptake, which were by no means optimal in view of the difficulties in preparing suitable emulsions of the substrate. This interaction appears to be at the

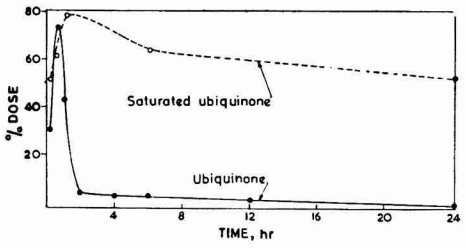


Fig. 7 — Retention of intracardially administered ubiquinone or saturated ubiquinone (0·25 μC./rat) by rat liver [After administering the tracers, the rats were killed at various time intervals, livers removed and radioactivity in the unsaponifiable lipids was estimated. The percentage values of the dose retained in the liver at various time intervals are given in the figure (data from ref. 20 and 21)]

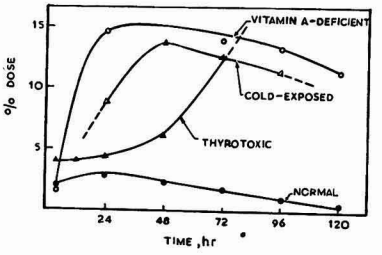


Fig. 8 — Retention of orally administered ubiquinone-10-$^{14}$C by livers of rats under different experimental conditions [Rats were dosed 0·5 μC. of the tracer and killed at different time intervals. The radioactivity in the unsaponifiable lipids of the liver was measured. The experimental conditions of vitamin A deficiency[27], cold exposure[28] and thyrotoxicosis[50] were described in the respective publications from which the data are collected]

flavoprotein level, since such reductases using exogenous lower homologues of ubiquinone have now been characterized[52].

These experiments showed that there were other sites in the mitochondria where considerable quantities of exogenous ubiquinone, added *in vitro*, were sequestered and made unavailable for reduction by succinate but could be released for the purpose by treatment with isooctane[51]. By the amount of mitochondrially-bound ubiquinone available for reduction, we can have a measure of equilibration of endogenous and exogenous forms. Exogenous ubiquinone absorbed via oral route gave completely ' equilibrated form ' whereas intracardial administration gave more than 50 per cent in the ' sequestered form '[21]. This way labelled mitochondria with radioactive ubiquinone can easily be prepared.

## Ubichromenol-Ubiquinone Interrelationship

Ubichromenol accompanies its structural isomer, ubiquinone, in several animals and in most tissues of the rat[16,53]. Changes in their concentrations parallel in altered nutritional conditions of vitamin A deficiency[54], vitamin E deficiency[53] and protein deficiency[26]. But the relative proportion of the two compounds was not constant. In some tissues, particularly heart, ubiquinone was high, but ubichromenol was low. Kidneys showed largest amounts of ubichromenol[55].

The physiological significance of ubichromenol is of particular interest. It was reported that the chromanol can replace vitamin E in gestation-resorption test in the rat[56], the only test in this animal which is specific for vitamin E, while other deficiency symptoms can be cured by antioxidants and selenium. Ubichromenol concentration decreased in vitamin E deficiency[53]. The vitamin status of tocopherol in some species, including human beings, is not established. It is tempting, therefore, to speculate that the possible reason may be that endogenous ubichromenol in the body might take over these functions.

*Natural occurrence of ubichromenol* — However, doubts were expressed on its natural occurrence as it was found to be formed from ubiquinone on treatment with alumina[57] and by alkali[58] which were involved in the isolation procedure. It was soon recognized that although these effects were real, they could not account for the presence of large amounts of ubichromenol found in some tissues and low amounts or absence in others. The proportion of ubichromenol/ubiquinone was not constant in every tissue as would be expected of an isolation artifact. Partial conversion of ubiquinone obtained under experimental conditions, normally employed, could not account for more than 10-15 per cent of ubichromenol present in rat liver[59]. This was further substantiated by using radioactive ubiquinone. Ubiquinone administered orally was absorbed into the liver, equilibrated with the endogenous form, but was not converted to ubichromenol to any significant extent even after long time intervals[31]. Raman and Rudney[60] observed small non-enzymic conversion of ubiquinone to ubichromenol in the presence of serum albumin or heat-denatured kidney mince, but concluded that this was not facile as surmised by Olson[61] and could not account for the total ubichromenol found.

*Biosynthesis of ubichromenol in the rat* — We attempted to solve the problem of the origin of ubichromenol from a biosynthetic approach. The rationale was simple; if ubichromenol is natural it should be biosynthesized in the animal like ubiquinone. The same problems had to be faced to exclude the possibility of artifacts at every stage of experiments. Mevalonate-2-$^{14}$C was found to be incorporated into ubichromenol isolated from liver, kidneys and intestines. The purification involved fractionation on deactivated alumina column followed crystallization to constant specific radioactivity[62]. These values for ubichromenol were higher than ubiquinone and, therefore, it was argued that the latter could not be the source (Table 2). Green *et al.*[63] confirmed incorporation of mevalonate-2-$^{14}$C at a late time interval, but reported lower values for specific radioactivity. We further found that the initial high values for ubichromenol decreased at late time intervals and the specific radioactivity curves of ubichromenol and ubiquinone show a striking precursor-product relationship[64]. The possibility of ubichromenol being a precursor of ubiquinone was dismissed, since such conversion could not be demonstrated[64]. Therefore, we interpreted that the incorporation data are suggestive of the conversion of a common intermediate into the two products, this being more distal to ubiquinone[43]. Later, Raman and Rudney[60] carried out similar experiments with kidney mince and found that at one early time interval the specific radioactivity of ubichromenol was higher, but not as high as we reported. Their study further revealed that the ring of ubichromenol was not as rapidly synthesized as the side chain, that the two sections may have independent turnover and that the ring seemed to be derived via ubiquinone physiologically but not as an artifact. We later realized that the second set of our experiments which used only adsorbent chromatography on alumina (both column and thin layer) had not separated some high-counting cogeners, probably prenyl alcohols, and, therefore, gave higher values, which are now considered invalid. Nevertheless, this does not alter the support to the natural status of ubichromenol, since after extensive purification by alumina column, thin layer chromatography and cocrystallization with added carriers (Joshi, V. C. & Jayaraman, J., unpublished data) and after powerful reverse phase thin layer chromatography (Rudney, H., personal communication) ubichromenol was found to be labelled by mevalonate-2-$^{14}$C, much more than could be accounted for by the artifact formation.

## Ubiquinone and Vitamin K in Microorganisms

*Importance of vitamin K synthesis in mycobacteria*— Microorganisms have fewer types of lipids and high proportion of lipid-quinones, practically the only known isoprene compounds in bacteria, making them ideal organisms for studying the biosynthesis of lipid-quinones. We first started the study of biosynthesis of the isoprene side chain of vitamin $K_2$ in *Mycobacterium phlei* and *tuberculosis* in 1960 at which time no information was available on the subject.

Vitamin $K_2$, a natural constituent of these bacteria and a vitamin to the animals, by implication, must be synthesized in the bacteria and not in the host. We hoped that understanding of its biosynthetic pathway might offer a possibility of finding a suitable inhibitor of the exclusively bacterial synthesis acting as a therapeutic agent. To our dismay, all our attempts to incorporate acetate-1-$^{14}$C or mevalonate-2-$^{14}$C into vitamin $K_2$ have not met with success[65]. While no other known isoprene compound existed in these bacteria, mevalonate-2-$^{14}$C was significantly incorporated into the cell residue and into an unknown lipid having polarity characteristics of prenyl alcohols.

*Synthesis of lipid-quinones in microorganisms* — Extending these studies with other bacteria, it was recognized that the non-incorporation of acetate or mevalonate into ubiquinone or vitamin K is a common feature in four bacteria tested: *Azotobacter vinelandii* (Q-8), *Escherichia coli* (Q-8 and $K_2$), *Pseudomonas* sp. (Q-9) and *Agrobacterium tumefaciens* (Q-10)[66]. But these tracers were readily incorporated into ubiquinone and sterols of moulds: *Aspergillus niger* (Q-9), *Neurospora crassa* (Q-10), *Penicillium chrysogenum* (Q-9) and *Gibberella fujikuroi* [Q-10($H_{10}$)]. This led to the conclusion that "the mevalonate pathway for the synthesis of ubiquinone is operative only in those organisms which also contain other isoprene compounds, such as sterol and carotene". Since then considerable information on the bacterial synthesis of lipid-quinones is available, but we still do not know how the isoprene chains are built. At the moment the experiments do not completely exclude the acetate-mevalonate pathway, although these tracers were not readily incorporated, since small amount of acetate incorporation into vitamin $K_2$[67] and ubiquinone[68] of *Esch. coli* had been found. Also, pyruvate-2-$^{14}$C and pyruvate-3-$^{14}$C were found to be incorporated, but not pyruvate-1-$^{14}$C, into ubiquinone of *Esch. coli* consistent with 'active acetate' being used for isoprene synthesis (Sharma, B. V. S., unpublished data).

*Experiments with benzoate-7-$^{14}$C* — It is instructive to record that early in 1963, Dr T. S. Raman tested in our laboratory the possibility of benzoate being the precursor of the ring of ubiquinone in *A. niger* and a benzoate-dependent yeast-like organism. As chance would have it, only benzoate-7-$^{14}$C was available with us and it gave negative results. Later that year, the laboratories of Rudney[69] and Olson[70] announced that ring-labelled *p*-hydroxybenzaldehyde or *p*-hydroxybenzoate (or benzoate) were efficient precursors of ubiquinone with carbon-7 being eliminated. Had we used ring or uniformly labelled benzoate we would have simultaneously discovered this intermediate.

*Polyprenyl alcohols* — In most of our experiments with microorganisms, mevalonate-2-$^{14}$C was incorporated into compounds having the same polarity characteristics as prenyl alcohols (Fig. 9)[66]. These have attained prominence lately after the brilliant identification by Robbins *et al.*[71] of the $C_{55}$-polyprenyl alcohol as the lipid-intermediate involved in the synthesis of Salomenella O-antigen. The universal occurrence of polyprenyl alcohols in microorganisms was already indicated by the earlier work. These

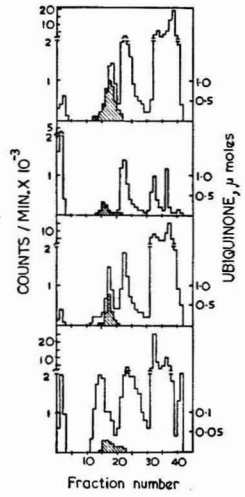


Fig. 9 — Radioactivity patterns of column eluents of the unsaponifiable lipids from moulds grown with mevalonate-2-$^{14}$C (10 μC./flask) as the tracer [In each case the lipids were fractionated on deactivated alumina column (20 g.); 10 ml. fractions were collected and radioactivity was measured. The eluents used in succession were petroleum ether (fractions 1-10), 5 per cent (vol./vol.) ethyl ether-petroleum ether (fractions 11-20), 10 per cent ether-petroleum ether (fractions 21-30), 20 per cent ether-petroleum ether (fractions 31-35), ethyl ether (fractions 36-40), ethanol (fraction 41) and ethanol-6 N HCl (4/1) (fraction 42). The shaded area corresponds to the content of ubiquinone. The radioactivity peak in fractions 22-30 corresponds to polyprenyl alcohols (data from ref. 66). The four figures from top to bottom correspond to *Aspergillus niger*, *Neurospora crassa*, *Penicillium chrysogenum* and *Gibberella fujikuroi*]

compounds are under study with respect to the chain length and their possible participation as lipid-intermediates.

*Ubiquinone in a bacterial reduced NAD oxidase system* — In this screening programme, we came across the interesting phenomenon of occurrence of the homologue, ubiquinone-10, in a bacterium *Agrobacterium tumefaciens*, the crown gall tumor-inducing plant pathogen, whereas lower homologues are normally found in the microbial world, Q-8 being the most common one in bacteria[72]. In bacteria, vitamin $K_2$ was considered to participate in the oxidation of reduced NAD, and ubiquinone in the oxidation of succinate[73]. *A. tumefaciens*, devoid of vitamin K, possessed an active reduced NAD oxidase system, sensitive to antimycin A, an unusual feature in bacterial systems. Antimycin A sensitivity is usually associated with ubiquinone-mediated systems — electron transport in mammalian mitochondria[74] and in photophosphorylation in chromatophores of *R. rubrum*[75]. Our investigations gave evidence for the participation of ubiquinone in reduced NAD oxidase system of this bacterium and the following sequence of electron transport had been suggested:

## Ubiquinone and Microsomal Oxidative Enzyme Systems

Accepting the constitutive nature of ubiquinone in the microsomes, what is its functional role in this cell component important in protein synthesis and some oxidations? In view of the oxidation-reduction of the quinone, we tested three oxidative enzyme systems in microsomes for possible participation of the ubiquinone present: reduced NAD-cytochrome *c* reductase[76], reactivation of reduced ribonuclease[77] and sulphite oxidase[78]. Although these probes turned out to be negative, valuable information was obtained on the properties of these systems.

*Reduced NAD-cytochrome c reductase system* — Microsomal ubiquinone can be partially reduced by reduced NAD and also by reduced NADP and succinate. Both ferricyanide and cytochrome *c* could oxidize the reduced form of ubiquinone in microsomes[79]. These results support a role for ubiquinone in reduced NAD-cytochrome *c* reductase, but direct proof could not be obtained, because acetone extraction to remove ubiquinone irreversibly inactivated the enzyme system. It is, however, possible that the quinone may be in a side pathway equilibrated with the main components. In contrast to mitochondria, however, the externally added ubiquinone was not reduced or oxidized by microsomes. Menadione was capable of interacting with the reduced NAD-cytochrome *c* reductase system both for reduction to quinol by reduced NAD and its reoxidation by cytochrome *c* and these reactions were completely insensitive to antimycin A.

*Formation of tertiary structure of proteins* — In the late stages of protein synthesis, the transformation of the random coil polypeptide to the highly ordered secondary, tertiary and quaternary structures which give the protein its three-dimensional conformation and its active sites, seems to take place at the microsomal level itself, since fully active enzymes were found on the ribosomes. The disulphide bridges which conform the tertiary structure and biological activity to some proteins, in addition to hydrogen bonding and ionic and hydrophobic interactions, have to be formed by oxidation of sulphydryl groups of cysteine residues, which must also be taking place on the microsomal membranes, and the electron transport activities and potential oxidants, therefore, assume importance.

The brilliant work by Anfinsen and coworkers[77] demonstrated that four disulphide bridges of bovine pancreatic ribonuclease could be reduced by β-mercaptoethanol in the presence of 8*M* urea with concomitant loss of enzyme activity. This preparation could be reactivated by dilution and aeration and the process was accelerated by the combined presence of washed microsomes and a supernatant factor derived from rat liver, the two factors being ineffective individually. We extended this work and found that depending on the homogenizing medium, the microsomal preparations alone could reactivate the enzyme[80]. Ferricyanide enhanced the microsomal reactivation, possibly by counteracting the inhibition of mercaptoethanol present in the reactivation system. Sulphite, sulphydryl and dithiol agents showed marked inhibition. The activity was sensitive to cadmium, indicating the involvement of a dithiol protein[81]; this, in fact, was found to be true after purification of the microsomal enzyme by Anfinsen and coworkers.

It was later shown that dehydroascorbate was probably the supernatant factor which oxidized the reduced ribonuclease to an inactive product[82]. The incorrect pairs of disulphide bridges were then reshuffled to the correct pairs by the above purified enzyme from microsomes. It was concluded that the 'disulphide-exhanging enzyme' did not catalyse oxidation of sulphydryl groups[83]. It is not yet certain whether in the process of protein synthesis, the oxidation of the sulphydryl groups takes place simultaneous with the growth of the polypeptide chain or will occur after the completion of the chain (with random disulphide bridges as implied above) (Fig. 10). In any case, oxidation must occur at some place while the polypeptide is still on the microsomes. This oxidation was, however, not due to ubiquinone, since acetone-extracted microsomes devoid of ubiquinone were active in the reactivating system.

*Haemoproteins as models for the catalysis of disulphide bridge formation* — The natural oxidant in the above system could be dehydroascorbate, as shown by Straub and Venetianer[82], which gives an indirect function to vitamin C at molecular level. Our work gave another interesting clue on this aspect. It was found that the oxidized form, not the reduced form, of cytochrome *c* and haemoglobin were capable of reactivating the reduced ribonuclease without addition of microsomes or other oxidants[81]. This is not merely an effect of a haemoprotein — catalase was ineffective — but one having bound form of iron capable of being oxidized and reduced. In haemoglobin it is possible that the redox state of iron may involve Fe-S covalent linkages. Obviously, disulphide exchange is not the mechanism here, since cytochrome *c* does not have disulphide bonds.

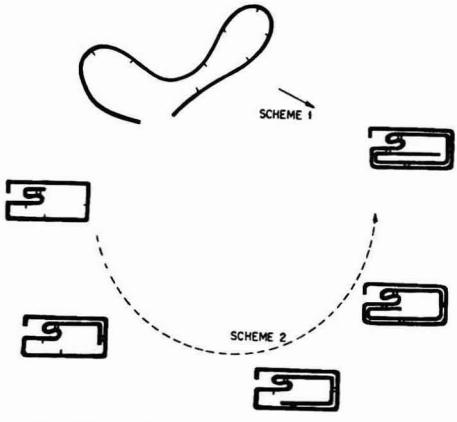


Fig. 10 — Scheme depicting two possible ways of forming the four disulphide bridges of ribonuclease [Scheme 1 shows the formation of active ribonuclease from the fully formed unfolded polypeptide by the 'reactivating system'. Scheme 2 shows the simultaneous formation of the disulphide bridges between cysteines 1-6, 2-7, 3-8 and 4-5 from N-terminal in the growing polypeptide]

We believe that this may serve as a model for the natural mechanism, which may involve the abundant non-heme iron in the microsomal membranes where proteins are being assembled.

*Sulphite oxidase* — A widely distributed microsomal enzyme catalysing specifically the oxidation of inorganic sulphite using cytochrome *c*, oxygen, ferricyanide or dichlorophenol indophenol as electron acceptor, was found in the rat[78]. Acetone extraction of microsomes removed all ubiquinone, but not sulphite oxidase activity and, therefore, ruled out the involvement of ubiquinone in the reaction. However, it was found that the acetone extracted residue had several-fold increased activity of the enzyme than the original microsomes. Such increased activity was also found in aged microsomes or on treatment with deoxycholate. Therefore, this enzyme is apparently present in a cryptic state when associated with the lipids of the microsomal membranes[79]. It appears some of the active sites of the existing enzyme protein may have been masked by the lipids which are released on reshuffling or removing the lipids. Another microsomal enzyme, glucose-6-phosphatase[84], is also known to be activated by deoxycholate and also by other treatments, e.g. triton X-100, not in common with the activation of sulphite oxidase. Sulphite oxidase assumes importance since an inborn error of absence of this enzyme in a human infant had been found recently[85].

The interesting features of the three systems are summarized in Table 3.

## Ubiquinone and Embryonic Development

*Ubiquinone in the rat embryo* — An important observation was made in the case of pregnant rats that ubiquinone-10-$^{14}$C was incorporated into foetuses both on oral and intracardial administration of the tracer. This is somewhat astonishing because of the lack of such absorption by tissues other than the liver and the spleen. It appears that ubiquinone in the blood can pass through the placental barrier and would be available for the foetus like other related lipid components, vitamins A, E and K and also cholesterol. Dietary ubiquinone is the only source of ubiquinone in the blood in view of the lack of its internal transport in the body; therefore, exogenous ubiquinone will have an important role as a source for the foetus. It is not clear whether the rat embryo at an early stage can synthesize its own ubiquinone or will depend on the blood supply and thereby indirectly on the diet. Ubiquinone-free diet, however, did not affect the development of the foetuses. It was difficult to decide whether small quantities of ubiquinone of intestinal or uterine origin were available in such animals.

*Ubiquinone and the developing chick embryo* — The chick embryo is an ideal system for such a study. It has an 'extra-uterine' existence during development. It is a 'closed system' free from any microbial contamination, with all the nutrients stored within the egg. Analysing a large number of eggs, it was found that sufficient ubiquinone-10 was stored in the yolk and none in the white portion[86]. These results were amply confirmed by others[87]. Increasing quantities of ubiquinone, along with sterols and tocopherol were laid into developing follicles in the hen's oviduct during their maturation to the yolk of the egg[88]. Studying the changes in the ubiquinone concentration in the egg yolk and the embryo during different stages of development, it was found that during the initial stages, there was a decrease of ubiquinone concentration in the yolk up to the fifth day, after which stage the embryonic ubiquinone increased several-fold (Fig. 11)[89]. The total ubiquinone content per egg also increased after the tenth day of development, indicating *de novo* synthesis.

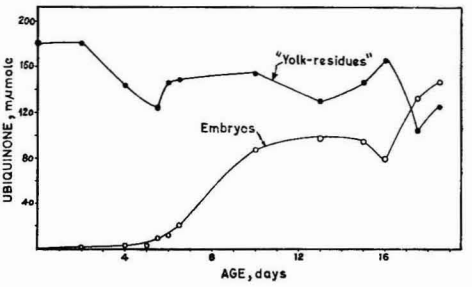


Fig. 11 — Changes in the content of ubiquinone of the developing chick embryo and the yolk residue [Each value is an average of at least three independent determinations. The experimental details were given in ref. 88 and 89]

TABLE 3 — FEATURES OF SOME MICROSOMAL OXIDATIVE ENZYME SYSTEMS IN THE RAT*

| Treatment or source of microsomes | Reduced NAD-cytochrome *c* reductase | Reduced ribonuclease reactivating system | Sulphite oxidase |
|---|---|---|---|
| Ageing of microsomes | No effect | No effect | Increased 6-fold; cryptic activity exposed |
| Acetone-extracted microsomes | Irreversibly inactivated | Decreased | Increased 10-fold; cryptic activity exposed |
| Extracted lipids added to above system | No effect | Activity restored | No effect |
| Deoxycholate treatment | Partial inhibition; requires high concentration for solubilization | Partial inhibition; easily solubilized | Increased 10-fold; cryptic activity exposed; easily solubilized |
| Feeding cholesterol or ubiquinone to rats | No effect | — | Native activity increased exposing part of the cryptic activity |
| Developing embryo | Present | Low | Low; cryptic activity absent |

*Information from ref. 79, 81 and unpublished work.

Exogenous ubiquinone-10-$^{14}$C, injected into the egg, was picked up by embryo at the early stages. Incorporation of mevalonate-2-$^{14}$C into ubiquinone could only be found at 10 days and not at earlier stages. These experiments indicate the following facts: (a) preformed ubiquinone can be utilized by the embryo, (b) ubiquinone synthesis is not elaborated in the early embryonic tissue and (c) yolk ubiquinone may serve as the important supply for the needs of the embryo in the early stages of development. So far no egg was found to be deficient of ubiquinone to test whether embryonic development will be impaired. The evidence, indirect as it may be, supports the view that ubiquinone may have a significant role in early embryonic development[90].

## Ubiquinone under Nutritional Conditions

A large number of experimental conditions, such as nutritional deficiencies, dietary supplements of hormones, toxic substances and inhibitors of cholesterol synthesis, and environmental stress conditions, have been tested in this laboratory for their effect on the concentration, synthesis and catabolism of ubiquinone in the liver of rats. These results are summarized in Table 4.

*Vitamin A deficiency* — Vitamin A deficiency and ubiquinone have an interesting historical association. The earliest observation on the compound with absorption peak at 275 mμ was made by Moore and Rajagopal[91] in lipids of livers of rats fed insufficient

TABLE 4 — CHANGES IN THE CONCENTRATION, SYNTHESIS AND CATABOLISM OF UBIQUINONE IN THE LIVER OF RATS UNDER DIFFERENT EXPERIMENTAL CONDITIONS

(The upward arrow represents increase and the downward arrow decrease, relative to the corresponding control animals)

| Experimental condition | Concentration | | Synthesis* | | Catabolism* |
|---|---|---|---|---|---|
| | mμmole/g. liver | % of normal (= 100) | Counts/min./ g. liver | % of normal (= 100) | |
| **NUTRITIONAL DEFICIENCIES** | | | | | |
| Vitamin A deficiency | | | | | |
| 10 days on deficient diet | 250 | 156 ↑ | 525 | 345 ↑ | Lowered |
| Plateau stage | 290 | 181 ↑ | 80 | 40 ↓ | do |
| Folate deficiency (2% sulphaguanidine, 33 days) | 170 | No change | 100 | No change | No change |
| Biotin deficiency (11% egg white, 60 days) | 123 | do | 100 | do | do |
| Protein deficiency (0% protein diet, 30 days) | 90 | 48 ↓ | 140 | 64 ↓ | Lowered |
| Starvation (no diet, 4 days) | 90 | 68 ↓ | 390 | 700 ↑ | Not known |
| **DIETARY SUPPLEMENTS** | | | | | |
| Thyrotoxicosis (0·3% iodinated casein, 34 days) | 490 | 290 ↑ | 186 | 240 ↑ | Lowered |
| Thyroxine supplement (30 mg./kg. diet) | | | | | |
| 10 days | 214 | 167 ↑ | 90 | 180 ↑ | do |
| 20 days | 250 | 172 ↑ | 20 | 50 ↓ | do |
| Thiouracil (0·3%, 20 days) | 110 | 76 ↓ | 30 | 83 ↓ | Not known |
| Cortisone (2 mg./day, 20 days) | 210 | 127 ↑ | 130 | 250 ↑ | Lowered |
| Adrenaline (50 μg./day, 20 days) | 220 | 133 ↑ | 60 | 176 ↑ | No change |
| Noradrenaline (100 μg./day, 15 days) | 140 | No change | 40 | No change | do |
| Hypervitaminosin A (5 mg. vitamin A/ day, 15 days) | 86 | 68 ↓ | 70 | 75 ↓ | Probably enhanced |
| Chlorophenoxy-isobutyrate (CPIB) (0·5%, 10 days) (inhibitor of cholesterol synthesis) | 196 | 245 ↑ | 250 | 285 ↑ | Lowered |
| SKF 525A (1%, 10 days) (inhibitor of cholesterol synthesis) | 68 | 61 ↓ | 20 | 7 ↓ | Not known |
| W398 (1%, 10 days) (inhibitor of cholesterol synthesis) | 65 | 58 ↓ | 30 | 10 ↓ | do |
| α-Phenyl butyrate (1%, 10 days) (inhibitor of cholesterol synthesis) | 78 | 70 ↓ | 100 | 35 ↓ | do |
| Dinitrophenol (0·3%, 7 days) | 160 | 150 ↑ | 70 | 107 ↑ | Lowered |
| Ethionine (0·5%, 15 days) | 68 | No change | 40 | No change | No change |
| Cholic acid (0·5%, 5 days) | 158 | 145 ↑ | 870 | 350 ↑ | Not known |
| Cholesterol (1%, 30 days) | 153 | 114 ↑ | 1270 | 690 ↑ | do |
| Ubiquinone (1·5 mg./day, 10 days) | 232 | 182 ↑ | 180 | 42 ↓ | do |
| **ENVIRONMENTAL CONDITIONS** | | | | | |
| Cold exposure (0-5°C.) | | | | | |
| 10 days | 330 | 210 ↑ | 150 | 430 ↑ | Lowered |
| 40 days (acclimatized) | 460 | 290 ↑ | 25 | 74 ↓ | do |
| Low atmospheric pressure (½ atm., 6 hr) | 160 | No change | 15 | 30 ↓ | Not known |

*Mevalonate-2-$^{14}$C (2 μC./rat) was orally dosed and the incorporation of radioactivity into ubiquinone in the liver, 2 hr after administration was taken to represent ' synthesis ' and retention of radioactivity relative to the control at 72 hr was taken to represent ' catabolism '.

vitamin A in the diet. The absorption peak became prominent because of its increased intensity and the lowered interference due to the absence of vitamin A[16]. Ubiquinone concentration increased progressively with deficiency in vitamin A[54]. It was found that this effect was characteristic of the liver and no other tissue in the rat[16], and was not found in chickens[92] and guinea-pigs[54] and, therefore, was not a necessary part of avitaminosis A. The increased liver ubiquinone could be prevented by supplying vitamin A alcohol and vitamin A acid[93] and by vitamin A analogues, retinene epoxide and retinene which support growth in the rat but not by furanoid-retinol (unpublished data from this laboratory). Partial lowering on administration of vitamin C was claimed by Malathi and Ganguly[94] but this could not be confirmed by Phillips[95].

The increased ubiquinone was considered to be due to increased synthesis since incorporation of mevalonate-2-$^{14}$C into liver ubiquinone increased[96] and this was explained to be due to the increased mevalonate pool available owing to the block at squalene in sterol synthesis[42]. In all these experiments, analysis was done at 24 hr, or longer, after the administration of the tracer, at which time interval the incorporation of the tracer would represent balance of synthesis and degradation. Incorporation of mevalonate-2-$^{14}$C at early time interval would represent 'synthesis' and retention of larger amounts of radioactivity at late time interval would represent lowered 'catabolism'[40]. We introduced the new concept that increased liver ubiquinone in vitamin A-deficient rats was due to impaired catabolism based on the following observations: firstly, the incorporation of mevalonate was lower in deficient animals at the early time interval; secondly, a large amount of incorporated radioactivity was retained at late time interval compared to normal animals (Fig. 12); and thirdly, absorbed exogenous ubiquinone was not degraded to the same extent in deficient animal[27]. We also confirmed the lack of increased synthesis in vitamin A-deficient livers in studies *in vitro* using tissue slices (Joshi, V. C., unpublished data). The effect on catabolic block could also be reversed by supplying vitamin A or its analogues which reduced the ubiquinone levels. Also, evidence obtained was in favour of the hypothesis that the accumulation of liver ubiquinone represents the impairment of the process of turnover of membrane structures with which ubiquinone is associated[27].

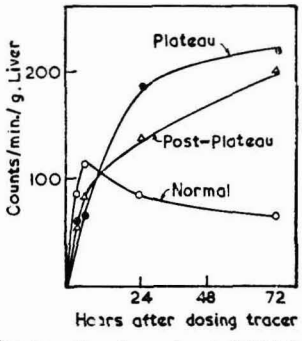


Fig. 12 — Incorporation of mevalonate-2-$^{14}$C into ubiquinone of livers of normal and vitamin A-deficient rats [Rats were orally dosed with the tracer (5 μC./rat) and killed after specified time intervals. The average values from two independent determinations are given. 'Plateau' and 'post-plateau' refer to the stages of deficiency of vitamin A in the rat (data from ref. 27)]

Recent experiments demonstrated that during the development of the deficiency, there was an intermediate stage where synthesis of ubiquinone increased, but it reverted to the initial low level when the animal was depleted of vitamin A (plateau stage). Also under toxic doses of vitamin A, both synthesis and liver concentration decreased. The above effects appear to be more due to the stress condition, similar to the responses obtained in other nutritional and environmental stress conditions, and not due to direct involvement of vitamin A or its absence (Joshi, V. C. & Laxman, M. R., unpublished data).

*Methyl groups of ubiquinone* — Other fat-soluble vitamins and most water-soluble vitamins were found to have no effect on ubiquinone. Biotin and folate were tested in this laboratory to complete the list, and found to be ineffective in influencing ubiquinone metabolism.

In the rat, it is not well established that all the three methyl groups (two O-methyl and one C-methyl) of ubiquinone were derived from methionine. It was shown that O-methyl groups were labelled by methionine-methyl-$^{14}$C[30]. The unusual C-methylation reaction was found to occur in animals as judged by the incorporation of formate-$^{14}$C[70], via probably folate coenzymes. The possibility of the intermediate participation of methionine was not directly verified. In view of the failure of folate deficiency to influence ubiquinone synthesis, it can be presumed that the abundant methionine present in the diets of these animals would have supplied the C-methyl group as well.

Ethionine is known to compete with methionine by transferring ethyl groups in the place of methyl groups. It was of interest, therefore, to see whether on feeding ethionine to rats the normal homologue of ubiquinone will be replaced by the ethyl homologue. This experiment will be of added significance since this will offer a natural method of obtaining mitochondria in which a homologue replaced the normal ubiquinone without resorting to the procedure of solvent-extraction followed by addition of the exogenous homologue. Previous studies using this technique by Folkers and associates[97] showed that the alkoxy group was essential for activity and could not be replaced by amino group or methyl group while 2-desmethyl ubiquinone was fully active. Our experiments showed that liver mitochondria of rats fed ethionine possessed full succinate oxidase and succinate-neotetrazolium reductase activities. Ubiquinone isolated from the liver and the kidney of such rats showed apparent discrepancies from the normal homologue in having a low melting point, lower extinction value at 275 mμ and streaking behaviour on reversed phase paper chromatography. But, it appears, these differences are due to some accompanying impurities that could not be removed from these samples in contrast to samples from

normal rats, since mass spectra (kindly carried out by Prof. T. W. Goodwin at the University of Liverpool) revealed only the normal methyl homologue of ubiquinone-9 (mol. wt 794) and no variant forms. Therefore, the methyl group transfer in ubiquinone biosynthesis is refractory to competition by ethionine.

*Effect of some hormones* — Under conditions of thyrotoxicosis induced by feeding 0·3 per cent iodinated casein to rats the liver ubiquinone concentration was known to increase[98]. We showed that the reason for this accumulation was increased synthesis and, in part, decreased catabolism[50]. Thyroxine supplement at sub-toxic doses also produced these effects. But the influence of thyroxine on ubiquinone metabolism appears to be indirect, since added thyroxine to a tissue-slice system synthesizing ubiquinone did not elicit any response. Also, in the normal animals, thyroxine apparently has no control on ubiquinone, since the lack of it in hypothyroid conditions produced no significant change in ubiquinone metabolism. Administration of exogenous cortisone and adrenaline, but not noradrenaline, increased the synthesis and concentration of ubiquinone in the liver. It appears that adrenaline may be the primary agent responsible for the increased synthesis of ubiquinone observed under a variety of stress conditions (Inamdar, A. R., unpublished data).

*Protein status and ubiquinone metabolism* — Since phenylalanine is needed for the formation of the quinone ring of ubiquinone, it is logical to expect that changes in concentration of dietary protein will affect the ubiquinone in the animal. As expected, protein deprivation in the diet of adult or weanling rats resulted in marked lowering of ubiquinone of liver and heart[26] and its synthesis[40]. This is the only well-accepted condition which decreased ubiquinone to very low levels. Deficiency of ubiquinone was not produced even when the animals were kept on protein-deficient diet for 30 days (Fig. 13). This work had been fully confirmed by Williams[99]. Being an essential component, ubiquinone appears to be maintained at a minimum concentration along with other vital oxidation reactions, probably by the breakdown of the tissue protein and using phenylalanine therefrom. Conservation of the important energy-yielding reactions seems to be part of the survival mechanism in such animals.

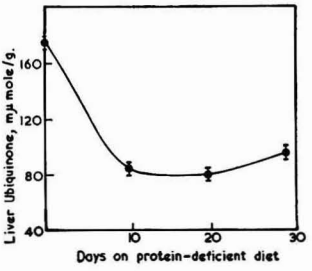


Fig. 13 — Changes in ubiquinone concentration in livers of rats maintained on protein-deficient diet [The values are mean and range of independent determinations from six rats (data from ref. 26)]

*Starvation and ubiquinone concentration* — Starving the rats for a period of 2-7 days by complete deprivation of the diet also depressed ubiquinone contents of the tissues. This is in disagreement with the reports of earlier workers[100,101]. In fact, decrease in synthesis and content of ubiquinone as observed by us should be expected in view of the reported lowering of the important enzyme in isoprene synthesis, HMG-CoA reductase under conditions of starvation[102].

*Effect of rancid fat on tissue vitamin E and ubiquinone contents* — A different type of experiment on nutritional alteration was carried out by Acharya and Jayaraman[103] in this laboratory. Rancid fat destroyed ubiquinone and vitamin E on incubation. Feeding this to normal or pregnant rats did not decrease the concentrations of ubiquinone or vitamin E in the tissues, including uterus and contents. But the foetuses were resorbed at about the fourteenth day, giving an example of resorption in spite of the presence of vitamin E and thereby suggesting that the action of vitamin E in fertility is indirect.

## Environmental Stress Conditions

Our interest in the effects of low invironmental temperature and low atmospheric pressure on the ubiquinone and oxidative metabolism had originated out of the topical importance of the understanding of the biochemical process attendant on exposure and acclimatization to high altitude conditions.

*Effect of exposure to low environmental temperature* — Several theories have been proposed to explain the increased heat production necessary to maintain the body temperature of the cold acclimatized rats. The basic mechanisms by which the energy is liberated in the form of heat still remain to be established. Rapid transfer of electrons by way of calorigenic shunt pathways in mitochondria as well as microsomes appears to be the most likely means of achieving the production of the excess heat[104]. To this end, components of such pathways have to be activated and increased in concentration. The possibility of increased ubiquinone obtained in cold exposed animals playing such a role is worthy of consideration[105]. Several dehydrogenases and reductases are now found to be activated and an integration of such systems may account for the excess heat produced. Ubiquinone appears to be an ideal compound to participate in such shunt pathways.

There were three stages in the process of accumulation of ubiquinone in the livers of cold exposed rats: firstly, at 5 days of exposure, there was lowered catabolism which continued throughout the cold exposure conserving thereby the normally synthesized ubiquinone; secondly, at 10-20 days, there appeared to be increased demand for ubiquinone, which was provided by increased *de novo* synthesis; and thirdly, at 40 days when the ubiquinone concentration was high, the rate of synthesis returned to the normal level. Feeding ubiquinone to these rats during cold exposure eliminated the increased synthesis at the intermediate stage (Fig. 14)[28]. Progressive with increase in ubiquinone in such animals, ubiquinone-dependent mitochondrial succinate-neo-

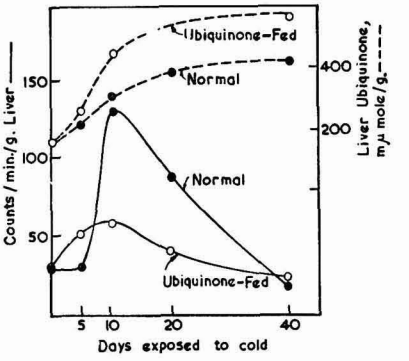


Fig. 14 — Changes in concentration and synthesis of ubiquinone in livers of rats exposed to low environmental temperature (0-5°C.) [Synthesis was measured by incorporation of radioactivity into ubiquinone 2 hr after orally dosing mevalonate-2-$^{14}$C (2 μC./rat). Ubiquinone was fed at a level of 1·5 mg./day/rat (data from ref. 28)]

tetrazolium reductase[106] also increased. Increased activity of this enzyme system could also be obtained in animals at room temperature on increasing the liver ubiquinone by absorption from dietary supply (Aithal, H. N., unpublished data). These findings, it is hoped, may turn out to be of practical importance.

*Effect of exposure to low atmospheric pressure* — Of the metabolic adjustments in the animals exposed to low atmospheric pressure and consequent hypoxia, those of oxidative metabolism will be of prime interest. Synthesis of ubiquinone requiring molecular oxygen and ATP was indeed lowered, since oxygen was limiting when rats were exposed for brief periods to half atmospheric pressure in a decompression chamber.

Some interesting observations were made on the changes in enzymes in such animals. Succinate oxidase activity in the liver increased and this was found to be due to the specific increase of succinate dehydrogenase — the rate-limiting enzyme in the multienzyme system. Cytochrome oxidase and succinate-neotetrazolium reductase activities were unaffected. The differential response of succinate-neotetrazolium reductase points to the fact that this system does not share the flavoprotein of succinate dehydrogenase of the oxidase system or has an alternate active site of the same protein[107].

Simultaneous exposure to low temperature and low pressure as obtained under conditions of high altitude would set in serious complications. The excess oxygen needed for the increased heat production is unavailable and, therefore, the process of acclimatization to the cold will be affected. Similarly, increased synthesis of ubiquinone necessary in cold exposure will not be possible in hypoxic conditions. The hyperthyroid condition obtained in cold exposure is detrimental to the process of adjustment to low pressure. Thus, the processes of adaptation to the individual conditions are opposing each other. Consequently, a balance of the two processes will have to be attained in acclimatization to high altitudes. Study of these aspects at the metabolic level is of fundamental importance.

## Regulation of Steroidogenesis by Ubiquinone

The pathways of synthesis of cholesterol and ubiquinone (side chain) share a common sequence of reactions between acetate and mevalonate and at least up to isopentenyl pyrophosphate. These two compounds can be considered as the end products of isoprene synthesis found in the liver. It is known that the biosynthesis of cholesterol in the rat was suppressed under conditions such as feeding cholesterol[108-110], and starvation[110,111] and was enhanced when the animals were treated with triton or X-irradiation. The reason for these changes was shown to be the alteration of the rate-limiting enzyme, β-hydroxy-β-methylglutaryl CoA (HMG-CoA) reductase[110,112]. As this enzyme appears to be shared by ubiquinone, it is important to know whether undesirable side effects occur in the production of this compound. Conversely, can ubiquinone exert a similar regulation and inhibit isoprene synthesis? Our experiments carried out during the last three years have revealed exciting new information of immense potential value on the regulation of steroidogenesis.

The incorporation of acetate-1-$^{14}$C and mevalonate-2-$^{14}$C in digitonin-precipitable sterols (mostly cholesterol), hydrocarbons (mostly squalene) and ubiquinone in the livers of rats fed either cholesterol (1 per cent in the diet) or ubiquinone-9 (1·5 mg./day/rat) was measured. Incorporation of the tracers was established to represent the true synthesis of these compounds and decrease thereof as due to inhibition of their synthesis.

*Effect of feeding cholesterol on isoprene synthesis* — As previously reported by other workers, cholesterol feeding inhibited endogenous cholesterol synthesis as expected of the established inhibitory effect on the microsomal HMG-CoA reductase. Also a secondary site of inhibition appears to be present after squalene, possibly the microsomal squalene hydroxylase. The existence of the secondary control point in the branched pathway would be necessary to divert the available small mevalonate pool, already lowered on account of the primary inhibition, towards the synthesis of ubiquinone, which explains the preservation of the small quantities of ubiquinone essential for vital oxidations[50].

*Effect of dietary ubiquinone on steroidogenesis* — The most interesting observation was the inhibition of sterol synthesis from acetate in rats fed small quantities of ubiquinone[50]. The site of this inhibition appeared to be before mevalonate and after acetyl CoA, since the incorporation of neither mevalonate into sterols nor acetate into fatty acids was affected. In several experiments the inhibition of sterol synthesis was in the range 30-50 per cent, despite long periods of feeding ubiquinone or increasing the dose. Nevertheless, liver cholesterol levels remained fairly constant[113]. These effects happened to be identical to those obtained on administering the plasma cholesterol depressing drug, *p*-chlorophenoxyisobutyrate (CPIB), also called by the trade name Atromid-S[114]. Liver being the source of cholesterol

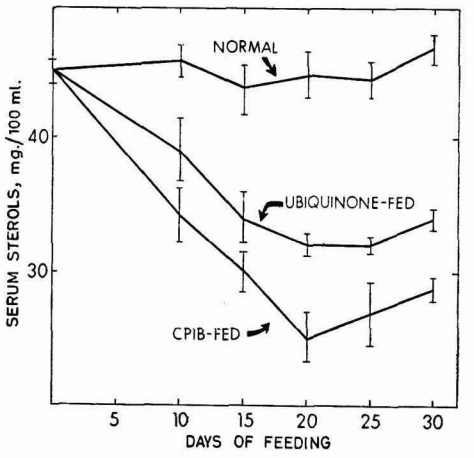


Fig. 15 — Concentration of serum sterols in normal rats fed ubiquinone (1·5 mg./rat/day) or CPIB (0·5 per cent in the diet) [Each value represents mean and range of independent determinations from six rats (data from ref. 115)]

in the serum, inhibition of its synthesis in the liver should reflect in lowering of serum sterol concentration. To our great satisfaction, this indeed turned out to be true. Feeding ubiquinone reduced serum sterols in a parallel manner as CPIB did (Fig. 15)[115].

It may be recalled that ubiquinone feeding increased liver ubiquinone concentration exclusively. The action of CPIB was considered indirect and by alteration of some metabolic reactions[116], and it was found that the drug increased the synthesis and the concentration of ubiquinone in the liver to the same extent as obtained by dietary ubiquinone[115]. Therefore, we believe that increased liver ubiquinone concentration, obtained either way, was responsible for the observed inhibition of sterol synthesis in the liver.

*Secondary control point in ubiquinone synthesis* — Exogenous ubiquinone also inhibited endogenous synthesis of ubiquinone after the mevalonate stage, without affecting the other branched pathway. A secondary site of inhibition appears to exist at the stage of synthesis of polyprenyl side chain, presumably after isopentenyl pyrophosphate[113]. Its quantitative significance is yet to be understood, but it appears to be present wherever ubiquinone concentration in the liver increased, such as cold exposure, vitamin A deficiency and hyperthyroid condition. The observed effects are represented in Fig. 16 and the characteristics are summarized in Table 5.

*Rate-limiting step in isoprene synthesis in the life cycle of the rat* — It is known that the rate-limiting step of isoprene synthesis is between acetyl

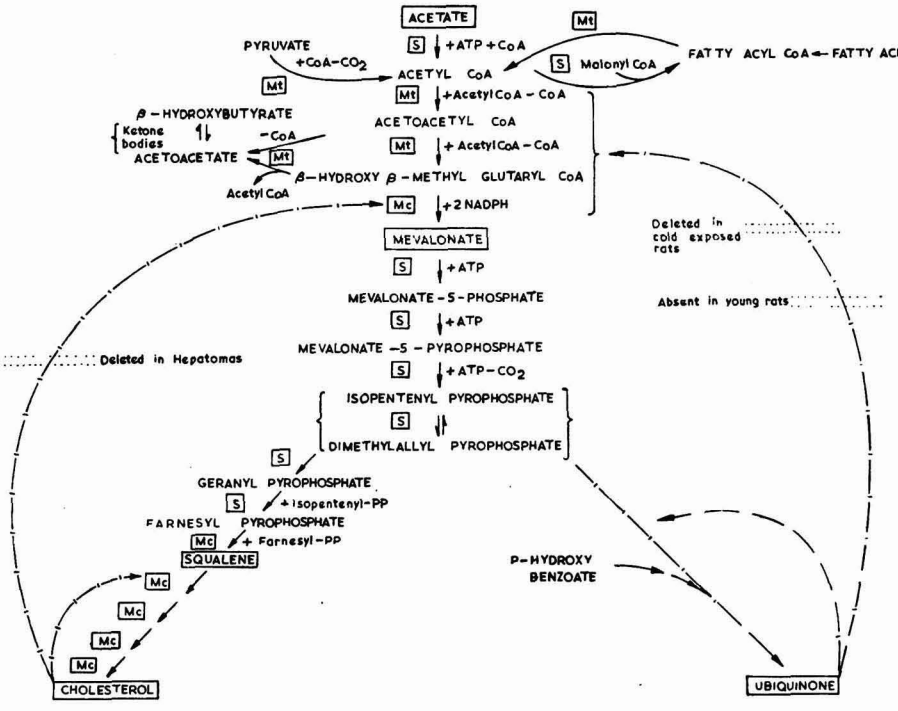


Fig. 16 — Schematic representation of isoprene pathway for the synthesis of cholesterol and ubiquinone and the sites of end product inhibition (shown by broken arrows) [S, Mt and Mc enclosed in blocks represent the sites of the enzymes, soluble supernatant, mitochondria and microsomes respectively]

TABLE 5 — CHARACTERISTICS OF THE END PRODUCT INHIBITION OF STEROL SYNTHESIS BY DIETARY UBIQUINONE AND CHOLESTEROL IN THE RAT

| | Ubiquinone-fed | Cholesterol-fed |
|---|---|---|
| Inhibition of synthesis in liver (acetates terols) | 50% or under | 95% or more |
| Serum sterol concentration | Decreased | Increased |
| Specificity of homologues | Only the natural, major homologue, Q-9 but not Q-10, Q-7 or Q-6 | Specific; derived bile acids also show similar effect (ref. 117) |
| Site of inhibition | | |
| Primary | Acetyl CoA → → → mevalonate | HMG CoA → mevalonate |
| Secondary | Isopentenyl-PP → → ubiquinone | Squalene → lanosterol |
| Feedback or repression | Not decided; ubiquinone concentration increased in nucleus, mitochondria and microsomes whenever such inhibition was found; exception: CPIB feeding in which microsomal Q did not increase; could be either feedback or repression of mitochondrial enzymes forming HMG CoA or repression of microsomal HMG CoA reductase, but not similar to the cholesterol feedback mechanism | Feedback inhibition of the microsomal enzyme, HMG CoA reductase (ref. 112) |
| Tissue specificity | Liver, but not kidney or intestines; other tissues not tested | Liver but no other tissue |
| Tumour cells | Not known | Deleted in hepatomas (ref. 118) |
| Young animals (livers) | Absent till weaning [CPIB effect also absent in young suckling rats (ref. 119)] | Present |
| Cold exposure (0-5°) (livers) | Absent in cold acclimatized animals | No effect |

CoA and mevalonate and this is identified as HMG-CoA reductase. Recent experiments demonstrated that while incorporation of radioactivity into sterols on the basis of unit liver weight from mevalonate-2-$^{14}$C was unaltered, that from acetate-1-$^{14}$C was high in young animals and decreased in the adult animals, showing that a rate-limiting step had developed after the initial growth phase (Krishnaiah, K. V., unpublished data).

*Significance of the control mechanisms* — Inhibition of endogenous synthesis of cholesterol by dietary cholesterol does not achieve any reduction of total body sterols; instead it is usually much higher, because the exogenous cholesterol is absorbed to a large extent and replaces the endogenous form. With respect to serum sterol concentration, this mechanism offers no possibility of reduction. Also, the existence of feedback inhibition by dietary cholesterol in the man has been in doubt[120]. But ubiquinone feeding does reduce the total body sterol content and the serum levels in the rat. Ubiquinone is a natural constituent of the animal tissues and is being ingested in the diets regularly. In the rat, only a small fraction of the dietary supply was found in the liver with more than 50 per cent being rejected in the faeces. Work in this laboratory also demonstrated the presence of ubiquinone-10 in a human faeces sample and in domestic sewage[121]. At the concentrations fed (about 10 mg./day/kg. body weight), there were no deleterious effects on the rats when ubiquinone supplements continued for extended periods. By natural metabolic alterations such high concentrations as necessary for the inhibitory effect could be produced within the body. Therefore, we consider the inhibitory effect by excess ubiquinone represents the natural regulatory mechanism for the maintenance of sterol synthesis in the liver. Liver supplies cholesterol through serum to other tissues, notwithstanding their endogenous synthesis. It will be necessary to know how much the tissues like adrenal and uterus that produce ' active steroid hormones ' depend on the liver supply for steroid nucleus before an assessment can be made on the complete physiological responses of the inhibitory effect of ubiquinone on steroidogenesis. In this connection it is noteworthy that ubiquinone, when intravenously injected in dogs, was found to inhibit steroidogenesis and to lower the secretion of aldosterone by the adrenals[122]. It seems likely that this effect might have been obtained by the above mechanism.

Atherosclerosis is one of the largest ' killers ' in the affluent societies. This involves hardening of the arteries by way of fat deposits, of which cholesterol forms the bulk. While the consequent failure of heart and excess cholesterol are not yet decidedly connected, reduction of the high serum sterol concentration is considered by the clinicians to lessen the danger. Encouraged by our experiments with rats, we tested the effect of ubiquinone on the serum sterol concentration in a clinical case of hypercholesterolemia. We could carry out this trial with the excellent cooperation of the patient, an employee of this Institute, and under the supervision of the Resident Medical Officer, Dr T. B. Subbarao. Ubiquinone-10, the natural homologue in the man, was taken daily in the morning mixed with coffee (30 mg. in a suitable placebo; formulation supplied to us by Hoffmann-La Roche). We were delighted to find the anticipated response, with absolutely no side effects. The serum cholesterol was brought down to 200 mg./100 ml. from the initial 260 mg./100 ml., when ubiquinone was consumed and returned to the high level when it was withdrawn (Fig. 17). Trials with more cases are under way yielding hopeful results.

## Retrospect and Prospect

The molecule of ubiquinone has fascinating characteristics and properties. While intensive work is going on in several laboratories it has still remained elusive in two aspects: its function and its bio-

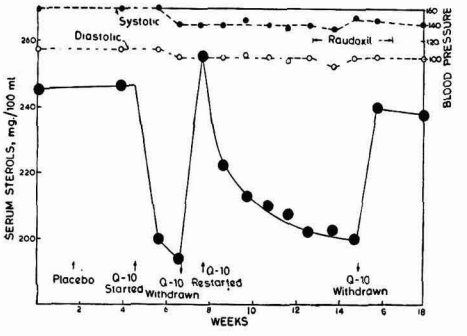


Fig. 17 — Response of serum sterol concentration to ubiquinone feeding in a hypercholesterolemic man [Ubiquinone mixed in a placebo, ¼ tablespoonful containing about 30 mg. of Q-10, was taken daily mixed in a cup of coffee in the morning. It was discontinued for a week in the middle during which period the patient went out of station, attended a marriage and had rich, fatty food. The serum sterol levels were depressed when Q-10 was consumed and they returned to higher initial levels on withdrawing it. The blood pressure values are also shown; note the trend of decrease while on ubiquinone treatment]

synthesis. Nevertheless, the progress on this group of compounds had been enormous much to the credit of workers in England, America and Switzerland and our own contributions form a minor part. The scope of this article had been intentionally limited to the areas of interest of this laboratory and, therefore, several important discoveries and references have been regretfully omitted. The knowledge gained on the lipid-quinones has been extensively covered in two recent reviews[7,9].

Ubiquinone, the major interest of our laboratory, had kept us busy and yielded good dividends. It had afforded us exercises into various aspects of biochemistry and allied disciplines. The underlying theme of a large volume of our work revolved around exogenous ubiquinone: absorption and distribution in tissues and cell components, interaction and equilibration with endogenous form, participation in and activation of electron transport systems and regulation of endogenous synthesis of isoprene compounds. One interesting sideline had developed out of the main theme. Ubiquinone and cytochrome *c*, the two 'mobile' electron transport components, were found to be capable of functions outside the scope of electron transport, viz. cytochrome *c* in the formation of tertiary structure of a protein and ubiquinone in the inhibition of steroidogenesis.

In the not too distant future, biosynthesis of ubiquinone will be completely understood and the possibility for the aerobic and anaerobic pathways being different seems well set. Being a constituent of membrane structure, the role of ubiquinone in membrane function will be on the focus. The very fact that ubiquinone is a widely occurring cellular constituent qualifies it for a role in cellular function according to Folkers[123], who had been advocating the role of ubiquinone homologues in muscular dystrophy and reticulocytosis of a type of haemolytic anaemia. He had also voiced optimism on the prospective role of ubiquinone in 'biology and medicine', and referred to the Japanese attempts on the extensive clinical trials. Our discovery on the regulation of steroidogenesis by ubiquinone will soon hopefully be exploited for its therapeutic use.

## Summary

The work carried out at the Department of Biochemistry, Indian Institute of Science, Bangalore, on ubiquinone has been summarized and the literature in these areas of interest has been reviewed, identifying the achievements and the unsolved problems. It has been established that ubiquinone is distributed in all the cell components of the liver. Exogenous ubiquinone is absorbed exclusively into the liver and is not mobilized by other tissues. All the tissues have independent capacity for the biosynthesis of ubiquinone except developing embryo at the early stages, which seems to require exogenous ubiquinone. Ubichromenol, the structural isomer of ubiquinone, has been shown to be synthesized independently, thereby supporting its natural occurrence. The mevalonate pathway for the synthesis of isoprene compounds is operative in animals, plants and moulds, but apparently not for the synthesis of ubiquinone in some bacteria, and vitamin K in mycobacteria. A general concept has emerged that under some stress conditions, such as vitamin A deficiency, cold exposure or thyrotoxicosis, liver ubiquinone accumulates owing to lowered catabolism and partly due to activation of synthesis. Excess ubiquinone in the liver inhibits the synthesis of cholesterol and consequently reduces serum sterol concentration. This discovery assigns a new function to ubiquinone as a regulatory molecule in steroidogenesis.

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# REVIEWS

PHYSICS OF THE MOON, edited by S. Fred Singer (American Astronautical Society, Washington DC), 1967. Pp. xi+248

This book is the thirteenth volume of the AAS Science and Technology Series and contains the proceedings of a symposium held by the American Astronautical Society at Washington DC during 26-31 December 1966.

The moon has captivated human imagination since the earliest times. Sanskrit poets were very fond of comparing the faces of damsels with the surface of a full moon. A Sanskrit scholar was shocked when he saw the lunar surface through a simple telescope and he would be further shocked after seeing the excellent photographs of the lunar surface provided by the Rangers, Surveyors, Orbiters, Luniks, Lunas and the Zonds, which provide the material for some of the papers presented at the symposium.

The available evidence indicates that many of the lunar craters are of impact origin, while others are the result of some form of volcanism. The large 'marias' on the lunar surface facing the earth are apparently the result of a flow of liquid material. Infrared measurements indicate that about a tenth of the lunar surface are covered with rocks, which reveal themselves as 'hot spots' during lunar nights and lunar eclipses. The remainder of the lunar surface appears to be covered with dust of particle size considerably less than a millimetre. This 'dust' is not soft.

The observational data also provide indirect information about the lunar interior and the origin of the moon. The paper by Singer on the 'Origin and dynamical evolution of the moon' is very thought-provoking. He concludes that the moon was initially a co-planet and that it was captured by the earth as the two approached within a couple of earth-radii, about four billion years ago. The capture was catastrophic in the sense that a huge amount of rotational energy was converted into heat within the body of the earth and the moon, sufficient to melt the moon as well as to defluid the crustal rocks of the earth. The latter event led to the early formation of oceans and the atmosphere. The discussions also speculate how a study of moonquakes may lead to an increase in our knowledge of earthquakes.

The book contains eleven papers and two panel discussions. The papers are on: Orbiter observations of the lunar surface; Crater statistics and erosion; Review of lunar infrared observations; Optical properties and infrared emissions of the moon; Radar properties of the moon; Remarks on lunar photography; Present knowledge about the thermal history of the moon; The geometric and dynamical figures of the moon; Lunar seismic exploration; Lunar gravity investigation; and The dynamical evolution of the moon. The first panel discussion is on the lunar surface while the second is on the lunar interior.

The book is well produced and is a good addition to public libraries, although a luxury for individuals.

P. R. PISHAROTY

INFRARED SPECTROMETRY OF INDUSTRIAL POLYMERS by C. J. Henniker (Academic Press Inc., New York), 1967. Pp. ix+229. Price $ 10.00

The book is divided into eight chapters of which the first five chapters discuss the instrumentation and the techniques of infrared spectroscopy in general. The adaptation of these techniques to the study of polymers has been lucidly discussed. By far the most outstanding feature of the book under review is the discussion on the techniques of sample preparation and special spectrometric techniques for polymer work. This book would give very valuable guidance for practical difficulties in these fields.

The basic theory of the infrared spectra is too inadequate and the author assumes the basic knowledge of spectroscopy and polymers. There is still a gap left because the application of the basic theory to crystalline and amorphous solids is quite involved and spectroscopists working on polymers have necessarily to be acquainted with these. Since this aspect is not discussed in the present book, a detailed thorough analysis of the polymer spectra has not been given. The last three chapters dealing with qualitative identification, quantitative analysis and special applications to polymers are rather short account of important contributions by various workers including those from the authors' laboratory. The bibliography in this part is undoubtedly valuable.

The expression in the first three chapters is rather unusual and the terminology uncommon in some places. This part of the book does not make an easy reading. It seems more like a translation of the original text. The expression, however, is better after the first two or three chapters. The book is well produced and practically free from printing mistakes.

The book on the whole is a good addition from the point of view of an experimentalist working on polymer spectra.

M. R. PADHYE

METALS HANDBOOK: Vol. 3 — MACHINING, edited by Taylor Lyman (American Society for Metals, Ohio), 1967. Pp. xvi+552. Price $ 35.00

It is one of the most comprehensive and extensive treatises on machining, covering the entire range of machining processes from turning and shaping through milling, grinding, etc., to the newest and exotic processes like electrochemical grinding, ultrasonic machining, plasma machining and laser machining. Extended sections are devoted to cutting tools and the influence of their geometry, surface finish and final treatments on their performance as regards metal removal rates, tool life,

surface finishes, etc. These cover today's tool materials in all their diversity — high speed steels, carbides, cermets and ceramics. The machining of all the engineering materials is dealt with separately in another section of the treatise. Material coverage starts from common place cast iron and extends down to the space age materials like titanium and beryllium. Here again it is the difficulties encountered in the machining of these materials which are given greater prominence and which are of greater interest to an engineer.

The most important aspect of the coverage is the limiting possibilities of the different processes when working with different materials under different heat treat conditions and the metal removal rate, tool life, tolerance and surface finish obtainable under those limiting conditions, data which are so very necessary at this time of fast growing technological requirements.

The entire coverage is supplemented by a large number of actual field examples on operation sequences, machining set-ups, production timings and influence of the working parameters of the operation, tool geometery, tool finish, etc.

The extensive coverage by some of the most prominent men in the field and based on the latest research findings make this volume an invaluable guide to any manufacturing facility and professional engineer.

CHANDRA MOHAN

HETEROATOM RING SYSTEMS AND POLYMERS by H. R. Allcock (Academic Press Inc., New York), 1967. Pp. xi+401. Price $ 16.50 or 132*s*.

There has been a marked activity in the field of heteroatom ring systems and polymers, particularly the skeletal systems containing boron, silicon, phosphorus, aluminium or carbon bonded to oxygen, sulphur or nitrogen. These new derivatives cover the spectrum from the classically 'organic-type' compounds to those which are essentially 'inorganic' in their characteristics. Although a number of recent reviews are available dealing with individual series of compounds such as siloxanes, phosphazenes and polyphosphates, this bock appears to be the first attempt to develop the whole domain of this highly diverse subject in a systematic manner, bringing out their similarities and differences and offering plausible explanations thereof. The growing information about a variety of compounds has been thus systematized under the headings such as nomenclature, bonding theory, pseudoaromaticity, ring-polymer equilibration, synthesis, polymerization reactions and polymer chemistry. The result is a logical and highly refreshing treatment that adds much to both our knowledge of the above nonmetals and of the characteristics which distinguish the various heteroatom frameworks.

The textual material is written in a clear and lucid style. Plausible reaction pathways have been generally illustrated by the use of graphic formulae. In addition, a considerable amount of information has been collected either in the form of numerous tables or graphs which summarize as well as illustrate the trends in properties in a very clear and concise fashion. The book is extensively and selectively referenced and the literature up to the middle of 1966 appears to have been covered well. The bond angles and interatomic distances for various heteroatomic systems have been compiled together at the end of the volume in the form of an appendix. This has added much to the value of the publication as a source of reference material. The whole book has a very clean and attractive appearance and the reviewer could come across only a minor printing error ($NaPO_4$-II) on the latter half of page 299.

The book would be very useful to general students for understanding the broad chemistry of heteroatom ring systems and polymers and is full of information even for those research chemists who are specially interested in any one of the specific areas included in the present volume. The author is to be congratulated on this excellent effort in this fastly growing area on the border-land of inorganic and organic chemistry.

R. C. MEHROTRA

ELEVENTH SYMPOSIUM (INTERNATIONAL) ON COMBUSTION (The Combustion Institute, Pittsburg, USA), 1967. Pp. xxi+1200. Price $ 42.00

The proceedings of the Eleventh Symposium on Combustion is a rich collection of 117 scientific papers on various aspects of combustion science. The volume has covered a wide range of specialities in the field in a pleasant manner. It begins with a plenary lecture by Prof. A. G. Gaydon on 'The use of shock tubes for studying fundamental combustion processes', a useful paper on a recent technique on the investigation of combustion phenomena. There are 14 papers on chemical kinetics and energy transfer; 10 on heterogeneous combustion; 6 on charged species in combustion processes; 6 on carbon in flames; 5 on combustion of ammonium perchlorate; 10 on fire research; 5 on radiation from rocket exhaust plumes; 10 on detonation; 11 on combustion and flow; 10 on flame mechanisms and structures; 8 on flame structure and properties; 6 on engine and engine-related combustion; 10 on explosion and ignition kinetics; and 5 on flame spectroscopy.

The chapter on chemical kinetics and energy transfer is interesting as it contains considerable amount of new and valuable material on a variety of chemical kinetics and vibrational relaxation topics. The chapter on heterogeneous combustion contains seven review articles and does not contain much new material. The section on combustion of ammonium perchlorate and charged species in combustion processes comprises some lively contributed papers. The papers grouped under combustion and flow are extremely interesting, specially a contribution on supersonic combustion studies and three papers on turbulent flames. Flame mechanisms and structure section contains an interesting paper on shock-tube study of the ammonia-oxygen reaction and cool flames in addition to eight other papers on the mechanism of flames using different fuels. The papers on ignition and flame propagation related to engines seem to be extremely useful.

Concluding, it should be mentioned that the volume, compiling such a large and complex work,

has been presented in a pleasing style and is praiseworthy. The Combustion Institute deserves congratulations from the combustion scientists and engineers for producing this excellent work.

D. BHADURI

SHELL ANALYSIS by A. Paduart, translated from the French by F. H. Turner (Oxford & IBH Publishing Co., Calcutta), 1967. Pp. 97. Price Rs 26.00

Here is an excellent introduction to shell roof analysis written primarily for the beginner. Though the volume is slender, its coverage is comprehensive. The author has achieved this result by omitting the rather lengthy derivations associated with shell theory and presenting only end results. The coverage includes folded plates, cylindrical shells and shells of double curvature. Important aspects of shell design such as elastic stability and the influence of prestressing are also discussed. Besides being a Professor, Dr Paduart is also an eminent consultant. He has, therefore, attempted a balanced presentation by paying adequate attention to such practical details as the choice of shell dimensions, proportioning and placing of reinforcement.

The author's treatment of folded plates is rather brief. In the chapter on cylindrical shells, he has done well to refer briefly to all the well-known theories, viz. Finsterwalder, D-K-J, Aas Jacobsen, Flügge, Dischinger, Lundgren, Vlasov van der Eb, ASCE manual, etc. The shortcomings of the membrane theory have received adequate treatment. The beam method, its validity and usefulness are also discussed.

In the chapter on doubly curved shells, the author has developed the membrane theory and its applications to shells of revolution, the hyperbolic paraboloid, the conoid, calottes and surfaces of translation. The reference to the bending theory is extremely brief. The author might perhaps have made the treatment more complete by including a few critical observations about the extent to which bending stresses penetrate in different types of doubly curved shells.

The principles of hanging roofs are briefly developed in a chapter devoted to this subject. The chapter on instability is extremely well written. The concluding chapter is devoted to the application of prestressing. Prestressing of both edge members as well as the shell edge are discussed. The treatment relies on physical reasoning rather than mathematical analysis.

Selected photographs of outstanding shell structures built in different parts of the world are included in the book. The selected bibliography appended to the book will help the reader to make a more penetrating study of this specialized subject.

The author deserves all praise for his simple and lucid presentation of the rudiments of shell analysis and design.

G. S. RAMASWAMY

ADVANCES IN ENZYME REGULALION: Vol. IV, edited by George Weber (Pergamon Press Ltd, Oxford), 1966. Pp. xiv+387. Price $ 15.00 or 105*s*.

The volume is a compilation of original papers presented at the Fourth Symposium on Regulation of Enzyme Activity and Synthesis in Normal and Neoplastic Tissues held at Indiana University School of Medicine on 4 and 5 October 1965. The editor must be congratulated for bringing together specialists in various fields and for choosing excellent areas on regulation.

In the session on 'Regulation of lipid metabolism', E. G. Ball showed the interrelationships among lipogenesis, Krebs cycle and HMP in adipose tissues. Nearly 45 per cent of $NADPH_2$ required for fatty acid synthesis is supplied by the citrate-malate cycle and the remainder by the HMP. Gellhorn and Benjamin reported decreased fatty acid oxidation and free fatty acid release under stress in old rats. This may be a cause of obesity in old age.

Rivlin and Langdon, in the session on 'Action of hormones at the enzyme biosynthetic level', showed that in hypothyroidism, FAD levels of rat liver decreased due to a lower level of flavokinase, suggesting regulation of coenzyme synthesis by thyroid hormone. Weber *et al.* reported the stimulation of biosynthesis of three glycolytic enzymes, glucokinase, phosphofructokinase and pyruvate kinase, by insulin, while four gluconeogenic enzymes, F-6-Pase, FDPase, PEP carboxykinase and pyruvate carboxylase, were suppressed at the same time. Glucocorticoid induced the four gluconeogenic enzymes but not the three glycolytic enzymes. So the two sets of opposing enzymes may be controlled by two separate sets of genic units. The model they propose is of great value for the understanding of glucose metabolism.

In the session on 'Enzyme regulation and cancer', Weber and Lea reported a decrease in the synthesis of carbohydrates and lipids and of degradation of proteins and nucleic acids as the hepatoma enlarged which may be due to alterations in key, rate-limiting enzymes. They compare these changes with those of normal and regenerating liver with an interesting model. Boxer and Shonk found a decrease in glycerol dehydrogenase and FDPase in human and rodent hepatomas. Hepp, Prüsse, Weiss and Wieland reported a lower level of acetate thiokinase in tumour tissues which may account for the higher acetate levels seen in these tissues.

Kuntzman, Welch and Conney in the session on 'Enzyme induction' reported that steroid hydroxylases of liver microsomes were affected by insecticides. Ville, Leusden and Zelewski showed that the synthesis of sterols in the placenta was different from that of other tissues. Inhibition of vitamin K induced prothrombin synthesis by actinomycin was reported by Olson. This was attributed indirectly to the action of vitamin K at the genetic level through allosteric binding with a regulator molecule. More work should be done on this interesting hypothesis.

In the section on 'Metabolic regulation', Peraino, Lamar and Pitot reported that glycogen, when deposited, gets associated with endoplasmic reticulum which is devoid of ribosomes. Such changes in the membrane may be of regulatory significance in carbohydrate metabolism. Struck, Ashmore and Wieland showed that oxidation of fatty acids in mitochondria may be responsible for the supply of $NADH_2$ for the reduction of oxaloacetate to malate

which is then transported to cytoplasm for the synthesis of glucose. Herrara, Kamm, Ruderman and Cahill showed modulation of glycogenolysis and gluconeogenesis by altering the levels of glucose in the perfusate of rat liver.

The session on 'Mechanism of adaptation at enzyme level' included the work of Gibson, Hicks and Allmann who described an increase in Acetyl-CoA carboxylase and fatty acid synthetase in the liver of starved, fat-free rats on refeeding. This induction was inhibited by actinomycin and puromycin. Potter, Gebert and Pitot showed that the levels of several enzymes undergo cyclic alterations in a manner different from normal rats fed *ad lib.* Ville's work showed an increased utilization of progesterone and decreased activity of 3β-hydroxy-steroid dehydrogenase isomerase in organ culture.

In the session on 'Regulation and isozymes', Knox showed that tryptophan pyrrolase accumulates in the liver of hydroxycortisone treated rats as an inactive enzyme. Very low concentrations of tryptophan activated the apoenzyme both *in vivo* and *in vitro.* Schwartz and Nisselbaum showed that the cationic component of aspartate aminotransferase in the serum is associated with neoplastic disease. Katunuma, Okada and Nishi described the presence of a new metabolic pathway of $NADH_2$ and $NADPH_2$ to nicotinamide in mitochondria. This pathway was accelerated by ammonium ion.

The special lecture by Sir Hans Krebs is of special interest. He describes an increase in ketone bodies during starvation due to 'calorigenic homeostasis'. In severe ketosis, gluconeogenesis is stimulated since oxaloacetate, an intermediate of gluconeogenesis, is diverted to citric acid cycle due to an increase in the activity of PEP carboxykinase.

The volume would have been more complete if the paper of J. Changeux of Pasteur Institut, who was present, could be included. Otherwise, the papers published in this volume provide a wealth of information on the regulation of enzymes of different metabolic pathways. The information content and the synthetic approach of several authors will be of great use to general biochemists, those interested in enzyme regulation and the cancer specialists. The publishers have done an excellent job in the get-up of the volume.

M. S. KANUNGO

## PUBLICATIONS RECEIVED

THE STRUCTURE AND STRENGTH OF METALS by A. R. Bailey (Metallurgical Services Laboratories Ltd, Betchworth, UK), 1967. Pp. viii+122. Price 32s.

LIPIDS AND LIPIDOSES, edited by G. Schettler (Springer-Verlag, Berlin-West), 1967. Pp. xiv +622. Price $ 30.00

CARTESIAN TENSORS by Nils O. Myklestad (D. Van Nostrand Co. Inc., Princeton), 1967. Pp. xiii +141. Price $ 3.95

HEAVY ORGANIC CHEMICALS by A. J. Gait (Pergamon Press Ltd, Oxford), 1967. Pp. xvii+249. Price 35s. or $ 6.00

SOURCE BOOK ON ATOMIC ENERGY by Samuel Glasstone (D. Van Nostrand Co. Inc., Princeton), 1967. Pp. vii+883. Price $ 9.25

FOOD FLAVORINGS — COMPOSITION, MANUFACTURE AND USE by Joseph Merory (The AVI Publishing Co. Inc., Westpost, USA), 1968. Pp. ix+478. Price $ 10.00

CHEMISTRY IN THE UTILIZATION OF WOOD by R. H. Farmer (Pergamon Press Ltd, Oxford), 1967. Pp. viii+193. Price $ 6.00 or 35s.

REINFORCED CONCRETE DESIGN HANDBOOK by C. Chandra & R. Narayanan (Today & Tomorrow Book Agency, New Delhi), 1967. Pp. xv+171. Price Rs 17.50

THE PLANETARIUM AND ATMOSPHORIUM—AN INDOOR UNIVERSE by O. Richard Norton (Naturegraph Publishers, Healdsburg, California, USA), 1968. Pp. viii+176. Price $ 2.75

PERFUMERY AND FLAVORING SYNTHESIS by Paul Z. Bedoukian (Elsevier Publishing Co., Amsterdam), 1967. Pp. xiii+395

HIGH ENERGY PHYSICS: Vol. II, by E. H. S. Burhop (Academic Press Inc., New York), 1967. Pp. xi +483. Price $ 24.00 or 192s.

THERMAL PERFORMANCE OF BUILDINGS by J. F. van Straaten (Elsevier Publishing Co., Amsterdam), 1967. Pp. xiii+311

PETROLEUM MICROBIOLOGY by J. B. Davis (Elsevier Publishing Co., Amsterdam), 1967. Pp. xiv+664

# NOTES & NEWS

## A new scale of optical radiations

A new scale of optical radiation has been developed in terms of which sources can be calibrated for radiant intensity and detectors for radiant sensitivity. The scale is based on the type of absolute detector in which a blackened receiver is heated alternately by absorption of the radiation to be measured and by a built-in electrical element. The electrical power is adjusted until no variation in the receiver temperature is observable on changing from one form of heating to the other. The irradiance $E$ at the limiting aperture of the detector is then given by

$$E = P/A\alpha$$

where $P$ is the electrical power input at balance; $A$, the area of the limiting aperture; and $\alpha$, the absorption of the receiver.

The advantage of this method is that the parameters involved, e.g. electrical power and area or length, are much more accurate than the usual radiation parameters. The scale is accurate to within $\pm 0{\cdot}2$ per cent [*Aust. J. Phys.*, **20** (1967), 567].

## A novel semiconductor switch

A new light-sensitive solid state diode which operates as a light-activated switch has been developed. The schematic structure of the GaAs *p-n-i-n* diode, with four differently doped regions, is shown in Fig. 1. The semi-insulating *i*-region is compensated GaAs, prepared by diffusing Cu, Fe, or Cr into low-doped *n*-GaAs.

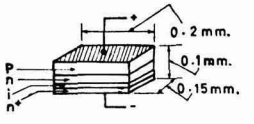


Fig. 1 — Schematic structure of the *p-n-i-n* GaAs diode

The electrical and optical properties of the diode as well as its switching behaviour have been tested. It exhibits a current-controlled negative resistance region with ratio of off- to on-resistance of the order of $10^6$. The turn-on voltage is between 10 and 200 V. and the turn-on time, with sufficient overvoltage, can be even 10 nsec. At higher currents the light emission at the *p-n* junction causes photoconduction in the *i*-region which is enhanced by the trapping of holes at acceptor levels. In the low resistance mode all the traps are filled and remain that way because of the high efficiency of light emission from the junction at the high currents [*J. appl. Phys.*, **38** (1967), 111].

## Determination of fluoride

Despite the numerous procedures described in the literature, the estimation of fluoride, specially in the presence of interfering ions, is very difficult. Two methods, based on the thermometric titration and potentiometric titration, have been developed, which open up some possibilities for handling certain situations more expeditiously. For example, the thermometric titration is less time-consuming if fluoride retarding elements like boron and aluminium are present and avoids interference due to sulphate, phosphate, borate and silicate.

Thermometric titration is quite useful for observing the course of metal-fluoride reactions, especially the endothermic reactions. The titration curve is also useful in inferring various phenomena like delayed precipitation, complex formation, etc. A number of cations have been found to react favourably with fluoride and useful as titrants in aqueous and/or alcoholic media. The four cations thorium, cerium(III), calcium and aluminium have been found to characterize most of the types of behaviour observed.

The titration has been carried out by adding 0·1-0·5 ml./min. of the titrant (0·2-1·0*M*) to 50 ml. of the test solution at controlled *p*H. Temperature is indicated by a 10,000-ohm thermistor in a modified Wheatstone bridge circuit.

Of the four selected titrants, thorium with the best temperature sensitivity and also least tolerance to the presence of other ions, gives the sharpest breaks at a *p*H of about 2·5. Cerium has the poorest temperature sensitivity but greater tolerance for most anions. Cerium has almost equal precision over the *p*H range 2-5, with least interference from borate and phosphate at higher *p*H. Aluminium, which gives better results in non-aqueous medium, has high tolerance for sulphate and phosphate and gives relatively smaller relative errors in the presence of borate and silicate. Calcium has a high tolerance for borate and can be used in the presence of considerable amounts of silicate and phosphate (after treatment with zinc acetate and not removing the zinc phosphate-silicate precipitate).

In the potentiometric method described, tetraphenylantimony sulphate has been used as an extractive titrant for fluoride. Fluoride has been extracted from an aqueous sample into chloroform as tetraphenylantimony fluoride. The fluoride ion activity in the aqueous phase is measured with a fluoride sensitive electrode. From the Sigmoid potentiometric titration curves obtained, the amount of fluoride is estimated.

Equal initial volumes of aqueous phase (*p*H 4-5) and extractant have been used. Thirty seconds of intimate phase contact by stirring and 15 sec. for phase separation are required between additions of titrant. Phosphate, sulphate, arsenate and arsenite do not interfere. Other multi-charged anions do not react with the titrant and can be expected not to interfere. The singly charged anions are also extracted by the titrant but can be removed by simple procedures [*Analyt. Chem.*, **39** (1967), 1771, 1776].

## *Soviet Physics-Semiconductors*

The American Institute of Physics is publishing the English translations of this Russian journal. The publication year is July 1967-June 1968. Translations would appear about six months after the Russian original. The price of

Vol. 1 (translation of the July 1967 Russian originals) and Vol. 2 (translation of the 1968 Russian originals) is $ 70 in USA and $ 74 abroad for each volume.

## Weizman Institute of Science

The annual report of the Weizman Institute of Science, Rehovoth, Israel, for the year 1966 records the scientific activities and achievements of the Institute.

*Applied mathematics* — The Department has completed the construction of the second GOLEM computer; by the end of the year three computers, two GOLEMs and a CDC 1604, were put to use. Work on the theory of ocean tides was continued using both numerical and analytical methods. It was found feasible to work out a solution for the tides in a model of the world oceans using computational grid of only one degree involving the solution of 170,000 simultaneous complex equations. Measurement of seismic noise was carried out with a view to locating a seismically quiet site for the geophysical observatory of the Weizman Institute.

*Biochemistry* — The work carried out in this Department is concerned mainly with (i) the elucidation of biochemical reactions involved in photosynthesis, (ii) mechanism of protein and nucleic acid synthesis, and (iii) interrelationship between algae and bacteria and the bacterial degradation of polysaccharides.

The existence of Emerson enhancement effect during NADP (nicotinamide adenine dinucleotide phosphate) photoreduction has been confirmed in isolated chloroplasts; the extent of enhancement, within certain limits, is a direct function of the ratio of reduction rates induced by the two monochromatic lights by themselves. Little or no enhancement is found during ferricyanide photoreduction, suggesting that ferricyanide photoreduction, in contrast to NADP photoreduction, is activated mostly, if not exclusively, by photosystem II. Studies with ribonuclease-1 indicated that the binding of ribonuclease-1 by ribosomes is more stable to high salt concentrations when the enzyme and the ribosomes come from the some species than when they are from different species, suggesting that there might be a selective advantage in preserving this affinity during evolutionary change.

*Biodynamics* — Work on the mechanism of nidation was continued. Shelesnyak's hypothesis of the mechanism of nidation (in rat) has been improved to consider the process synonymous with progestation involving a sequence of phases, viz. (i) priming, corresponding to the time of ovulation and mating including semen-uterine interactions, ovum fertilization and tubal transport of fertilized ovum through the fallopian tube; (ii) sensitization, occurring over the period beginning with mating and ending with the time of blastocyst entrance into uterine lumen; (iii) stimulation, induction of sensitized endometrium to decidualize; (iv) decidualization, proliferation and differentiation of endometrial tissue into decidual tissue forming the nidus; and (v) blastocyst attachment or implantation, the final stage of nidation. The experimental approach to this study involves designed and controlled alterations of selected conditions of various components of blastocyst-uterine system during different phases of progestation.

Studies on the relation of histamine release to oestrogen action in the pregnant rat indicated that histamine release in uterus does not appear to be an obligatory response to oestrogen action and is independent of oestrogen action in the pregnant rat uterus; both oestrogen and histamine, acting in sequence, are essential for decidualization and nidation. Investigations on the origin of eosinophilic granulocytes in the rat indicated that they appear periodically in the uterus of rat in certain physiological states (e.g. estrus of normal cycle) originating from haematopoietic sites in bone marrow and are not produced locally from cells within the uterus itself. A rapid method for the isolation of ova from uteri of rats by flushing the uterus with the medium and collecting the ova in the rinse fluid *per vaginam* has been evolved. Another method was evolved for the quantitative estimation of urinary androsterone, aetiocholanolone and dehydroepiandrosterone, employing enzymatic hydrolysis for glycuronide conjugates, solvolysis for sulphate esters, ascending thin-layer chromatography, elution and spectrophotometric quantitation of eluted steroids as Zimmermann chromogens.

Other projects undertaken include immunological studies of human gonadotropins, mechanism of drug interference with nidation and insulin blockade as a tool in endocrine physiology.

*Biological ultrastructure* — Investigations on red blood cells, the ideal systems for the study of maturation and ageing of mammalian cells, have been continued. Red cell differentiation and nuclear expulsion in erythroid clones grown in spleen of irradiated mice have been studied employing optical and electron microscopy. Maturation of reticulocyte and the decline in their capacity for protein synthesis have been studied both biochemically and with electron microscope. Experimentally induced deterioration of red cells *in vivo* and *in vitro* is directed towards elucidating the mechanism by which red cells are sequestered in senescence and in post-traumatic and other anaemias.

Human erythrocytes have been separated into age groups depending on their specific gravity using the two-phase centrifugation technique, and their enzyme activity assessed. Decline in enzyme activity as a function of red cell age is found to be similar for all enzymes except hexokinase, which is relatively low even in young red cells. The other studies undertaken in this Department include influence of oxygen toxicity on the rate of red cell ageing and polyphenols in the preventive treatment of post-traumatic anaemia.

*Biophysics* — Proteins and synthetic polypeptides as well as other polymers of biological and technological importance continued to be the main subjects of study in the Department. Anticoagulant properties of heparin-like acid mucopolysaccharide isolated from rat kidneys were compared with those of standard heparin preparation. Both materials showed antithrombin activity and inhibition of thrombin formation, supporting the results of chemical characterization of rat kidney mucopolysaccharides. The role of solvation in stabilizing the poly-L-proline II

helix as well as the mechanism of action of neutral salts in destabilizing the poly-L-proline II conformation have been investigated. The results indicate that water does not have a unique structural role in the stabilization mechanism. Soybean oil meal was found to contain 4 distinct haemagglutinins separable chromatographically on column of DEAE cellulose. The four haemagglutinins are all glycoproteins containing mannose and glucosamine. A method for the determination of the sequence of amino acid residues in di- and tripeptides using NMR technique has been developed. The method is based on the directions of shifts of the spectral lines as a function of *p*H and amino acid sequence. The influence of microenvironment on the activity of proteolytic enzymes, trypsin and chymotrypsin, has been investigated embedding them in charged polymers. The size of the active centres of proteases has been determined by the use of substrates containing both the natural and unnatural D-isomers of amino acids of a known distance from the point of action of enzyme. Structural studies have been carried out on bacterial cell walls and disulphide bonds of a trypsin inhibitor.

*Cell biology and chemical immunology* — In these two Departments, the work carried out was concerned mostly with the different aspects of antigen and antibody reaction. In the Cell Biology Department, an analysis of the control of cell differentiation with the mechanism of antibody production and immunological tolerance has been made. Studies on the kinetics of tolerance induction to protein antigens in X-ray treated rabbits revealed that the inducibility of tolerance depends on the schedule of antigen administration rather than on the amount administered *per se*. *In vitro* differentiation of muscle cell has been further investigated with a view to testing the relative stabilization of RNA in differentiated cells. Studies on the feedback mechanisms of erythropoiesis have also been continued.

In the Chemical Immunology Department, work was continued on the synthetic antigens. Synthetic polypeptides and peptidyl proteins have been used as models in studies on the (i) intracellular fate of antigens, (ii) genetic control of immune response, (iii) delayed hypersensitivity, (iv) immunological tolerance, and (v) antigen competition. Methods for more sensitive detection of antibodies and for their immunospecific isolation have been developed.

*Electronics* — Mössbauer spectroscopy studies in magnetism have been extended to $^{119}Sn$ and $^{151}Eu$ in addition to the work on $^{57}Fe$. Work in the biomedical field has led to the discovery that the ferromagnetic material covering the radula of some chitons is essentially magnesium ferrite and it is not magnetized in the natural state. Using the laser for measuring small vibrations, it was found that the impedance characteristic of the middle ear of frogs is highly peaked at around 1200 cycles, which is also their main carrier frequency. A new method of cardiac pacemaking, which combines transvenous catheter with a radiofrequency pacemaker has been developed. Another method for the conversion of light into electrical energy for the purpose of tissue stimulation of cardiac pacemaking has also been developed.

*Experimental biology* — The long-term projects undertaken in this Department relate to (i) the complex factors involved in spontaneous and induced leukaemia and related diseases in mice; (ii) characterization of RLP (factor capable of inhibiting radiation leukaemogenesis in mice) and its mode of action; (iii) recognition of cell-free thyamic factor implicated in functional potentialities of that organ, the study of its biological properties and its relevance to carcinogenic process; and (iv) the study of organ-specific differences in nucleic acids (especially in their methylated bases) as related to cell differentiation and neoplasia.

The inhibitory effect of RLP on radiation leukaemogenesis in mice studied under different biological conditions indicated that RLP fails to inhibit the spontaneous development of lymphatic (thymic) leukaemia in mice, suggesting that the inhibitory action of RLP is an anti-radiation effect with respect to leukaemogenesis rather than an antagonist against the leukaemogenic process proper.

*Nuclear physics* — The work in this Department covers a broad spectrum of activities ranging from abstract field theory, through theoretical and experimental work in elementary particles, nuclear, solid state and chemical physics to the development of various types of instruments. Considerable progress has been made in the study of massless particles, Heisenberg versus Schrödinger formalism, and various scattering theorems. Work on nuclear theory covered shell model calculations in nuclear spectroscopy, the role of core excitation in structure and reactions, transfer reactions and hyper nuclei. Progress in instrumentation has been highlighted by the completion of the television scanning and measuring system for bubble chamber photographs. Other projects undertaken in this Department include studies on biochemical statistics and use of Mössbauer effect for studies on mechanism of hearing.

*Organic chemistry* — Compounds of theoretical as well as of biological interest have been studied in this Department. Several photochemical reactions have been investigated; the alkylation of a variety of systems with olefins has been extended to amino acid derivatives and peptides. The photoinduction reaction of pyrimidines and photolysis of conjugated diacetylenes as well as of enol trichloroacetates and tosylates have been studied.

The hitherto unsolved problem of synthesizing aminosugar disaccharides with hexosamine as the non-reducing moiety has been studied. The synthesis of $1 \rightarrow 3$ and $1 \rightarrow 4$ glucosaminyl-galactose fragments of many naturally occurring complex glycolipids has been accomplished. A new type of chemotherapeutic agents, nitrated hydrazothiazoles, with strong antiprotozoal activity, have been synthesized and screened.

A new Department of Chemistry has been formed by the merger of organic chemistry, X-ray crystallography and photochemistry units.

*Plant genetics*—Growth, differentiation and nucleic acid metabolism of two species of Lemnaceae have been studied. Autoradiographic studies involving the mitotic synchronizing agent 5-amino uracil (5AU) indicated that increasing periods of 5AU treatment

reduce mitotic division and progressively reduce the rate of nuclear and nucleolar RNA synthesis of interphase cells. Breeding work on wheat, barley, castor bean, melons and cucumbers has been continued. The other projects undertaken include studies on drought resistance in field crops and experimental morphogenesis of sex organs and other plant organs.

## Seminar on analysis

A Seminar in Mathematical Analysis was held at the Institute of Mathematical Sciences, Madras, from 20 December 1967 to 6 January 1968, with Profs. W. H. J. Fuchs of Cornell University, L. A. Rubel of the Institute for Advanced Study, University of Illinois, and K. R. Unni of the Institute of Mathematical Sciences, Madras, as the principal lecturers. The seminar was inaugurated by Shri V. R. Nedunchezhian, Chairman of the Board of Governors of the Institute.

Prof. Fuchs's lecture was on 'Meromorphic functions of lower order less than one', Prof. Rubel's on the 'Vector space of analytic functions', and Prof. Unni's on the 'Bernstein's approximation problem'.

More than 50 participants from all parts of the country attended the seminar which was the first of its kind in India. The seminar was supported by a special grant from the Department of Atomic Energy, Government of India.

## Announcements

▪ *The Eighth International Congress on High-speed Photography* will be held at Stockholm during 23-29 June 1968. The congress would review and explore the recent advances made in high-speed photography and, among others, topics like the use of laser as a light source and holographic imaging with reconstructed wave fronts would be discussed. Further details regarding the congress may be obtained from the Secretariat, 8th International Congress on High-speed Photography, Box 23, Stockholm 80, Sweden.

▪ *The Second International Symposium on Radiosensitizing and Radioprotective Drugs* will be held in Rome under the auspices of the European Society for Biochemical Pharmacology, during 6-8 May 1969. Details regarding the symposium can be had from Dr H. Moroson, Sloan-Kettering Institute for Cancer Research, Donald S. Walker Laboratory, 145 Boston Post Road, Rye, NY, USA or Dr M. Quintiliani, Istituto Superiore di Sanità, 299 Viale Regina Elena, Roma 00161, Italy.

**FORTHCOMING INTERNATIONAL CONFERENCES, 1968**

| *Date* | *Conference* | *Place* |
|---|---|---|
| 4-8 June | International Congress on Research in Photosynthesis | Freudenstadt, W. Germany |
| 9-13 June | Canadian Nuclear Association, International Conference and Exhibition | Toronto |
| 12-14 June | International Congress of the Federation of Associations of Textile Chemists and Colourists | Paris |
| 12-14 June | International Communications Conference | Philadelphia |
| 17-21 June | Fourth International Materials Symposium (Structure and Chemistry of Solid Surfaces) | Berkeley, USA |
| 17-30 June | International Council of Scientific Unions — General Assembly | Paris |
| 18-22 June | Fifth International Light Metals Congress | Leoben, Austria |
| Summer (late) | Eighth International Congress on Tropical Medicine and Malaria | Tehran |
| 23-29 June | Eighth International Congress on High-speed Photography | Stockholm |
| 23-29 June | Fourth International Congress on Catalysis | Moscow |
| 24-28 June | Sixth International Plansee Seminar on High Temperature Materials | Reutte, Austria |
| 25-28 June | International Symposium on Gas Chromatography | Copenhagen |
| 30 June-5 July | International Congress on Endocrinology | Mexico, DF |
| 1-5 July | Eighth International Congress on Glass | London |
| 7-13 July | Eighth International Congress on Gastroenterology | Prague |
| 8-13 July | Fifth International Symposium on the Chemistry of Natural Products | London |
| 8-20 July | Eleventh International Congress on Photogrammetry | Lausanne |
| 10-20 July | International Conference on Large Electric Systems | Paris |
| 14-20 July | Twelfth International Symposium on Combustion | Poitiers, France |
| 15-19 July | International Congress on Vehicle Mechanics | Detroit |
| 22-25 July | International Congress on Animal Reproduction and Artificial Insemination | Paris |
| July | International Conference on the Electron Capture and the Higher Order Processes in Nuclear Decays | Debrecen, Hungary |
| July | Second International Symposium on Pharmaceutical Chemistry | Munster, W. Germany |
| July | Second International Symposium on the Chemistry of Argonic Silicon Compounds | Bordeaux |
| 2-9 Aug. | International Congress on Entomology | Moscow |
| 5-10 Aug. | Fourth Congress of the International Federation for Information Processing | Edinburg |
| 6-16 Aug. | Ninth International Congress on Soil Science | Adelaide |
| 16-23 Aug. | Third International Congress on Histochemistry and Cytochemistry | New York |
| 19-28 Aug. | Congress of the International Mineralogical Association | Prague |
| 19-28 Aug. | Twelfth International Congress on Genetics | Tokyo |
| 19-28 Aug. | International Geological Congress | Prague |

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