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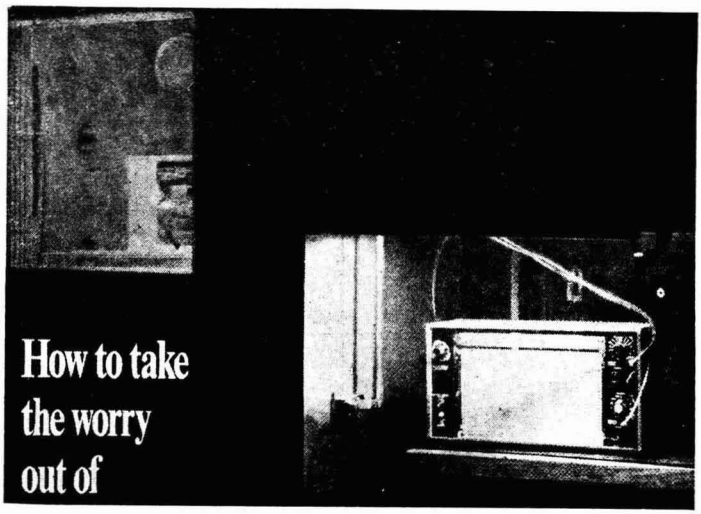

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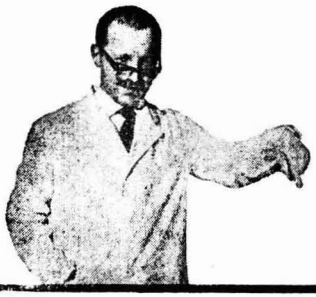

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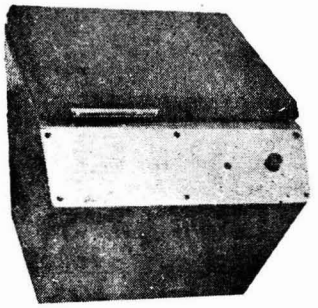

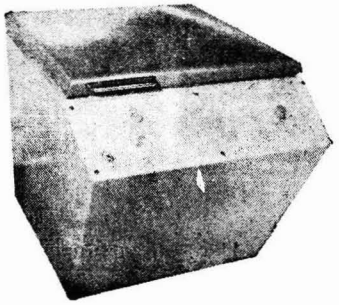

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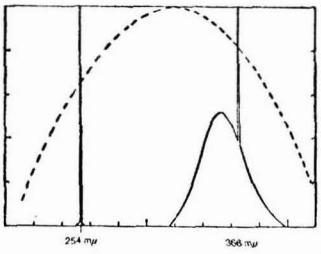

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Current Topics

Twenty-five Years of CSIR

THE Council of Scientific & Industrial Research (CSIR) celebrated its Silver Jubilee at a simple ceremony at the National Physical Laboratory, New Delhi, on 10 August 1968. The celebrations were inaugurated by the President of India, Dr Zakir Husain, and Shrimati Indira Gandhi, Prime Minister and President, CSIR, presided over the function. Among others who participated in the function were Dr Triguna Sen, Union Minister for Education and Vice-President, CSIR; Dr A. Ramaswami Mudaliar, Founder President, CSIR; Mr Rene Maheu, Director-General, Unesco; and a large number of delegates from various countries attending the Conference on the Application of Science and Technology to the Development of Asia (CASTASIA), which was currently in session in New Delhi. Dr Triguna Sen welcomed the large gathering of invitees and staff members from CSIR establishments in Delhi, and Dr Atma Ram, Director-General, Scientific & Industrial Research, proposed a vote of thanks. Simultaneously, the national laboratories and other CSIR establishments celebrated the occasion through similar functions, open days and radio programmes. The All India Radio organized a Science Week to commemorate the occasion. A souvenir entitled *25 Years of CSIR* was released on the occasion. Silver Jubilee awards were presented to 27 employees of CSIR who completed 25 years of service on 1 April 1967. Several of the CSIR laboratories also participated in an exhibition 'Science in Everyday Life' organized by the Directorate of Advertising and Visual Publicity in connection with the CASTASIA.

Of the 30 CSIR laboratories in existence today, only eight have been in existence for 15 years or more and ten others are hardly ten years old. However, there is unmistakable evidence of the important position the organization has come to occupy in the life of the nation. Looking at the conditions prior to the establishment of CSIR, it is evident today that the credit for bringing about the upsurge in science and technology during the two decades since Independence goes to CSIR. While its laboratories represent all that is progressive and modern in science and technology, its contribution in raising the level of research in other organizations, particularly the universities, through financial and other assistance, has been considerable.

There are few parallels for the amazing speed and thoroughness with which the work of planning, organizing and equipping the research establishments of CSIR in diverse fields of science and technology was carried out. This achievement gets added significance when viewed in the light of the fact that, being the first of its kind in a developing country, CSIR had no model to follow or experience to draw upon, and it had the difficult task of working out its own guidelines, training and recruiting personnel to staff its institutions and equipping laboratories with tools required for scientific and technological research. What has been achieved speaks of the boundless energy, resourcefulness, foresight and organizing capacity of the chief architect of this organization, Dr Shanti Swarup Bhatnagar. It was also a matter of extreme good fortune for the country that in the initial days when the forces of national reconstruction and resurgence were set in motion, CSIR had a man of Nehru's vision at the helm of affairs.

The projects and plans of the constituent laboratories of CSIR today embrace practically all the basic sciences and specialized applied fields like aeronautics, buildings and roads, electronics, geophysics, glass and ceramics, mechanical, metallurgical and structural engineering, oceanography and scientific documentation. There are regional laboratories concerned with technological problems in their respective regions. There are besides 65 field stations of various laboratories set up in different parts of the country for survey and evaluation of the industrial raw material resources of the particular areas and for undertaking extension and liaison work. The annual budget of CSIR today is of the order of Rs 2000 lakhs compared to Rs 6·7 lakhs in the year of its inception.

The basic principle underlying the formulation of the research programmes of the CSIR laboratories is the fulfilment of the broad objective assigned to it at the time of its inception, viz. 'to serve as a central organization for the promotion of science and its application to national development'. In specific terms, the applied research programmes have been concerned primarily with the survey, evaluation and upgrading of industrial raw materials, development of new technologies or adaptation of known technologies to the conditions and materials obtaining in India, development of substitutes for scarce and imported raw materials and finished products, design, development and fabrication of pilot plants, testing facilities, etc. The work of survey and evaluation of industrial raw materials has been gigantic in proportions and at the same time of the widest possible diversity. Among the materials covered are coal, limestones, graphite, glass sands, clays, foundry moulding sands, puzzolanic materials and medicinal plants. The quality of about 7000 million tons of coal from different coalfields has been assessed and new coal sources with reserves estimated at 750 million tons of coking and blendable coals have been located. Extensive coal washing studies conducted over the last two decades or more have been of tremendous economic value by way of rationalizing the use of coal from different areas. An assessment of the exact economic benefits accruing from these and other similar researches is an almost impossible task. Know-how for about 400 processes has been developed and several of these have led to or have the potentialities of leading to the evolution of new patterns of industrial utilization and innovation. Typical examples

are the development of know-how for the manufacture of optical glass and the process for the production of infant food from buffalo milk, leading to the establishment of a thriving industry and almost complete independence from imports of infant food. Substitutes for a variety of products, such as mica and ceramic capacitors, ferrites, processes for the production of a wide variety of ferro-alloys have been worked out, as a result of which large-scale production of these products has become possible.

CSIR has all along given due consideration to the concept that planning of research includes both research promotion and research utilization, and for ensuring enhanced industrial utilization of results of research it has been assisting the industry through technical information, design engineering and consultancy services and analysis and testing facilities. The sustained efforts of CSIR in establishing close links and communication with industry has helped in securing the utilization of a significant number of processes and products developed through research. By the end of 1966-67, 232 processes had been released to industry. Of these, 103 processes are in commercial production and 129 are in various stages of exploitation. Seventeen other processes released free of charge have also been utilized by industry. Based on the know-how developed in the laboratories, 58 processes are now in semi-commercial or pilot production in the laboratories themselves.

Another direction in which CSIR has done yeoman's service to Indian industry is by initiating cooperative industrial research movement in the country. By providing funds and expertise, CSIR has helped in the establishment of 9 cooperative associations covering tea, textiles, jute, cement and plywood industries; these research associations have become important centres for both basic and applied research germane to the problems of the concerned industries; they also provide technical consultancy, industrial liaison, information and extension services to member firms.

The CSIR laboratories can boast of a very solid contribution to the country's industrial development through their collaboration with the Indian Standards Institution, the national standards body. Over 500 CSIR scientists participate in the work of some 1800 ISI committees and have contributed in a big way to India's standardization effort over the past two decades. All this work has helped to establish a meaningful rapport between industry and science, with immense benefits to the national economy in the shape of savings in material and component costs, higher productivity, better process control and quality of products, import substitution, promotion of exports and greater overall utilization of indigenous resources.

CSIR's contribution in organizing scientific information facilities in the country, including storage, retrieval and communication of information has been of a pioneering nature. Beginning with a quarterly journal in 1942, the *Journal of Scientific & Industrial Research*, the CSIR now publishes five original research periodicals which have attained international standing. The *Journal of Scientific & Industrial Research*, converted to a general science periodical in 1963, can rightly claim to be India's leading general science periodical, catering to a wide variety of subjects. During 1967, the various journals published over 900 papers compared to 38 papers in the first volume of the *Journal of Scientific & Industrial Research*. The CSIR has been engaged on a gigantic national project concerning the compilation of an encyclopaedic dictionary — the *Wealth of India* — on the raw material and industrial resources of the country. To date 7 volumes in the raw materials series and 6 in the industrial products series have been brought out. The Indian National Scientific Documentation Centre (Insdoc) established in 1952 with the assistance of Unesco has grown into a national documentation facility equipped for procurement and supply of documents published in any part of the world; the centre also provides translation and bibliographic services. An integral unit of Insdoc is the National Science Library, a cooperative acquisition facility locating scientific documents in the places of their use, with only bibliographic control of holdings through a Union Catalogue.

CSIR's record in the promotion of science and technology in the country is an impressive one indeed. Its varied activities have had a profound and far-reaching impact on the country's development in various directions. The following figures provide ample evidence of its solid contributions: research papers published, 11,000; research schemes sanctioned, 1800; patents taken out, 1500; and processes developed and released, 340. A still more significant contribution has been the creation of science-consciousness in the country as a whole and research-consciousness in industry. As mentioned by the President of India, Dr Zakir Husain, in his inaugural address, " the major contribution of CSIR has been in the creation of science-consciousness among the people and making industry realize the need for helping themselves by promoting research cells within their organization. It has also invested the scientists with the necessary self-respect and stature for contributing significantly to the nation's growth."

In an organization like CSIR there is no room for the feeling of ' resting on oars ' at any time. The country's ever expanding economy will provide the scientists and technologists with hundreds of problems to grapple with. The country's economic growth depends to a large extent on the efficiency with which national research bodies like CSIR function. It is to be hoped that the coming years will see more and more fruitful results coming out of CSIR laboratories. There is obvious need for greater attention in respect of utilization of research results. As pointed out by Dr A. Ramaswami Mudaliar on the occasion of the Silver Jubilee celebrations, the main responsibility of a scientist is to make inventions and develop new processes. He is not expected to undertake design and development work relating to the translation of the results of his research to commercial scale production. This is the responsibility of industry. There is obvious need for the creation of the requisite climate for greater rapport between research and industry so that the results of industrial research emanating from the laboratory are exploited quickly. This is the task to which CSIR must address itself in the coming years.

Silicon Solar Cells & Their Terrestrial Applications

S. C. JAIN*, H. K. SEHGAL & R. B. GANGADHAR
Department of Physics, Indian Institute of Technology, New Delhi 29

THE first silicon solar cell (SSC) was made in 1954 at the Bell Telephone Laboratories, USA[1]. Since then the art and technology of the fabrication of SSC have made tremendous advances. The work on SSC can be divided into two main areas: (i) for terrestrial uses of SSC and (ii) for use of SSC in outer space. Some aspects of technology of fabrication, improvement in performance and terrestrial applications of SSC are reviewed in the present paper. Results of the unpublished work done in this laboratory are also reported.

Fabrication of Silicon Solar Cells

The conventional method of making the shallow *p-n* junction SSC by diffusing the desired *n* or *p* type impurity from the vapour phase in *p* or *n* type single crystal silicon slices has been described by Gangadhar *et al.*[2]. They used low resistance contacts by electroless plating of nickel followed by sintering. The nickel contacts are mechanically strong, but during sintering, nickel penetrates the shallow diffused layer, degrading the cell characteristics. Plated gold contacts usually used[3] for the *p/n* cell have the same limitation. To overcome this difficulty, modified silver-titanium contacts[4] were developed at the Bell Telephone Laboratories. Very recently, still better silver-cerium contacts[3] have been developed and used. These contacts are later discussed in detail. The fabrication processes for *p/n* and *n/p* SSC based on diffusion technique are summarized in Table 1.

Recently, the ion implantation technique has been used for the fabrication of *p-n* junctions[5-7]. An ion implantation apparatus developed by Burrill *et al.*[8] for *n/p* SSC production is shown in Fig. 1. The apparatus consists of a high current, low density plasma ion source, a pre-acceleration analysing magnet and a sample implantation chamber. The source is run on a phosphorus containing gas. A large diameter ^{31}P beam comes from the source at 5 keV. and passes through the analysing magnet, which removes all the impurities. The ions are then accelerated on to the sample at energies up to 150 keV., by voltage applied to the sample holder. The impurity profile programming can either be done by rotating the samples in the path of the ion beam, or by varying the input voltage to the high voltage supply and the implantation time. The latter method is more suitable and convenient in practice. In this process, the incoming ion beam impinges on the cells at an angle of 7° to avoid channelling. The sample holder can be maintained at any desired temperature. The *p* type slices mounted on the holder are brought to face the impinging ion beam limited to an area of 2×6 cm.^{2}. The ^{31}P ions are incorporated in the surface layer of silicon slices forming an *n/p* junction. After implantation, the samples are annealed at 750°C. for about 16 hr to decrease the sheet resistance of the implanted layer to a minimum. Contacts are placed on the front with Ti-Ag and on the back with alloyed Al. The antireflection coating is now applied to the cells. Finally, integral cover slips of silicon monoxide are grown by an ion beam high vacuum sputtering process. Even with poor starting material an efficiency better than 9 per cent is easily achieved by this technique. This method also enables the fabrication of solar cells with very little variation in cell to cell performance. Thus, this process will help in the production of good solar cells at relatively low costs.

Performance of Solar Cells

Conversion efficiency — The conversion efficiency of a solar cell is defined as the percentage of the incident energy converted into electrical energy. The maximum conversion efficiency can be determined from the ratio of the area of the biggest rectangle that can be accommodated under the *V-I*

TABLE 1 — FABRICATION PROCESSES FOR *n/p* AND *p/n* SILICON SOLAR CELLS

	n on *p*	*p* on *n*
Material	*p* type 0·5-1·2 ohm-cm., silicon	*n* type 0·3-1·5 ohm-cm., silicon
Diffusion process		
Source	P_2O_5	BCl_3
Temperature	875°C.	1060°C.
Time	30 min.	10 min.
Contact process	Evaporated; silver-titanium, sintered at 600°C.	Plated; gold-nickel
Antireflection coating process	Evaporated SiO	Formed automatically in diffusion process

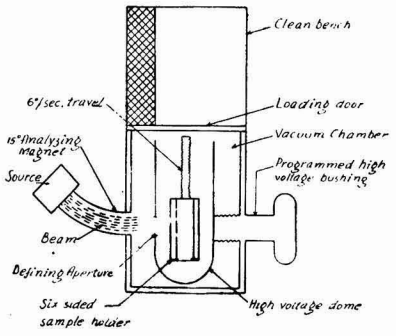

Fig. 1 — Ion implantation machine: schematic diagram[8]

*Also at the National Physical Laboratory, New Delhi 12.

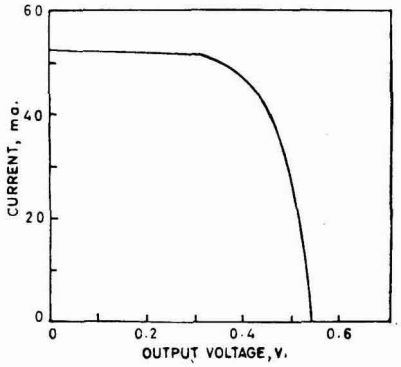

Fig. 2 — *V-I* characteristic of a SSC fabricated at the Indian Institute of Technology, New Delhi

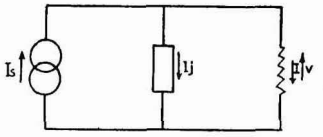

Fig. 3 — Equivalent circuit for the solar cell[9] (cell series resistance = 0)

characteristics of the SSC to the light energy incident on it. The *V-I* curve is drawn by varying the load resistance and measuring the voltage and current across it for a fixed illumination level of incident light. A typical *V-I* curve for an *n/p* SSC (conversion efficiency 10-11 per cent), fabricated at the Indian Institute of Technology, New Delhi, from 10 ohm-cm. *p* type (boron-doped) silicon is shown in Fig. 2. The shape of the *V-I* plot is represented by a parameter called the ' curve factor ', defined as the ratio of the area of the largest rectangle that can be accommodated under the *V-I* plot to the product of the short circuit current and open circuit voltage. The load corresponding to the point on the *V-I* plot, where the biggest rectangle under the curve can be drawn, is called the optimum load for the solar cell for that illumination level. A value of unity for the curve factor is the ideal case, but cells with curve factor 0·70-0·75 are now possible[3]. The efficiency of the cell is a sensitive function of physical parameters like temperature, series resistance of the cell, intensity of illumination and thickness of the semiconductor slice. The utility of SSC for high speed a.c. applications depends on its capacitance and response time.

For solar radiation, which has its maxima in the blue region, a cell more sensitive to blue radiations would give more output power for the same incident intensity. In other words, the power output of the cell depends on its spectral response and the spectral composition of the incident light.

Temperature effect on the conversion efficiency — The conversion efficiency of the solar cell depends on the temperature of the cell[9]. The factors which make the conversion process temperature dependent are introduced by the properties of semiconductors and the behaviour of the *p-n* junction. Let a simple equivalent circuit[9] (Fig. 3) be considered for the ideal solar cell with load resistance R_L. The cell is assumed to have zero series resistance, so that the short circuit current is equal to the light generated current. The load current density for such a junction will be the difference of the short circuit current I_s and the junction current I_j. The total junction current I_j is the sum of the current arising from the ideal junction current, a recombination current and a leakage current. To study the temperature dependence of the power supplied to the load resistance by the cell for a particular incident light flux, the temperature variation of I_s and I_j must be considered.

For a suitably located junction and large lifetimes of the charge carriers, I_s can be expressed in terms of the diffusion lengths L_n and L_p for electrons and holes as

$$I_s = qg(L_n + L_p) \qquad ...(1)$$

where g is the generation rate of electron-hole pairs per cm.^{3} per sec. and q is the electronic charge. The dependence of I_s on temperature is mainly due to the effect of temperature on the diffusion lengths of the carriers. The diffusion length is given by

$$L = (D\tau)^{\frac{1}{2}} \qquad ...(2)$$

where D is the diffusion constant and τ is the lifetime of minority carriers. Temperature does not have an appreciable effect on D and τ and hence on the diffusion lengths. Another factor effecting I_s is mainly the generation rate g which increases with temperature due to decrease in the band gap E_g of the semiconductor. Both these effects are, however, small and I_s is not a rapidly varying function of temperature.

The temperature dependence of junction current can be visualized by considering its effect on the ideal junction current, i.e. the current flowing over the junction barrier and the recombination current arising from the recombination of charge carriers in the depletion layer. The leakage current through surface channels has the same temperature dependence as the recombination current and both can be described by similar equations.

The ideal junction current can be written as

$$I_j = I_0\left[\exp\left(\frac{qV}{kT}\right) - 1\right] \qquad ...(3)$$

where

$$I_0 = qn_i^2\left[\frac{1}{N_A}\left(\frac{D_n}{\tau_n}\right)^{\frac{1}{2}} + \frac{1}{N_D}\left(\frac{D_p}{\tau_p}\right)^{\frac{1}{2}}\right] \qquad ...(4)$$

Here n_i is the intrinsic carrier density; N_A and N_D, the net acceptor and donor impurities per unit volume; D_p and D_n, the respective hole and electron diffusion coefficients; and τ_p and τ_n, the hole and electron lifetimes respectively. The magnitudes of I_0 and hence of I_j are determined by the band gap of the semiconductor through its effect on n_i^2 and because the coefficient I_0 in Eq. (3) rises exponentially with temperature, it results in an exponential increase in I_j.

Similarly, the recombination current is given[10] by

$$I_j = \frac{qn_i}{(\tau_{p_0}\tau_{n_0})^{\frac{1}{2}}}\omega\frac{2\sinh(qV/2kT)\,f(b)}{(\phi-V)(q/kT)} \qquad ...(5)$$

where τ_{p_0} and τ_{n_0} are the lifetimes of holes and electrons in a material with all acceptor and donor levels filled completely; ω, the width of depletion layer; ϕ, the barrier height of p-n junction; and $f(b)$ is a function varying slightly with voltage. The magnitude of I_j varies as n_i and is determined by half band gap. The sum contribution from these factors gives the net dependence of I_j on temperature.

Due to temperature variation in I_j and a nearly constant I_s, the load current density becomes a function of temperature. For the case when both the components of junction current are effective, the dependence of I_j on V is given by

$$I_j = I_0[e^{qV/kT}-1]+\frac{qn_i}{(\tau_{p_0}\tau_{n_0})^{\frac{1}{2}}}\omega\frac{2\sinh(qV/2kT)\,f(b)}{(\phi-V)(q/kT)} \qquad ...(6)$$

Experimental V-I curves at different temperatures obtained with an SSC fabricated at the Indian Institute of Technology, New Delhi, are shown in Fig. 4. Figs. 5, 6 and 7 give the temperature dependence of short circuit current, open circuit voltage and maximum power output respectively for the same cell. The values of voltage are widely different at different temperatures, but there is no appreciable change in I_s as expected from the above considerations. For these measurements the cells were mounted in a thermostat and were illuminated through a window. Pt-Pt+Rh (13 per cent) thermocouples were attached to the cells to measure the temperature.

Effect of series resistance on conversion efficiency — Like all other known generators of electrical power, solar cells also possess some internal series resistance[11,12], which plays an important role in determining the current-voltage characteristics of most of the conventional power generators. This is, however, not the case with solar cells. The p-n junction of the cell determines the current-voltage characteristics of solar cells; the series resistance contributes only in a secondary way.

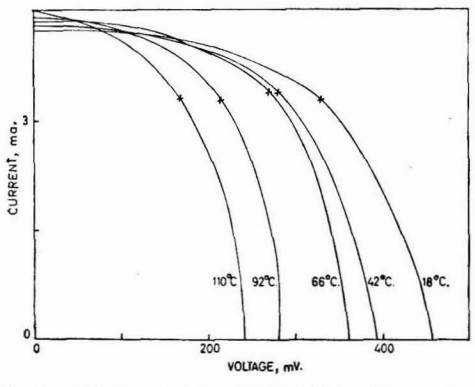

Fig. 4 — V-I characteristics of SSC at different temperatures

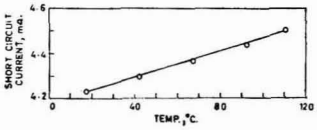

Fig. 5 — Short circuit current versus cell temperature

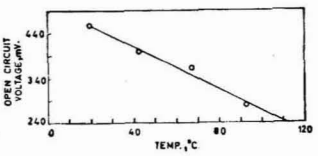

Fig. 6 — Open circuit voltage versus cell temperature

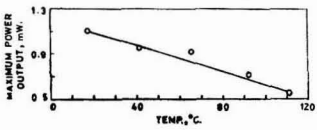

Fig. 7 — Maximum power output versus cell temperature

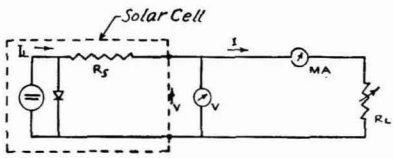

Fig. 8 — Equivalent circuit for the solar cell for series resistance determination[11]

Considering the equivalent circuit of a solar cell shown[11,13,14] in Fig. 8, the output current for a cell with series resistance R_S is given by

$$I = I_0\left[\exp\left\{\frac{q}{AkT}(V-IR_S)\right\}-1\right]-I_L \qquad ...(7)$$

where I_0 is the diode reverse saturation current determined by material properties; I_L, the light generated current; V, the voltage; and A is a dimensionless constant with a value usually between 2·5 and 3·0. As discussed earlier, the maximum power output of the solar cell is a function of the curve power factor. It is obvious from Eq. (7) that increased series resistance would result in rounding at the knee, the rounding being more pronounced for higher light intensities. This rounding at the knee results in a decrease in the curve power factor as well as in the conversion efficiency of the cell. The rounding becomes prominent even at lower intensities if the cell series resistance is very high. Typical curves of the cell output power into a matched load versus the solar irradiance for different values of series resistance R_S are shown[11] in Fig. 9. It is apparent that the output power decreases more prominently for increased illumination for cells with higher series resistance. The series resistance effect and the

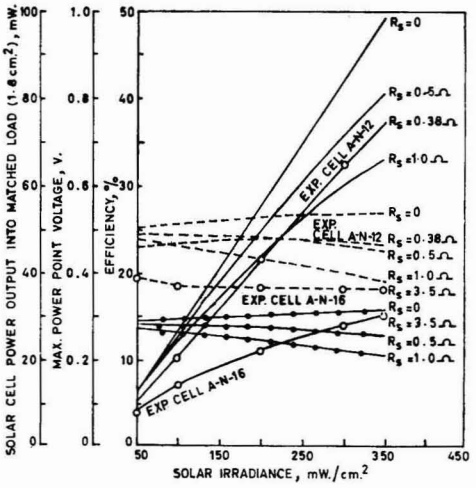

Fig. 9 — Cell output power into a matched load versus solar irradiance for different values of series resistance[11] [—, maximum power output; - - -, maximum power point voltage; and ●—●—●, efficiency]

exact evaluation of its magnitude is of great importance for device development and for proper evaluation of current-voltage characteristics.

The series resistance of a cell comprises mainly the resistance of the bulk material, sheet resistance of the diffused layer and the contact resistance of the electrical connections. The bulk resistance contribution to the series resistance of the cell can be made negligibly small by the proper choice of the semiconductor material. Sheet resistance can be reduced appreciably by improving the 'collection' of the charge carriers in the diffused layer. It is achieved by putting gridded contacts[15] on the active surface of the cell. The contact resistance resulting from the metallic gridded structure on the semiconductor substrate can be minimized by the deposition of the right type of contact material[3,4] followed by sintering and subsequent tinning as discussed later.

By optimizing these factors, it has become possible to fabricate solar cells with sheet resistances as small as 0·3 ohm. The method commonly used for series resistance measurement was suggested by Swanson[11]. It consists in measuring the *V-I* characteristics of the cell at two different (unknown) intensities of light illuminations. The two characteristics are translated against each other by the amounts ΔI_L and $\Delta I_L \times R_S$ in the current and voltage axes respectively. To determine the series resistance R_S of the cell an arbitrary interval ΔI is considered in such a way that the lines drawn parallel to the voltage axes from points $I_{s(1)} - \Delta I$ and $I_{s(2)} - \Delta I$ on the current axis cut the two characteristics in their respective knee as shown[11] in Fig. 10. The displacement parallel to the current and voltage axes corresponding to these two points will be ΔI_L and $\Delta I_L \times R_S$ respectively. From these data it is possible to calculate the series resistance R_S.

Effect of thickness of slice on conversion efficiency — If the thickness of the slice is too small, it also affects the conversion efficiency of the solar cell. A typical curve for SSC showing the effect of thickness of the slice on the efficiency is shown[16] in Fig. 11. In the typical example, the efficiency of SSC, made from 10 ohm-cm. material, falls from 11·5 to 8·5 per cent with the thickness of the cell varying from 16×10^{-3} to 4×10^{-3} in. The efficiency remains practically the same for slices with thickness $\gtrsim 20 \times 10^{-3}$ in. The loss in conversion efficiency for a very thin cell can be explained easily. A silicon solar energy converter shown[4] in Fig. 12 is representative of a class, in which the radiant energy is absorbed in the semiconductor material, upsetting the equilibrium condition and permitting electrical power to be utilized in an outer load. The light flux incident on the cell can be subdivided into the following groups: (i) an appreciable fraction of the incident light which is reflected away; (ii) photons absorbed near the surface creating electron-hole pairs which recombine and never reach the junction; (iii) photons absorbed near the junction, on both sides, generating hole-electron pairs which are separated by the built-in field near the junction giving rise to useful power; (iv) photons absorbed

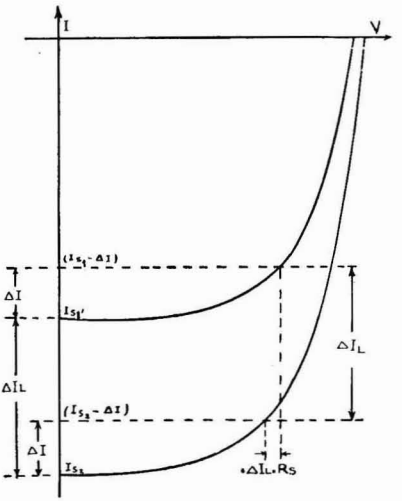

Fig. 10 — Series resistance as a function of *V-I* characteristics[11]

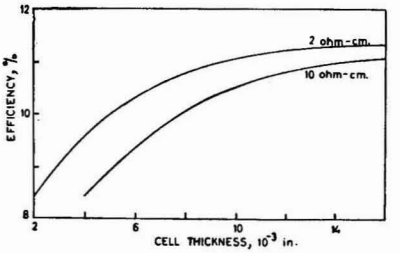

Fig. 11 — Efficiency versus cell thickness[16]

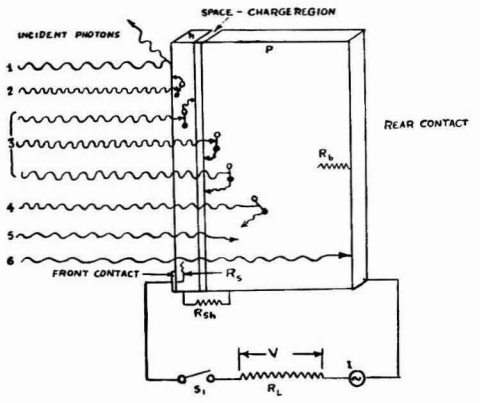

Fig. 12 — Effects of incident light of different wavelengths on SSC[4]

deep in the base region generating hole-electron pairs, too far from the junction to be useful; (v) photons, with insufficient energies to create hole-electron pairs, absorbed generating heat; and (vi) photons with lower energies which are usually not absorbed. If the base of SSC is very thin, some of the photons which generate useful hole-electron pairs in the base region [group (iii)] will pass through the cell, resulting in a lower output power for the same incident intensity, effectively lowering the conversion efficiency. On the other hand, if the thickness exceeds the optimum thickness of the cell, which is experimentally determined to be 0·5 mm., photons of groups (iv) and (v) will be absorbed, resulting in a rise in cell temperature. From this simple reasoning it is evident that the cell thickness should not be lower than the optimum value and also must not exceed this value to avoid higher cell temperature and hence lower conversion efficiency for higher photon fluxes. Thinner SSC with lower efficiency might, of course, find use in space crafts where power to weight ratio is an important consideration.

Spectral response of the cell — Spectral response of the photovoltaic cell[17] is a strong function of the wavelength of the incident radiation. The charge carrier pairs generated in the volume of the semiconductor at a distance x below the surface by the incident radiation of wavelength λ, whose absorption in the semiconductor obeys Lambert's law, can be written as

$$N(X) = N_0 e^{-\alpha X} \qquad ...(8)$$

Here α is the absorption coefficient; and N_0, the incident photon current density at $X = 0$. The photovoltaic response of a p-n junction, located at a distance l below the surface of the semiconductor wafer of thickness b, depends on the fraction of the liberated minority carriers that arrive at the junction.

Considering the properties of the junction and applying the appropriate boundary conditions, the hole contribution $I_p(l)$ and the electron contribution $I_n(l)$ to the current can be written as

$$I_p(l) = \frac{qN_0(1-R)}{\alpha[1-(\nu_p^2/\alpha^2)]}\left(\beta_p \nu_p e^{\nu_p l} - \gamma_p \nu_p e^{-\nu_p l} - \alpha e^{-\alpha l}\right) \qquad ...(9)$$

where

$$\beta_p \Delta_p = e^{-\nu_p l}(h+\alpha) - e^{-\alpha l}(h+\nu_p)$$
$$\gamma_p \Delta_p = e^{-\alpha l}(h-\nu_p) - e^{\nu_p l}(h+\alpha_p)$$
$$\Delta_p = e^{\nu_p l}(h+\nu_p) - e^{-\nu_p l}(h-\nu_p)$$
$$h = s/D_p$$
$$\nu_p = (D_p \tau_p)^{-\frac{1}{2}} = L_p^{-1}$$

and

$$I_n(l) = \frac{qN_0(1-R)}{\alpha[1-(\nu_n^2/\alpha^2)]}\left(\beta_n \nu_n e^{\nu_n l} - \gamma_n \nu_n e^{-\nu_n l} - \alpha e^{-\alpha l}\right) \qquad ...(10)$$
$$\beta_n \Delta_n = \alpha e^{-(\nu_n l + \alpha b)} - \nu_n e^{-(\nu_n b + \alpha l)}$$
$$\gamma_n \Delta_n = -\alpha e^{(\nu_n l - \alpha b)} - \nu_n e^{(\nu_n b - \alpha l)}$$
$$\Delta_n = 2\nu_n \cosh\left(\nu_n b - \nu_n l\right)$$

where $\nu_n = (D_n \tau_n)^{-\frac{1}{2}} = L_n^{-1}$, q is the electronic charge; R, the reflectivity of the surface; α, the absorption coefficient of the incident light; s, the surface combination velocity; and L_p and L_n, the diffusion lengths of holes and electrons.

The collection efficiency of the junction, denoted by Q, is then given in terms of hole and electron current as

$$Q = \frac{I_p}{I_{\max.}} + \frac{I_n}{I_{\max.}} = Q_p + Q_n \qquad ...(11)$$

where

$$I_{\max.} = qN_0(1-R) \qquad ...(12)$$

Plotting the above results with arbitrary values of the constants, as are applicable to the semiconductors, typical plots of Q_p versus α with νl as a parameter are shown in Figs. 13 and 14. From

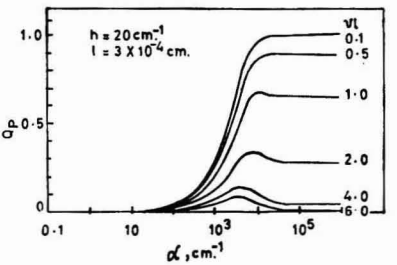

Fig. 13 — Q_p versus α for different values of νl (shallow junction)[17]

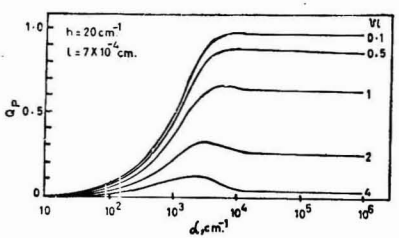

Fig. 14 — Q_p versus α for different values of νl (deep junction)[17]

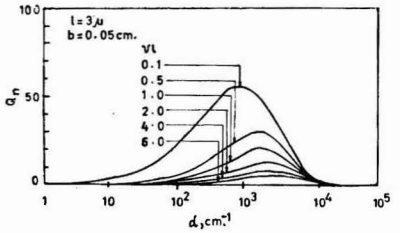

Fig. 15 — Collection efficiency of the rear part of the junction versus α for different values of νl[17]

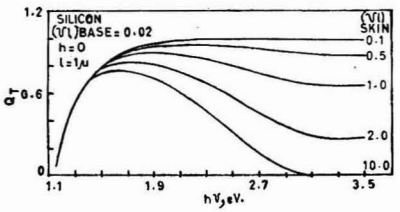

Fig. 16 — Total collection efficiency Q_T versus $h\nu$ for silicon[17]

a comparison of the two curves it can be seen that in the case specified in Fig. 14, the hole collection efficiency of the diffused skin of junction starts rising at lower values of α than it does for the shallow junction of Fig. 13. Thus infrared radiation is more efficiently used by deep junctions.

The contribution from the base of the junction, i.e. from the region $l<x<b$, is shown in Fig. 15. In most efficient solar cells a major contribution to Q is from this region.

The plot of $Q_{\text{total}} = (Q_n+Q_p)$ versus $h\nu$ of incident radiation in silicon, for different values of νl, is presented in Fig. 16. For shorter wavelengths, the collection efficiency of the junction increases for shallow junctions. Thus it is clear that to have an efficient solar cell in the blue region of the spectrum, we must have a very shallow junction. Very recently very shallow junction cells[18] with high conversion efficiency in the blue wavelength region have been developed. These have a junction depth of about 0·3 μ.

Effect of intensity of illumination on the conversion efficiency — Increase in the intensity of illumination of the cell results in a linear increase in the number of optically generated electron-hole pairs and, hence, the light generated current I_L also increases linearly with the intensity of incident light. For maintaining the same value of conversion efficiency of the cell for increased incident flux, it is essential to have an effective collection of charge carriers, to prevent them from recombining before they are separated. So a cell designed for a lower incident flux will show a lower conversion efficiency for higher flux values. Cells designed for higher light intensities show higher conversion efficiencies at higher illumination levels. Another factor contributing to the decrease in the efficiency of the cell, as a result of increased intensity of illumination, is the increase in the cell temperature. For very high intensities it becomes essential to cool the cell to maintain good conversion efficiencies.

Improvements in the Performance of SSC

The theoretical and experimental efficiency of SSC has been discussed by Shockley and Queisser[19]. The maximum efficiency is found to be 30 per cent for an energy gap 1·1 eV., assuming that the total recombination is radiative and that the source of light and the cell are black bodies at 6000° and 300°K. respectively. Silicon is element 14 in the periodic table and has a certain rate of unavoidable nonradiative transitions[20]. As actual junctions do not obey the predicted current-voltage relationship, it is not possible to reach this theoretical limit of conversion efficiency. However, it seems possible to increase the efficiency of the SSC further to approach the limit set by the material and junction characteristics.

Starting material — To obtain the best solar cell performance, it is essential to choose a good quality starting material with (i) good junction characteristics, (ii) high base region minority carrier lifetime and (iii) large diffusion length. Good starting material permits the fabrication of broad spectral response, extremely shallow junction, high efficiency cells with good yields.

Solar cells have been fabricated from 1 ohm-cm. *p* type boron-doped and *n* type phosphorus-doped material for quite some time. The characteristics of the solar cells made from these two materials are compared in Table 2. It is seen that the 1 ohm-cm. *n* type material is superior to the 1 ohm-cm. *p* type silicon used for the fabrication of solar cells. This relation between the two types was reversed after the bombardment of these cells with high energy particles[21]. The *p* type 1 ohm-cm. silicon was more radiation resistant than *n* type 1 ohm-cm. material. Better junction characteristics and improvement in long wavelength response for the 10 ohm-cm. *p* type over the 1 ohm-cm. *p* type boron-doped silicon were reported by Mandelkorn *et al.*[22]. Mandelkorn[23] reported that the solar cells made from 10 ohm-cm. aluminium-doped oxygen-free silicon were better than those fabricated from 10 ohm-cm. boron-doped material for the radiation properties. The characteristics of the radiation damaged cells made from aluminium-doped silicon improve on annealing, whereas in the case of cells made from boron-doped silicon they deteriorate further. Due to this quality the 10 ohm-cm.

TABLE 2 — TYPICAL CHARACTERISTICS OF SSC

	n on *p*		*p* on *n*	
	Typical	Best	Typical	Best
Open circuit voltage (V_{oc}), V.	0·57	0·595	0·585	0·615
Short circuit current* (I_s), ma.	57	61	63	68
Curve power factor (CPF), %	68	70	72	75
Efficiency, %	9	10	10·5	12

*Equivalent outer space current.

aluminium-doped oxygen-free silicon is ideal for cells employed for outer space applications. However, for terrestrial uses 1 ohm-cm. *n* type silicon is the most appropriate material.

Contacts — Around 1960 it was discovered that the $\frac{1}{2}\times 2$ cm.^{2} silicon solar cells had about 20 per cent higher conversion efficiencies than those obtained in the 1×2 cm.^{2} cells[15]. The contact on the active surface of the cell used to be a line contact parallel to the longer edge. For such a contact geometry, there is a great probability of recombination for the charge carriers at larger distances from the contact. Better collection efficiencies for smaller mean free path of the charge carriers was obtained by having more interconnected contacts (grids) on the active surface of the cell. For such contact geometries, larger values of sheet resistance can be tolerated. The efficiency of the cell for such gridded structures depends on the width of the contact lines and the separation between them. For a particular illumination level, it is maximum for a critical width of and separation between the grid lines. Wider grid lines would have smaller contribution to series resistance, but would reduce the active area available to the incident light, resulting in a reduction of the output power available from the cell. Narrower grid lines would increase the contact resistance and hence the series resistance of the cell, effectively reducing its useful output power. Similarly, closely spaced grid lines would reduce the active area of the cell. If the separation is too much, charge carriers would be lost by recombination, reducing the output power again. In practice, a compromise is made between these factors and an optimum spacing S and width T of the grid lines are calculated. Knowing the terminal voltage V near the maximum power point and the other data of the solar cell without grid structure, the values of S and T for the grid structure of Fig. 17 can be calculated from equations[15]

$$T = 2^{5/4}\frac{\rho_T^{3/4}}{\rho_p^{1/2}}(BCj_0e^{BV})^{\frac{1}{4}}W^{3/2} \qquad ...(13)$$

$$S = \sqrt[3]{\frac{2T}{BC\rho_p j_0 e^{BV}}} - \frac{2T}{3} \qquad ...(14)$$

where $B = q/AkT$, $C=1-(I_0/I_L)\,(e^{BV}-1)$, ρ_T is the sheet resistance of the contact strip; ρ_p, the sheet resistance of the diffused layer; and j_0, the saturation current density.

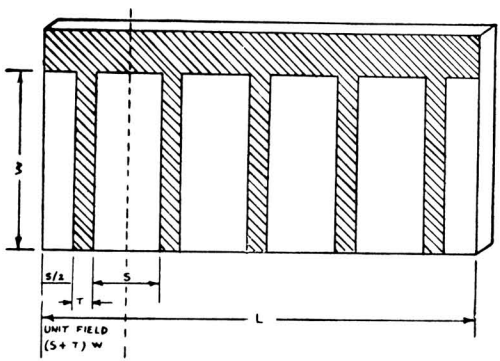

Fig. 17 — Configuration of a contact grid structure[15]

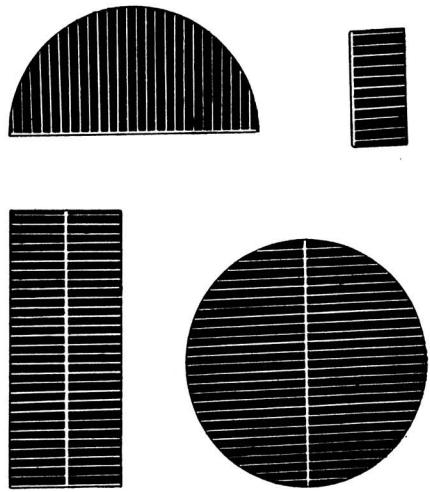

Fig. 18 — Typical grid structures for different solar cell geometries[28]

It is apparent from Eqs. (13) and (14) that for higher light intensities the width of the grid lines should increase and their separation should decrease. Typical grid structures on various cell geometries are shown in Fig. 18.

Good solar cells have highly polished surfaces and so the plated contacts made by electroless deposition of nickel or vacuum evaporation of gold do not adhere properly to the cell. To improve the adherence of these contacts, these are sintered to form an alloy with silicon. During sintering, the junction is penetrated, seriously degrading its characteristics. To avoid this, silver-titanium process was developed at the Bell Telephone Laboratories[4]. In this process, titanium is the reactive element and is applied to the silicon surface as grid structure of required geometry in vacuum. It is covered with a silver coating to protect it from oxidation. The silicon slices are then sintered. This leads to a low resistance contact without forming any liquid alloy and without any damage to the *p-n* junction. These contacts can be used for both front and back surface of the cell. However, the characteristics of extremely shallow junctions used for super blue cells are degraded by using the silver-titanium contacts also. It is now known to be due to the 'killer impurities' in titanium. Titanium has been replaced by cerium to overcome this difficulty. Such contacts have better electrical and mechanical characteristics. The contact characteristics are compared in Table 3.

Coatings and cover glass slides — For good silicon solar cells the slice is highly polished and, as a result, about 31 per cent of the incident light is reflected from the surface of the cell[24] (Fig. 19). Antireflection coatings are used to reduce the reflection losses of useful light from the cell surface. The proper

index of reflection for the coating material η_1 can be determined from the relation[25] $\eta_1 = \sqrt{\eta_0 \eta_s}$ where η_0 and η_s are the indices of refraction of the material on either side of the antireflection coating. Pure silicon has an index of refraction between 3·4 and 4 in the visible range; so the optimum value of refractive index should be around 1·9. Silicon monoxide has the desired properties for use as antireflection coating and it serves excellently well on bare silicon cells. A single layer interference antireflection film of SiO is vacuum-evaporated on to the surface of the solar cell. The quality of the film is checked by looking at the interference colours in diffused light after suspending these cells for 30 min. in steam over boiling water. Adherence of the film to the cell is tested by the scotch-tape test. Typical curves showing the reflectivity of the bare cell and the silicon monoxide-coated cell[24] are presented in Fig. 19. The spectral response of a n/p solar cell before and after SiO coating[24] is shown in Fig. 20. The effect of the thickness of silicon monoxide coating on the short circuit current is evident from the data presented in Table 4. SiO-MgF_2 coatings have been used for super blue cells, because of their higher utility for shorter wavelengths. The performances of the two coatings are compared in Table 5.

Because of the negative temperature coefficient for power output of SSC it would be desirable to keep the temperature of the cell as low as possible. Bare cells have total emittance of about 0·32 at room temperature, whereas for borosilicate glasses it is 0·85. So, if such a borosilicate glass slide is kept in thermal contact with the cell, the effective emittance of the cell would increase appreciably and the temperature of the cell will not rise so high. It is evident from the data presented in Table 6 that cover glass on uncoated cell brings about a slight improvement in the performance of the cell; on coated cell the reduction of the short circuit current is less than 1 per cent.

TABLE 3 — CONTACTS ON SHALLOW JUNCTION CELLS[3]

Characteristic	Silver-titanium	Silver-cerium
Slope* (R_c), ohm	0·25	0·2
A (junction constant value)	3-5	1·2-1·4
Diode reverse current† (I_0), μa.	>100	<20
Open circuit voltage (V_{oc}), V.	<0·52	0·54
Break load, g.	500	1500

*Slope of forward biased diode voltage-current curve in 300-400 ma. region.
†Diode reverse current for 0·6 V. bias.

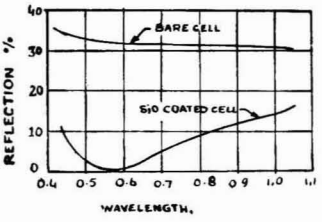

Fig. 19 — Effect of SiO antireflection coating on n/p solar cell reflection[24]

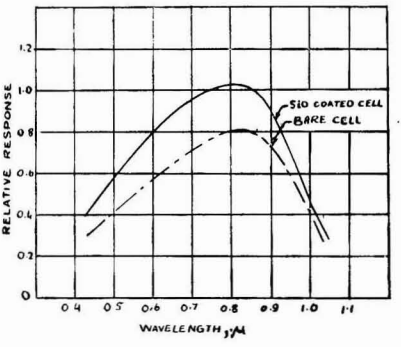

Fig. 20 — Typical spectral response of n/p solar cell before and after SiO antireflection coating[24]

TABLE 4 — DEPENDENCE OF COATING FACTOR* ON FILM THICKNESS[24]

(The antireflection coating was of SiO)

Antireflection coating thickness, A.	Antireflection coating colour	Coating factor %
Zero	Bare	100
1900	Pale blue	112
1700	Light blue	122
1600	Dark blue	122
1400	Dark purple	126
1300	Purple red	128
1200	Red yellow	127
1100	Yellow green	126

*Coating factor was calculated as $\frac{I_s \text{ coated}}{I_s \text{ noncoated}} \times 100$.

TABLE 5 — PERFORMANCE OF ANTIREFLECTION COATING IN SUPER BLUE CELLS[3]

Filter wavelength μ	Short circuit current* ma.			Coating factor for SiO %	Coating factor for SiO-MgF_2 %
	Non-coated	Coated with SiO	Coated with MgF_2-SiO		
0·95	4·0	5·0	4·96	125	124
0·9	8·7	11·1	10·9	128	125
0·8	9·5	12·5	12·4	132	130
0·7	9·2	13·1	12·5	142	136
0·6	7·1	10·2	10·1	144	143
0·5	4·4	5·8	6·95	132	158
0·45	3·4	3·8	5·5	112	162
0·4	3·8	3·5	6·77	92	178
Total	50·1	65·0	70·08		
Overall coating factor†				130	140

*Measured with filter wheel solar simulator (Mandelkorn, J., Broder, J. D. & Ulman, R. P., private communication).
†Coating factor (%) $= \frac{\text{coated cell current}}{\text{noncoated cell current}} \times 100$.

TABLE 6 — COATING FACTOR VALUES FOR DIFFERENT THICKNESSES[24]

(The values are in %)

Antireflection coating thickness, A.	Without cover glass	With cover glass
Bare	100	113
1700	125	122
1400	126	125
1200	124	123

For putting these glass covers epoxy resins are usually used. The resins become opaque on exposure to ultraviolet light. To avoid this, special reflective coatings were used to cut off these radiations[26,27]. Multilayer coatings have been developed to cut off the ultraviolet and infrared radiations and to improve the absorption of useful radiations[27]. A recent improvement of putting the glass cover avoids the use of epoxy resins. The cover glass is metallized in a pattern identical to the top contact pattern of the cell and is bonded to the metallized region of the cell by an appropriate type of solder to withstand temperature cycling. Metallized contacts provide paths for heat flow to the cover glass.

Concentrators — The intensity of illumination and hence the power output of SSC can be increased by using geometrical reflectors, known as concentrators. Light from a large area is concentrated on a small area SSC by using these concentrators[28]. A simple inexpensive system which could give a wide range of concentration ratios is a conical reflector concentrator shown in Fig. 21. It is economical and easy to manufacture from polished sheet metal or to mould from plastic. This design also gives a structurally stable shape, not easily damaged by handling or adverse weather conditions. For such reflectors the concentration ratio is a function of cone angle. The cone angle and the lengths of the sides are so adjusted that the light striking the uppermost portion of the conical collector always impinges upon the cell surface area after a single reflection only. The concentration ratio of a concentrator is determined by calculating the circular area corresponding to the top of the cone (the area intercepting sunlight) and dividing by the area of the target or cell surface. Concentration ratio is shown as a function of cone angle for 100 per cent reflection factors from the concentrator surface[28] in Fig. 22. The lower curve in Fig. 22 represents data obtained using a 50 per cent reflection factor. For 90° cone angle, the light reflected from the reflector does not strike the cell and a concentration ratio of unity is obtained. As the cone angle decreases, the concentration ratio increases. The maximum calculated length of the side of the cone, for which the incident light intercepted by the concentrator reaches the SSC after a single reflection, is shown in Fig. 22 as a function of cone angle (curve L).

These conical concentrators do not produce uniform intensity throughout the cell area, but give good distribution of light over the target area and localized hot spots are not produced. Inexpensive concentrators because of their distorted sides give better distribution of light on the cell area. It is more practical to use concentration ratios smaller than 5. Higher concentrations lead to excessive heating of the cell and are, therefore, advantageous only if external cooling is provided. With the use of the concentrator the light flux increases and it becomes essential to have wider grid fingers with smaller spacing between them as required by Eqs. (13) and (14).

Typical *V-I* characteristics of the cell, with and without a concentrator, obtained at the Indian Institute of Technology, New Delhi, are shown in Fig. 23. A power gain of 3·81 was obtained with a concentrator with cone angle 50°. This cone angle was preferred for two reasons: (i) with the concentration provided by this concentrator external cooling is not essential and (ii) it leads to a concentrator of moderate size. Smaller cone angles, however, give rise to too big concentrators as is apparent from Fig. 22

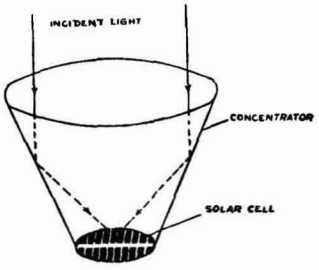

Fig. 21 — Conical reflector: schematic diagram

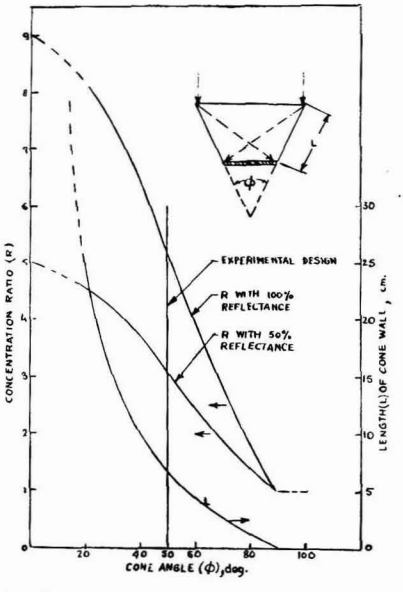

Fig. 22 — Concentration ratio and length of cone wall of the concentrator system versus cone angle[28]

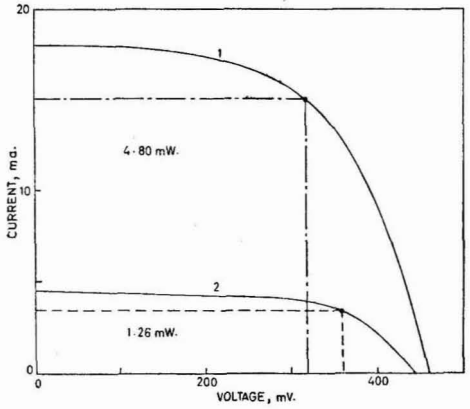

Fig. 23 — V-I characteristics with and without conical concentrator system [Cone factor for I_{sc} = 4·00; gain factor for output power = 3·81; 1, with concentrator; 2, without concentrator; and ●, maximum power point]

(curve L) and excessive heating becomes an additional problem.

Terrestrial Applications of Silicon Solar Cells

Silicon solar cells have found utility in light measuring instruments, large signal on-off applications and as power supplies for beacon towers, etc. The square shape of the V-I curve (Fig. 2) indicates that when SSC is used as a detector element, two types of operations, one into a high impedance load and the other into a low impedance load, should be possible.

For high load resistance, the voltage output approximates to V_{oc} and has logarithmic dependence on light intensity according to the equation

$$V_{oc} = \frac{kT}{q} \log \left(\frac{I_L}{I_0} + 1 \right) \qquad ...(15)$$

When the load impedance is lower than that for the maximum power output, the output current varies very slightly over a large range of impedance. Under these conditions the output current approximates to the short circuit current I_s and is directly proportional to the cell area and the light intensity. Because of extremely small dependence of short circuit current on temperature, the cell can be used satisfactorily with lower than optimum load impedance without any external cooling for very high light intensities.

When silicon solar cell, which is sensitive to wavelengths between 0·35 and 1·2 μ, is to be used as a detector of visible light only, some of its efficiency must be sacrificed by including a filter which cuts off infrared radiations. In these light measuring units[29] a wide range of meter movement design and SSC combinations are possible. Two cases, namely the linear and the logarithmic indication of light output, are more common. The design criterion involved is that for logarithmic indication the meter load should be at least 30 per cent greater than the matching load of the proposed cell and illumination combination. For linear indication, the meter load should not be greater than 70 per cent of the matching load.

For light proportional sensitive controls, as in automatic camera lens stop control[29], silicon solar cells are most useful because of their high efficiency. When SSC is used for switching, it has to distinguish between light and no-light or two different light fluxes incident on it. The output of the solar cell, when illuminated, performs some function which it would not perform in dark or at a lower light flux. For switching, the output from the cell operates a relay. The relay selected to operate with a particular SSC should have two important design specifications, namely the power P required to operate it and the relay coil resistance. If the relay is to be operated by one SSC without amplification of its output, the voltage required to operate the relay coil should not exceed 0·4 V. The remaining parameters of interest for the switching problem to specify the relay system are the level of illumination necessary to actuate the system and the cell area. It is always convenient to first have the level of illumination and the specifications of the available relay and then determine the cell area. For fast switching, small area solar cells are used. In such cases, a choice of the proper relay becomes more important. For this purpose, due to a very small power output available from the cell, it becomes very difficult to actuate the normally available inexpensive relays. In cases where power output from the cell is not enough to operate the relay, a transistor amplifying stage can be used. A typical amplifier circuit used for switching is shown in Fig. 24. R_1, R_2 and R_3 are adjusted to bias the transistor correctly in terms of the current available from the cell. Systems employing such SSC-operated relays have been extensively used in large signal on-off applications. A safety interlock using SSC can be achieved in hazardous machinery to protect the operators.

A photoelectric counter can be made by arranging a source and a photocell on the opposite sides of a conveyor belt. The articles coming along the conveyor momentarily cut the light and cause the counter to register. The same principle can be used for counting vehicles in traffic study, burglar alarms, smoke alarms, turning on-off street lights or beacon lights, opening of doors on approach of persons, etc. Typical uses to which the solar cells can be put are the reading of information from punched tapes, in teletypewriter encoding systems and for automatic control systems in industry.

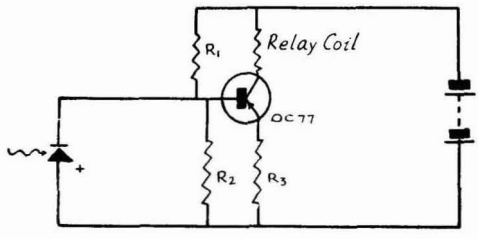

Fig. 24 — Solar cell transistor relay circuit[29]

Silicon solar cells are also used to convert sunlight into useful electrical power. For this purpose, these cells are assembled as small power packs and used for portable radio receivers, particularly the transistor variety, where the supply voltage can be kept small. These batteries are also useful for remote weather telemetering stations in inaccessible locations, or in telephone and telegraph repeaters located at sites where power lines are more expensive or as source of power supplies for beacon towers, etc. In cases where continuous operation is desirable, such as beacon towers, local storage batteries (nickel-cadmium or silver-cadmium) are employed. These batteries are charged during sunlight hours and supply power during nights or cloudy days.

It is evident from the above that considerable advances have been made in the fabrication of SSC at the Indian Institute of Technology, New Delhi. Several cells fabricated have been supplied to research workers in national laboratories, universities and other government departments. The limiting factor in developing this work further continues to be the lack of indigenous sources of semiconductor grade silicon material.

Summary

The recent advances in the technology of fabricating silicon solar cells and their various terrestrial applications are reviewed. Improvements in the cell conversion efficiency by optimizing the starting material, techniques of junction formation, cell thickness, series resistance (through improved contacts), depth of the diffused layer, spectral response and by reducing reflection losses (using special thin film coatings) are discussed in detail. A concentrator system for concentrating the incident flux on the cell surface to obtain more power from a single cell is described.

Acknowledgement

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Carotenoids

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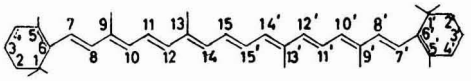
A NUMBER of excellent monographs and reviews have appeared on carotenoids. The present review is restricted to a survey of the recent advances on some specific aspects.

Pigments, due to their bright colours, attracted the attention of organic chemists quite early. Carotenoids are the most widely distributed pigments, in both plant and animal kingdom. They are present in almost all species, from the simplest unicellular protozoa to the highly organized multicellular mammals.

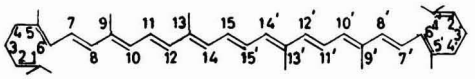
The first yellow pigment to be isolated in a crystalline form was carotene[1]; the composition of the carotene from carrot was determined by Willstatter and Mieg[2]. The relationship between the fat-soluble A factor[3] and carotene was suggested by Steenbock[4]. Karrer *et al.*[5] suggested similarity between the structures of β-carotene and vitamin A.

The first classification of carotenoids was proposed by Thudichum[6], who grouped the yellow pigments found in many tissues of both vegetable and animal origin under the name 'luteine' or 'luteins'. He suggested the name luteine for the reason that the characteristic yellow pigment of the corpus luteum on the ovaries of mammals, especially that of the cow, is one of the representatives of the 'luteine' pigments[6]. Later, terms like lipochrome, lipoxanthin and chromolipids were proposed to include a large number of plant and animal pigments. Tswett[7] suggested the name carotenoids for the various chromolipids which are chemically and genetically related to carotene. A further subclassification of the carotenoids into hydrocarbons and oxyhydrocarbons led Tswett to designate them as carotenes and xanthophylls respectively.

A number of definitions have been given for the carotenoid group of compounds in the past. The following is the latest definition of carotenoids given by the Commission on the Nomenclature of Biological Chemistry of the International Union of the Pure and Applied Chemistry[8]: "The carotenoids are chemical compounds of aliphatic or aliphatic-alicyclic structure, composed of partly dehydrogenated isoprene groups (3 or 4 to 8 or more). These groups are formed into a chain, in such a way that the alternate single and double bonds (conjugated double bonds) form the chromophoric system; these occur in such a way that in most of the carotenoids, two methyl side chains situated at the centre of the molecule or near the centre of the molecule are separated by five carbon atoms." The numbering of the carbon atoms of carotenoids (e.g. β-carotene with two β-ionone rings and lycopene with both β-ionone rings open) as suggested by the Commission, is as follows:



β-Carotene



Lycopene

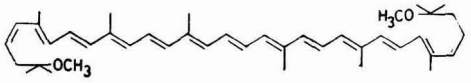
In the present review, the specific names of carotenoids, e.g. cryptoxanthin for β-carotene-3-ol and violaxanthin for β-carotene-5,6,5',6'-diepoxy-3,3'-diol have been retained and the terms 'oxygenated carotenoids' for epoxy-, keto- and the hydroxy-carotenoids, and 'xanthophylls' to designate the hydroxy carotenes and their epoxides only and not the epoxy- or keto-carotenes have been used. The carotenoids, like the steroids, plant terpenes and rubber, can be classified chemically under the name isoprenoid substances.

Approximately 100 carotenoids have been either isolated or synthesized and their structures elucidated either completely or partially. Generally, the carotenoids consist of 40 carbon atoms. A number of methods for elucidating the constitution and structure of a large number of carotenoids are given in literature[9].

Some of the carotenoids whose isolation and characterization have been carried out in recent years are mentioned in brief below.

Spirilloxanthin

Spirilloxanthin was first isolated from cultures of *R. rubrum* and from mixed cultures of *Rhodovibro* species[10]. Karrer and Koening[11] proposed that spirilloxanthin is 3,3'-dimethoxy carotenoid, but recently Jensen[12] showed that spirilloxanthin is 1,1'-dimethoxy carotenoid and established the structure of spirilloxanthin as



Capsanthin and Capsorubin

The occurrence of these pigments in paprika, *Capsicum annum*, is well known. The structures for these carotenoids were proposed by Barber *et al.*[13] and Faigel and Karrer[14] on the basis of studies on nuclear magnetic resonance spectra and confirmed by total synthesis[15].

These carotenoids constitute a new class of carotenoids containing a five-membered ring instead of a six-membered ring.

α- and β-Zeacarotenes

Petzold *et al.*[16] reported the presence of α- and β-zeacarotenes in yellow corn and showed that β-zeacarotene possesses vitamin A activity, whereas α-zeacarotene does not. It has been suggested that α-zeacarotene is 7′,8′-dihydro-δ-carotene and β-zeacarotene is 7′,8′-dihydro-γ-carotene.

Neoxanthin

Neoxanthin is one of the major carotenoids of the leaves and was isolated first by Strain[17]. The structure of neoxanthin has been proposed by Goldsmith and Krinsky[18] and confirmed recently by Curl[19].

Phoenicoxanthin, Phoeniconone and Phoenicopterene

The presence of phoenicoxanthin in marine fish species, *Phoenicoparrus andius*, *Phoenicoparrus jamesi* and phoenicopterene, and 4-keto-α-carotene in the plasma of *Phoenicopterus ruber* was reported by Fox and Hopkins[20]. Phoenicoxanthin can be converted to phoeniconone, 3-keto-canthaxanthin, by alkali-catalysed air oxidation. Fox and Hopkins[20] proposed the structure of phoenicoxanthin as 3-hydroxy canthaxanthin.

Distribution

The organisms which synthesize carotenoids can be divided into two groups: photosynthetic and nonphotosynthetic. The photosynthetic organisms, including higher plants, algae and purple bacteria synthesize carotenoids, which are incorporated into the photosynthetic apparatus together with the chlorophylls in the grana or the lamella of the chloroplasts or in the chromophores. The pigments found in the photosynthetic apparatus are remarkably distinct for each major group of photosynthetic organisms, though the photosynthetic bacteria appear to show some fundamental diversity[21].

Carotenoids are also present in the nonphotosynthetic tissues, e.g. petals, fruits, etc., of higher plants. The distribution and presence of carotenoids in the nonphotosynthetic tissue vary with a single genus or species. Details about the distribution of carotenoids in plants, micro-organisms and animals have been provided by Karrer and Jucker[9], Goodwin[22], Jensen[23] and Dougherty and Allen[24].

The fat-soluble nature of carotenoids had led some workers to suggest that they usually occur as oily droplets in the cell lipoids or in admixture with solid or semi-solid fats. While this is qualitatively true, there are evidences to suggest that the carotenoids exist in combination with proteins in the tissues.

To get an insight into the mechanism of photosynthesis, studies have been made on the photoreceptors of photosynthetic organisms and their behaviour compared with that of animal photoreceptors, e.g. the retinal rods or cones in vertebrates[25-27]. Depending on their shape and size, photoreceptors are known as chromatophores, grana, megaplasts and chloroplasts. The chloroplasts contain proteins (including cytochrome *f* and b_3), lipids, inorganic material, RNA, DNA, chlorophyll (8 per cent) and carotenoids (2 per cent). The carotenoids present in the chloroplasts are lutein, β-carotene and zeaxanthin. Red and blue algae contain, in addition to the above pigments, other photosynthetic pigments like phycoerythrin and phycocyanin. A simplified molecular model of the chloroplasts has been proposed by Wolken[27] on the basis of electron microscopic measurements and other studies. According to this model, the carotenoid molecules are spaced at the interstitial position between the chlorophyll molecules in a monolayer and for every one molecule of carotenoid, at least three molecules of chlorophyll are present in a network. A similar model in which the porphyrin head lies at an angle of 45° and one protein layer contains carbon dioxide reducing enzyme and the other oxygen evolving enzyme has been proposed by Calvin[28].

Isolation, Separation, Identification and Estimation of Individual Carotenoids

Isolation

The plant material (pulp, peel, flowers, etc.) or micro-organisms are immersed in ethanol for 3 days at room temperature. The ethanolic extract is filtered off and the plant residues or micro-organisms are blended in a Waring blendor successively with ethanol, acetone and petroleum ether (b.p. 40-60°)-diethyl ether (1 : 1, vol./vol.). The filtered extracts are combined and washed with water until free of acetone and ethanol. The remaining organic layer containing the carotenoids is dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The oil obtained after concentration is dissolved in ethanol and saponified with alcoholic KOH (10 per cent, wt/vol., 15 ml. for 1 g. of oil) at 60° for 30 min., and then left overnight at room temperature. The unsaponifiable material is extracted with petroleum ether and diethyl ether and washed thoroughly with distilled water to remove alkali, then dried over anhydrous sodium sulphate and concentrated to a volume of about 20 ml. under reduced pressure.

The concentrated solution of the unsaponifiable matter in petroleum ether is left overnight under nitrogen at −20°; the precipitated sterols are removed by filtration at −20°. By repeating this process a number of times, most of the sterols can be removed.

Animal tissues—The carotenoids can be extracted according to the procedure of Glover *et al.*[29]. The method consists in grinding the tissue with anhydrous sodium sulphate, followed by extraction with diethyl ether at 45°, filtration, reduction of the volume under reduced pressure and chromatography.

Separation and Identification

Physical methods — Chromatography: The carotenoids are resolved from the mixture of carotenoids into individual pigments by chromatography on magnesium oxide-celite (1 : 1 and 3 : 2, wt/wt) and water-deactivated (5, 3, 2, 1 per cent, vol./wt) alumina

columns. After separation and collection of individual components, the carotenoids can be further purified by rechromatography on the same or different chromatographic systems[9]. Depending on the position of the bands on the column, the various carotenoids can be tentatively identified[9,22]. However, the relative positions are not absolute and depend upon the type of adsorbent and the solvent system.

Tsukida and Zechmeister[30], making use of lime-celite columns and employing hexane/acetone as developing solvents, observed that the sequence of adsorption of β-carotene, β-carotene monoepoxide and β-carotene diepoxide was reverse of that reported by Karrer and Jucker[31] for calcium hydroxide columns.

The chromatographic adsorption behaviour of β-carotene, its epoxides, di- and polyhydroxy xanthophylls and their epoxides on alumina and magnesium oxide-celite columns was also studied by us[32] (Table 1).

From Table 1, it is evident that the adsorption affinity of carotenoids on an alumina column, using light petroleum containing varying amounts of ether and acetone as the developing solvents, increases with increase in the hypophasic character of the substance. However, no such relationship was manifested between the hypophasic character of carotenoids and their adsorption affinity on magnesium oxide-celite columns using light petroleum containing varying amounts of acetone as the developing solvents.

We have shown that magnesium oxide-celite column serves as a reverse-phase chromatographic column to alumina[32].

Carotenoids were identified by mixing them with authentic samples followed by co-chromatography on suitable adsorbents.

An ingenious method of separating minute amounts of carotenoids by thin layer chromatography was reported by Stahl[33]. Using different solvent systems and adsorbents, the separation was achieved by Bollinger[34].

Absorption spectra: Carotenoids having 7-12 double bonds exhibit a three-banded spectrum in the visible region. The absorption maximum is related to some extent to the constitution of the carotenoids. The relationship between the absorption maximum and the constitution of carotenoids has been discussed by Karrer and Jucker[9].

The absorption maximum depends on the *cis-trans* configuration[35] and on the solvent used. Absorption spectra in solvents like hexane, light petroleum, ethanol, chloroform and carbon disulphide were studied by a number of workers for the identification of carotenoids.

Melting points: Melting points and mixed melting points with authentic samples can be used for the identification of carotenoids

Partition ratios: The quantitative determination of partition ratios of about 35 carotenoids using hexane-95 per cent methanol and hexane-85 per cent methanol was carried out by Petracek and Zechmeister[36]. The method of determining partition ratios is essentially as follows: hexane and methanol are first saturated with each other. The sample is usually dissolved in the more effective equilibrated solvent in a concentration, so that direct reading at $\lambda_{max.}$ of absorbance A_1 could be done. The pigment is allowed to equilibrate between the two solvents by gentle shaking. The absorbance A_2 is then taken again in the phase used for the dissolution of the sample. The percentage of epiphasic or hypophasic character is given by $(A_2/A_1) \times 100$.

The partition ratios of a number of algae and bacterial carotenoids were determined by Goldsmith and Krinsky[18], Krinsky and Goldsmith[37], Jensen[38-40] and Nakayama[41]. Krinsky[42] defined the M_{50} value of a carotenoid as the percentage concentration of methanol at which the partition ratio of the carotenoid in question is 50 : 50. He reported M_{50} values of a number of carotenoids.

The partition ratios and M_{50} values of a number of carotenoids have been reported by Subbarayan *et al.*[32] (Table 2). The method of partition ratio determination is very useful in the identification of unknown carotenoids.

Cis-trans isomerization: The catalytic action of iodine on carotenoid solutions at room temperature and in the presence of light is the most frequently used method for ascertaining the *cis-trans* configuration of the carotenoids[43,44]. The test involves adding 0·05 ml. of 0·001 per cent iodine solution in hexane to 10 ml. of the purified carotenoid in hexane in a test tube and exposing to light from an incandescent lamp (100 W.) for 5, 10, 15 and 30 min. The ultraviolet and visible spectra are recorded before and after exposure to light (iodine is removed by the addition of sodium thiosulphate and filtration). After isomerization, a *cis*-carotenoid will absorb at slightly longer $\lambda_{max.}$, whereas a *trans*-carotenoid will absorb at shorter $\lambda_{max.}$.

Countercurrent distribution is also used in the separation and identification of carotenoids[45-47].

Chemical methods — The various chemical reactions like $SbCl_3$ colour test with conc. H_2SO_4, conc. HCl,

TABLE 1 — CHROMATOGRAPHIC BEHAVIOUR OF CAROTENOID PIGMENTS

(The pigments are arranged in the descending order of adsorption)

Alumina column, deactivated either with water or methanol	Magnesium oxide-celite column
β-Carotene	β-Carotene diepoxide
β-Carotene monoepoxide	β-Carotene monoepoxide
Mutatochrome	β-Carotene
β-Carotene diepoxide	Luteochrome
Luteochrome	Mutatochrome
Aurochrome	Aurochrome
Lutein	5,6-Monoepoxylutein
Zeaxanthin	Violaxanthin
5,6-Monoepoxylutein	Lutein
Chrysanthemaxanthin	Antheraxanthin
Flavoxanthin	Zeaxanthin
Antheraxanthin	Neoxanthin
Mutatoxanthin	Chrysanthemaxanthin
Violaxanthin	Flavoxanthin
Luteoxanthin	Lutcoxanthin
Auroxanthin	Mutatoxanthin
Neoxanthin	Auroxanthin

TABLE 2 — PARTITION RATIOS AND M_{50} VALUES OF CAROTENOIDS IN HEXANE AND METHANOL

Carotenoid	Functional group	Partition ratio*					M_{50} value (ref. 32)	M_{50} value reported by Krinsky[42]
		H:95%M	H:85%M	H:80%M	H:75%M	H:70%M		
Cryptoxanthin	One hydroxyl	86:14†	100:0	—	—	—	—	106·0
Cryptoflavin	One hydroxyl, one 5,8-epoxy	76:24	100:0	—	—	—	—	—
Rubixanthin	One hydroxyl	82:18	100:0	—	—	—	—	—
Lutein	Two hydroxyl	12:88†	44:56†	69:31	91:9	100:0	83·5	82·3
Zeaxanthin	Two hydroxyl	11:89†	40:60†	66:34	87:13	98:2	82·5	80·8
5,6-Monoepoxy-lutein	Two hydroxyl, one 5,6-epoxy	—	24:76	—	—	90:10	79·0	—
Chrysanthema-xanthin	Two hydroxyl, one 5,8-epoxy	8:92	21:79	50:50	72:28	90:10	80·0	—
Antheraxanthin	Two hydroxyl, one 5,6-epoxy	5:95	14:86	28:72	63:37	80:20	76·5	72·6
Mutatoxanthin	Two hydroxyl, one 5,8-epoxy	5:95	14:86	22:78	32:68	46:54	69·0	73·4
Violaxanthin	Two hydroxyl, two 5,6-epoxy	3:97	12:88	21:79	32:68	40:60	65·0	66·2
Luteoxanthin	Two hydroxyl, one 5,6-epoxy, one 5,8-epoxy	2:98	12:88	17:83	27:73	38:62	63·5	64·3
Auroxanthin	Two hydroxyl, two 5,8-epoxy	0:100	8:92	14:86	25:75	32:68	56·0	66·7
Neoxanthin	Three hydroxyl, one 5,6-epoxy	0:100	0:100	5:95	9:91	12:88	45·0	46·2
Astacin	Four keto	27:73	50:50	80:20	100:0	100:0	85·0	—

H, hexane; M, methane.
*The accuracy of the method is of the order of ±2·0 per cent.
†The values reported by Petracek and Zechmeister[36] are nearly the same as these.

boron trifluoride and N-bromosuccinimide have been discussed by Goodwin[43]. The following are the two other important chemical methods for the identification of carotenoids.

Reaction with conc. HCl: Carotenoids having epoxy groups give faint blue colouration on treatment with conc. HCl. This test can be performed by adding conc. HCl to an ethanol solution of a carotenoid[48].

Isomerization of a 5,6-epoxide to 5,8-(furanoid) epoxide can be achieved by adding a drop of ethanolic HCl. A shift of 15-20 mμ in the absorption maximum towards shorter wavelengths indicates the conversion of a 5,6-epoxide to 5,8-epoxide.

Reaction of epoxy carotenoids with mercuric chloride: Yamamoto *et al.*[49] reported the formation of a blue-green complex with a broad absorption maximum in the region 600-700 mμ, when a dry epoxy carotenoid is heated with dry mercuric chloride. They[49] prepared carotenoid-mercuric chloride complexes of violaxanthin, auroxanthin and neoxanthin and recorded the absorption maxima in acetone.

Mercuric chloride complexes of 14 epoxy carotenoids were prepared by us[32] according to the method of Yamamoto *et al.*[49]. In this method, the solvent-free dry epoxy-pigment is mixed with dry powdered mercuric chloride in approximately 1-5 weight ratio and heated on a boiling water-bath in a sealed tube for 1 min. The absorption spectra are recorded after dissolving the epoxy carotenoid-mercuric chloride complex in acetone. The results of the observations are presented in Table 3.

TABLE 3 — ABSORPTION MAXIMA OF MERCURIC CHLORIDE COMPLEXES WITH EPOXY CAROTENOIDS

Epoxy carotenoid	$\lambda_{max.}$ of the complex (in acetone), mμ
5,6-Monoepoxy-β-carotene	680-85
Mutatochrome	680-85
5,6,5′,6′-Diepoxy-β-carotene	655-60
Luteochrome	655-60
Aurochrome	655-60
Cryptoxanthin-5,6-monoepoxide	675-80
Cryptoflavin	675-80
Antheraxanthin	690
Mutatoxanthin	690
Chrysanthemaxanthin	680
Violaxanthin*	635
Luteoxanthin	635
Auroxanthin*	635
Neoxanthin*	655

*Values reported by Yamamoto *et al.*[49] were identical.

The mercuric chloride-epoxy carotenoid complexes in acetone are blue except for antheraxanthin, mutatoxanthin and chrysanthemaxanthin, which are blue-green.

The absorption maxima of 5,6- and 5,8-epoxy carotenoid-mercuric chloride complexes are identical. The formation of coloured mercuric chloride complexes with specific absorption maxima is a characteristic property of the 5,6- or 5,8-epoxy carotenoids as none of the other carotenoids forms complexes

with mercuric chloride and can be used for the identification of a number of epoxy carotenoids.

Estimations: Total carotenoids can be determined by dissolving the unsaponifiable portion in a known volume of light petroleum and measuring the E value at 445 mμ, assuming $E^{1\%}_{1\,cm.}$ for the crude extract to be the same as for β-carotene (2500) (ref. 43). Similarly, the concentration of the individual carotenoids can be determined by measuring $E_{max.}$ and comparing it with known $E^{1\%}_{1\,cm.}$ values at $\lambda_{max.}$ for pure pigments.

The $E^{1\%}_{1\,cm.}$ values for most of the known carotenoids have been given by Goodwin[43]. For unknown pigments, $E^{1\%}_{1\,cm.}$ at $\lambda_{max.}$ can be assumed to be the same as for β-carotene (2500) (ref. 43).

Chemical Synthesis of Carotenoids, Their Epoxides and Epoxides of Vitamin A

The total synthesis of β-carotene and lycopene was achieved simultaneously in the year 1950 by three group of workers[50-52]. Karrer and Eugster[52] synthesized α-carotene and ϵ_1-carotene. The synthesis of γ-carotene was reported by Garbers *et al.*[53]. Cryptoxanthin, zeaxanthin and isozeaxanthin and many other carotenoids were synthesized on an industrial scale by Isler *et al.*[54]. β-Apo-carotenals, the assumed intermediates in the conversion of β-carotene to vitamin A, were also synthesized. The total synthesis of the carotenoids of the C_{50} series, decapreno-β-carotene, and C_{60} series, dodecapreno-β-capreno-β-carotene was also achieved[55].

The synthesis of epoxides of β-carotene was first reported by Karrer and Jucker[31], and modified later by Tsukida and Zechmeister[30].

The conversion of β-carotene into carotenoids containing additional double bonds[56] was also achieved. A few reviews have appeared on the synthesis of carotenoids[57-60].

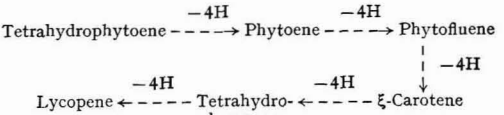
In 1965, Jungalwala and Cama[61] reported for the first time the synthesis and structure of 5,6-monoepoxyretinol, 5,6-monoepoxyretinyl acetate, 5,6-monoepoxyretinal and their corresponding 5,8-epoxy compounds.

Biosynthesis of Carotenoids

The biosynthesis of carotenoids in plants[62,63] and bacteria[23] has already been reviewed. Accordingly, in the present review only the salient points and some recent work on the biosynthesis of carotenoids are discussed.

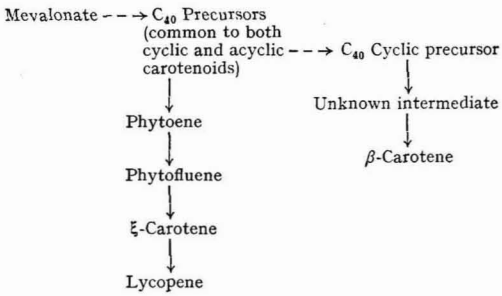
Braithwaite and Goodwin[64] showed that the biogenesis of the isoprenoid precursor of β-carotene is accomplished by the condensation of three moles of acetate with concomitant loss of one carboxyl group. The actual participation of acetoacetyl-CoA (C_4 unit), β-hydroxy-β-methyl glutaryl-CoA (HMG-CoA) and mevalonic acid in the biosynthesis of isopentyl pyrophosphate is now well known. It was shown by many workers[65-73] that mevalonic acid is the active isoprenoid compound in sterol and carotenoid biogenesis and the labelling pattern with labelled mevalonate in the isolated carotenoid was similar to that found in squalene. Varma and Chichester[74] showed that isopentenyl pyrophosphate and ^{14}C-geraniol are intermediates in carotenoid biosynthesis. ^{14}C-Farnesyl pyrophosphate[75-79], geranylgeranyl pyrophosphate-1-^{14}C (ref. 80) and ^{14}C-geranylgeraniol[74] were shown to be intermediates in the biosynthesis of carotenoids. It is proposed that the two C_{20} units, geranylgeranyl pyrophosphate and geranyl-linalool pyrophosphate, could condense to give the primary C_{40} unit (lycopersin) in analogy with squalene biosynthesis.

Depending on the distribution of carotenoids in tomatoes, Porter and Lincoln[81] were the first to propose the following biogenetic scheme for carotenoids:



The formation of other carotenoids involved ring closure and oxidation. However, at that time, the structures of most of the carotenoids were not clear.

The mechanism by which the ionone ring is formed in cyclic carotenoids is not yet understood. The following scheme for the formation of cyclic and acyclic carotenoids from C_{40} precursor is proposed by Goodwin[82]:

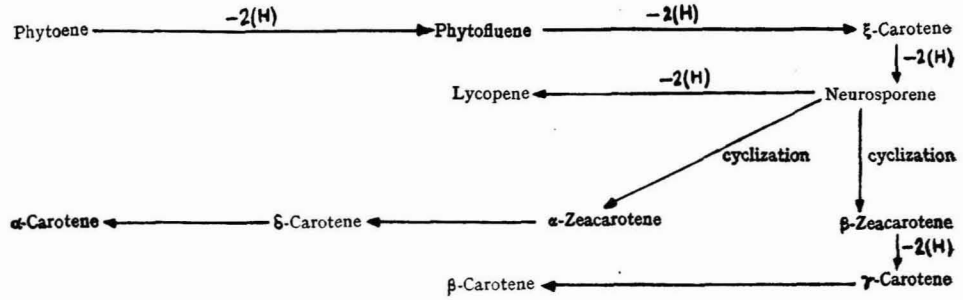


It is assumed that once the pathway branches, there is no interconversion. The formation of hydroxy and epoxy carotenoids directly from carotene hydrocarbons has been proposed by Glover and Redfearn[83] and Decker and Uehleke[84].

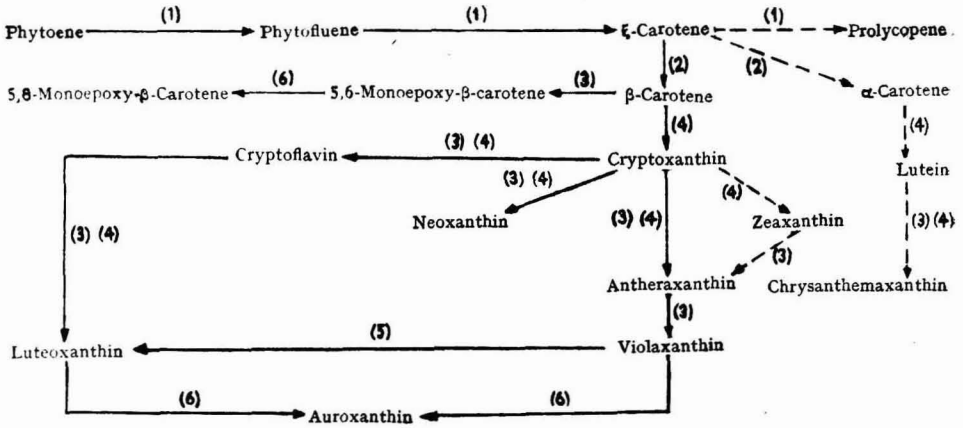
Jungalwala and Porter[85] established the structure of phytoene as 15,15′-*cis*-7,8,7′,8′,11,12,11′,12′-octahydrolycopene. The biosynthesis of phytoene from isopentenyl and farnesyl pyrophosphates by a partially purified tomato enzyme system has been reported recently by Jungalwala and Porter[86].

The biosynthesis of carotenoids in higher plants has been reviewed by Goodwin[87]. The formation of other carotenoids from phytoene[87] takes place according to Scheme 1. Xanthophylls are formed by the insertion of oxygen atom into either α-carotene or β-carotene at a later step.

Using stereospecifically labelled mevalonic acid with tritium at C-4 and C-5, Goodwin and Williams[88,89] elucidated the mechanism of ring closure to form α- and β-ionone rings in α-, β-, γ-, δ- and ε-carotenes. Williams *et al.*[90,91] postulated the mechanism of formation of the *retro* structure in carotenoids, such as eschscholtzxanthin. They[90,91]



Scheme 1



[⟶, main pathway; and - - →, minor pathway. The above scheme consists mainly of 6 steps: (1) dehydrogenation, (2) cyclization, (3) epoxidation, (4) hydroxylation, (5) partial furanization, and (6) complete furanization]

Scheme 2

also proposed that α-carotene may be converted to β-carotene and not vice versa and suggested that the α- and β-ionone rings of carotenes are synthesized separately from a common precursor.

Williams *et al.*[92], using stereospecifically labelled mevalonic acid, showed that sequential dehydrogenation from phytoene to β-carotene is stereospecific at each step.

Recently, the biosynthesis of cyclic carotenes using stereospecifically labelled mevalonic acid (^{14}C and ^{3}H) has been studied by Williams *et al.*[93]. They confirmed their earlier observations[91,92] that the α-ionone ring of α-carotene does not arise from the isomerization of β-ionone ring of β-carotene and the same is true for the α-ionone ring in δ- and ε-carotenes and α-zeacarotene. Similarly, the β-ionone ring in β-carotene does not arise by the isomerization of α-ionone ring of α-carotene. The α- and β-ionone rings are formed independently from the same carbonium ion intermediate[93].

Based on the distribution of carotenoids in the commonly occurring Indian fruits, papaya[94] and oranges (pulp and peel[95]), we tentatively proposed the biogenetic Scheme 2 for carotenoids in fruits starting from phytoene. The presence of significant amounts of zeaxanthin in the anthers of gul mohr (*Delonix regia*) flowers[96] indicates that Scheme 2 is valid for most of the fruits and may or may not be valid for flowers.

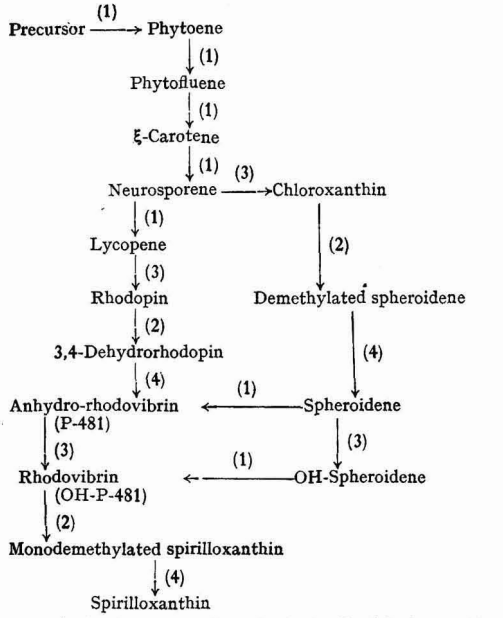
Based on structural considerations and the kinetics of appearance and disappearance of various carotenoids, Jensen *et al.*[97] and Jensen[23,98,99] put forward Scheme 3 for the biogenesis of carotenoids in purple bacteria.

In principle, a total of four enzymes might, therefore, be required to catalyse all the reactions in Scheme 3. But so far none of the enzymes mentioned has been isolated to confirm Scheme 3.

Functions of Carotenoids in Plants, Bacteria and Animals

Functions in plants and bacteria — The functions of carotenoids in photosynthesis, protection against photosensitivity, photophosphorylation, phototaxis, phototropism and reproduction were dealt with in detail by Goodwin[100]. Karrer *et al.*[101] and Cholnoky *et al.*[102] proposed a role for carotenoids in oxygen transport.

Recently, Krinsky[103] has suggested that the carotenoid pair, antheraxanthin-zeaxanthin, could fulfil the requirements of being 'chemical buffers' to protect cells against lethal photosensitized oxidations in chloroplasts.



[(1) dehydrogenation in the side chain, (2) dehydrogenation at C_3 and C_4 or C_3, and $C_{4'}$, (3) hydration, and (4) methylation]

Scheme 3

Functions in animals — Carotenoids are known to serve as precursors to vitamin A. Depending on their ability to give vitamin A, they are classified as biologically active and biologically inactive. The most important biologically active precursor of vitamin A known up till now is β-carotene. The chemistry and biochemistry of vitamin A and carotenoids have been discussed in detail by Moore[104]. Moore[105] was the first to show the conversion of β-carotene to vitamin A and proposed that the site for this conversion may be the liver. But Glover *et al.*[29,106], Mattson *et al.*[107] and Thompson *et al.*[108] showed that the small intestine is the site of formation of vitamin A from β-carotene. Two mechanisms — terminal oxidation and central fission — are proposed for the conversion of β-carotene to vitamin A.

Terminal oxidation: The biological potency of β-carotene in growth tests[109-111] indicates that β-carotene is only half as active as vitamin A on weight basis, showing the formation of only one mole of vitamin A from one mole of β-carotene. The biological activities of α- and γ-carotenes[112,113] also substantiated the hypothesis of terminal oxidation. Further support for terminal oxidation was provided by the fact that β-apo-8'-carotenal, the oxidation product of β-carotene has been found to be biologically active[114]. Festenstein[115] identified the presence of two apo-carotenals in the intestine of horse.

Again 16,16'-bis-homo-β-carotene is biologically active, although it does not possess a central double bond and the metabolism of cryptoxanthin, known to form vitamin A, has never led to the storage of the ' hydroxy-vitamin A ' corresponding to the inert half of the carotenoid. Pullman and Pullman[116] on theoretical grounds have favoured terminal oxidation.

The synthesis as well as the metabolism and biological potency of 5,6-monoepoxy-β-carotene and 5,6,5',6'-diepoxy-β-carotene were studied by us[117] to get an insight into the formation of 5,6-monoepoxy-vitamin A compounds *in vivo* and to throw some light on the mechanism of formation of vitamin A from β-carotene.

Attempts to synthesize 5,6,5',6'-diepoxy-β-carotene using 1·5 active oxygen atoms per mole of β-carotene[31] were unsuccessful[117]. Using 1 : 3 moles of β-carotene and monoperphthalic acid for 5,6-monoepoxy-β-carotene and 1 : 6 moles of β-carotene and monoperphthalic acid for 5,6,5',6'-diepoxy-β-carotene[30], the synthesis of these epoxides has been achieved. The absence of β-carotene after oral administration of either 5,6-monoepoxy-β-carotene or 5,6,5',6'-diepoxy-β-carotene to vitamin A deficient rats indicated that de-epoxidation of these epoxy carotenoids may not be likely. Time course studies indicated that the small intestine is the major, if not the only, site for the conversion of 5,6-monoepoxy-β-carotene to vitamin A. No 5,6-monoepoxy-vitamin A could be detected anywhere in the tissues after oral administration of either 5,6-monoepoxy-β-carotene or 5,6,5',6'-diepoxy-β-carotene. Only vitamin A could be detected after oral administration of 5,6-monoepoxy-β-carotene. 5,6-Monoepoxy-β-carotene has a biological potency 21 per cent that of all-*trans* β-carotene, whereas 5,6,5',6'-diepoxy-β-carotene has negligible biological potency.

From our results[117] it seems likely that the conversion of β-carotene to vitamin A may be from one of the β-ionone rings rather than at the central double bond. However, it should be emphasized that there may not necessarily be an analogy in β-carotene molecule compared to that in β-carotene-monoepoxide and β-carotene-diepoxide.

Central fission — Even though there are a few reports that β-carotene is only half as active as vitamin A on weight basis, Koehn[118] and Burns *et al.*[119] reported that β-carotene is as active as vitamin A on weight basis, i.e. two moles of vitamin A form one mole of β-carotene, indicating that the cleavage of the β-carotene molecule may be at the central double bond.

Olson[120], based on his studies on the conversion of radioactive β-carotene to vitamin A by the rat intestine, postulated that vitamin A is formed as a result of the central fission of the carotene molecule. The mechanism of conversion of β-carotene to vitamin A has been discussed in detail by Glover[121].

Using cell-free homogenates of rat intestinal mucosa, Goodman and Huang[122] demonstrated the conversion of β-carotene to vitamin A. They showed that virtually all the radioactive β-carotene that is not recovered unchanged was converted to retinol or retinal. The percentage of conversion of β-carotene to retinal[122] after 75 min. of incubation was only 30-50 per cent. From their results they proposed[122] that during the biosynthesis of vitamin A, β-carotene presumably reacts with molecular oxygen, followed by the cleavage of the central double bond to form two moles of retinal, and retinal is then

reduced to retinol, which is subsequently esterified, mainly with palmitic acid.

Olson and Hayaishi[123] found an enzyme in the supernatant solution of rat liver and intestine which converts β-carotene into retinal and retinol as its sole products. It was shown that oxygen is required for the reaction, and the intermediate product is retinal. The enzyme was named[123] tentatively as β-carotene 15,15′-oxygenase, which cleaves β-carotene molecule at the central double bond giving rise to two molecules of vitamin A. β-Apo-carotenols and carotenals were not detected by them[123] when β-carotene molecule was incubated with the enzyme.

Goodman *et al.*[124], using β-carotene uniformly labelled with ^{14}C throughout the molecule but specifically labelled with ^{3}H only at the central carbon atoms (C-15 and C-15′), showed that the biosynthesis of vitamin A from β-carotene is most likely a dioxygenase reaction (*in vitro*), in which molecular oxygen reacts with the two central carbon atoms of β-carotene, followed by cleavage of the central double bond of β-carotene to yield two molecules of retinal. The soluble enzyme, which converts β-carotene into two moles of retinal *in vitro*, from rat intestinal mucosa has been partially purified by precipitation with ammonium sulphate between 20 and 45 per cent saturation by Goodman and coworkers[124]. They also showed that in the absence of bile salt or synthetic detergent (sodium glycocholate, Tween-80) no enzyme activity to convert β-carotene to retinal could be seen. The enzymatic conversion of β-carotene to retinal is a dioxygenase type of reaction and the enzyme is inhibited by inhibitors of sulphydryl groups[124]. From these as well as our own observations[117], it may be correct to postulate that the cleavage of carotene hydrocarbons may occur at the central double bond, whereas in oxycarotenoids the cleavage may occur at the α- or β-ionone ring containing the epoxide group.

Crain *et al.*[125] showed that, when β-carotene is incubated with the soluble fraction of the intestinal mucosa, 90 per cent of the β-carotene degraded is found in the retinal fraction and the rest in retinol and the retinoic acid fraction. Incubating β-carotene with the soluble fraction of the intestinal mucosa in the presence of NADH, they[125] found 42 per cent of the degraded β-carotene in the retinoic acid fraction and 27 per cent in the retinal fraction. Thus they[125] proposed that the β-carotene molecule cleaves at the central double bond giving rise to retinal.

Very recently, McAnally and Szymanski[126] studied the metabolism of α-carotene and demonstrated the formation of α-vitamin A and vitamin A from α-carotene. Vitamin A and α-vitamin A formed after oral administration of α-carotene were stored in the liver. They proposed that these experiments neither support nor rule out the two proposed mechanisms of conversion of β-carotene to vitamin A.

Pro-3,4-*dehydroretinol from lutein* — Synthesis of 3,4-dehydro-α-carotene, 3,4-dehydro-β-carotene and 3,4,3′,4′-dehydro-β-carotene was achieved by Karmarkar and Zechmeister[127]. It was shown by them that 3,4-dehydro-β-carotene has a biological potency 15 per cent that of β-carotene when tested on rats. They proposed that 3,4-dehydro-β-carotene and 3,4,3′,4′-dehydro-β-carotene may be converted to 3,4-dehydroretinol.

Budowski *et al.*[128] isolated 3′-hydroxy-3,4-dehydro-β-carotene from acidulated soyabean soap stocks and identified the dehydro-β-carotene by chromatography, with a synthetic sample prepared by reaction of lutein with *p*-toluene sulphonic acid. The *in vivo* conversion of 3′-hydroxy-3,4-dehydro-β-carotene to 3,4-dehydroretinol in chicks and mouse was shown by Budowski *et al.*[129,130].

Association of Carotenoids with Proteins and Their Biological Function

The relationship between lipids and proteins is essential for maintaining the integrity of the cell and both lipids and proteins are found in many structures that present large surfaces. Again, the incompatibility of most lipids, including higher fatty acids, sterols, terpenes, carotenoids and vitamin A with the aqueous environment of plant and animal tissues demands specialized vehicles for their transport and metabolism. Lipids in the healthy cells, with the exception of those specialized for the storage of triglycerides, are highly dispersed and they are transported in the living cells as chylomicra, discrete lipid particles, or in the form of protein complexes, generally known as lipoproteins. Lipoproteins are present in every living cell.

The presence of complexes of lipids with proteins is usually demonstrated by the fact that they could not be extracted with diethyl ether and other non-polar solvents, whereas after drastic treatment with polar organic solvents[131,132] or in the presence of protein denaturing agents or after hydrolysis, the lipids could be extracted easily. Complexes of lipids and proteins which have solubility characteristics of proteins are called ' lipoproteins ' and those with solubility characteristics of lipids are known as ' proteolipids '[133]. The association of lipids with proteins and the natural occurrence of some lipoprotein systems have been reviewed by Gurd[134]. Criddle[135] has recently discussed the protein and lipoprotein organization in the chloroplast.

Palmer[136] postulated the existence of a carotene-albumin complex in cow and hen sera. It was observed[137] that carotene is bound to serum globulin. The presence of chlorophyll-protein complex in green leaves was shown by a number of workers[138-141]. The attachment of carotenoid pigments to proteins in photosynthetic bacterium, *R. rubrum*, has also been demonstrated by different investigators[142-144]. Nishimura and Takamatsu[145] isolated a coloured proteid containing β-carotene as its component from green parsley leaves. The coloured proteid[145] showed absorption maxima at 280, 498 and 538 mμ, with an inflection at 460 mμ and the pigment separated from the complex showed absorption maxima at 450 and 470 mμ. The association of carotenoid pigments with proteins in invertebrates has been reviewed recently by Cheeseman *et al.*[146].

A carotenoid-protein complex has been isolated by us[147] from mangoes (*Mangifera indica*) and purified threefold with respect to carotenoids by

ammonium sulphate fractionation. On the basis of physico-chemical studies, the complex has been established[137] as a lipoprotein and the carotenoid present in the complex has been shown to be β-carotene. It showed absorption maximum at 280 mμ due to proteins and peaks of absorption maxima at 445 and 475 mμ corresponding to β-carotene. There are no significant shifts in the $\lambda_{max.}$ of the pigment due to conjugation with proteins[147]. The complex has been partially characterized with respect to its electrophoretic mobility by disc electrophoresis.

These carotenoid-protein complexes may well represent one of the natural states of carotenoids *in vivo*, possibly, playing an important role hitherto unknown in the physiology and metabolism of carotenoids.

Summary

Recent advances in the chemistry and biochemistry of carotenoids are reviewed. The aspects covered include: distribution; isolation, separation, identification and estimation of individual carotenoids; chemical synthesis of carotenoids, their epoxides and epoxides of vitamin A; biosynthesis of carotenoids; functions of carotenoids in plants, bacteria and animals; and association of carotenoids with proteins and their biological functions.

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Oligonucleotides & Polynucleotides as Tools for Biochemical Studies

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A MAJOR achievement of modern biology is the recognition of the vital functions of the nucleic acids and of the close interrelationships between nucleic acids and proteins. The developments in this field have given rise to the 'information' theory, according to which the genetic information is contained in the DNA molecules within the chromosomes in the cell. The DNA can be replicated by an enzymatic process which involves specific pairing (A: T, G: C) of the four different types of nucleotide bases (A, T, G, C) so as to form exact copies of the original DNA, thus perpetuating and transferring the genetic information. It is the exact sequence of the four different types of nucleotide bases within the DNA molecules that carries the genetic information in a coded form. To read the coded message, the genetic information is transcribed in the form of messenger RNA, utilizing again the specific pairing (A: U; T: A; G: C) of the four types of nucleotide bases (A, U, G, C) in an enzymatic process. In the case of RNA viruses, the genetic information is stored and copied in the form of RNA. The coded information contained in the messenger RNA specifies the linear sequence of the 20 different kinds of amino acids in the protein product. This translation process is also carried out by the nucleotide base pairing mechanism[1] through the mediation of low molecular weight adaptor RNA (tRNA, sRNA) molecules which possess anticodons to read the codons on the messenger RNA. They carry specific amino acids to the site of protein synthesis — the ribosome, messenger RNA complex — and also recognize the correct codons, thus bringing about a very orderly protein synthesis. The genetic code is the dictionary used by the cell to translate the four-letter language of the nucleic acid to the 20-letter language of the proteins. It denotes which trinucleotide or trinucleotides specify a particular amino acid.

Chemically and enzymatically synthesized oligo- and polynucleotides of well-defined sequences are essential tools for the deciphering of the genetic code[1-7]. In this article, an attempt is made to review the methods used for the synthesis of oligo- and polynucleotides of defined sequences. Some of the important biochemical studies carried out using these compounds are also mentioned.

The structural formulae and the abbreviations for DNA and RNA chains are given in Chart 1.

Chemical Synthesis

Earlier literature pertaining to the chemical synthesis of polynucleotides has been reviewed by Khorana[8] and Michelson[9]. Both these workers have also reviewed the methods used for the synthesis of nucleosides and nucleotides. In this article, it is assumed that nucleosides and nucleotides are available, and the main concern is their proper modification and the formation of the $C_{3'}—C_{5'}$ phosphodiester (internucleotide) bond, starting from suitably protected nucleosides and nucleotides.

Polynucleotide chains (Chart 1) can be considered as composed of mononucleotide units and as such one can approach the synthesis of the smallest chain, a dinucleoside phosphate, in two ways as shown in Chart 2 for the synthesis of d-TpA and CpG.

Scheme I starts with a 3'-phosphate and Scheme II with a 5'-phosphate, but in both cases a $C_{3'}—C_{5'}$ internucleotide bond is shown to be formed with the appropriate hydroxyl function. But in these condensations, the phosphorylation reaction can occur at any of the hydroxyl functions on the sugar moiety or at the amino functions on the heterocyclic rings, giving a number of byproducts; hence the necessity for the protection (temporary derivatization) of the extra hydroxyl groups, extra phosphomonoester groups and amino groups. For the formation of a phosphodiester bond between a phosphomonoester and a hydroxyl group efficiently, special reagents are necessary. The glycosidic bonds between the deoxyribose or ribose and the bases are acid labile, extremely so, in the case of purine nucleotides. Because of the presence of the 2'-hydroxyl group, RNA is alkali labile. Any efficient method of synthesis has to take into consideration all the above problems.

Condensing Agents

The most widely used and the classical condensing agent in oligonucleotide synthesis is dicyclohexyl carbodiimide (DCC)[8]; until recently, DCC was the only reagent used for all practical purposes. Hindered aromatic sulphonyl chlorides like mesitylene sulphonyl chloride[10,11] and 2,4,6-tri-isopropylbenzene sulphonyl chloride[12] have proved to be very useful reagents in recent work on the synthesis of oligo- and polynucleotides[13-22]. Several other reagents have been proposed and listed for the activation of phosphate ester groups and internucleotide bond synthesis. Typical among these are: picryl chloride[23], trichloracetonitrile[24], substituted isoxazolium salts[10,25], dimethylformamide chloride[11,26], carbonyl-bisimidazole[27], ethylpolyphosphate[28,29] and mesitoyl chloride[30].

The mechanism of internucleotide bond synthesis using phosphomonoesters by the DCC method has been investigated[31], and it has been concluded that trimetaphosphates are the initial phosphorylating species. The mechanism of activation by aromatic sulphonyl chlorides seems to be different[11]. Monomeric metaphosphate or its complex with pyridine has been proposed[32] as the phosphorylating species in the case of DCC as well as aromatic sulphonyl chloride mediated condensations of phosphomonoesters with hydroxylic components.

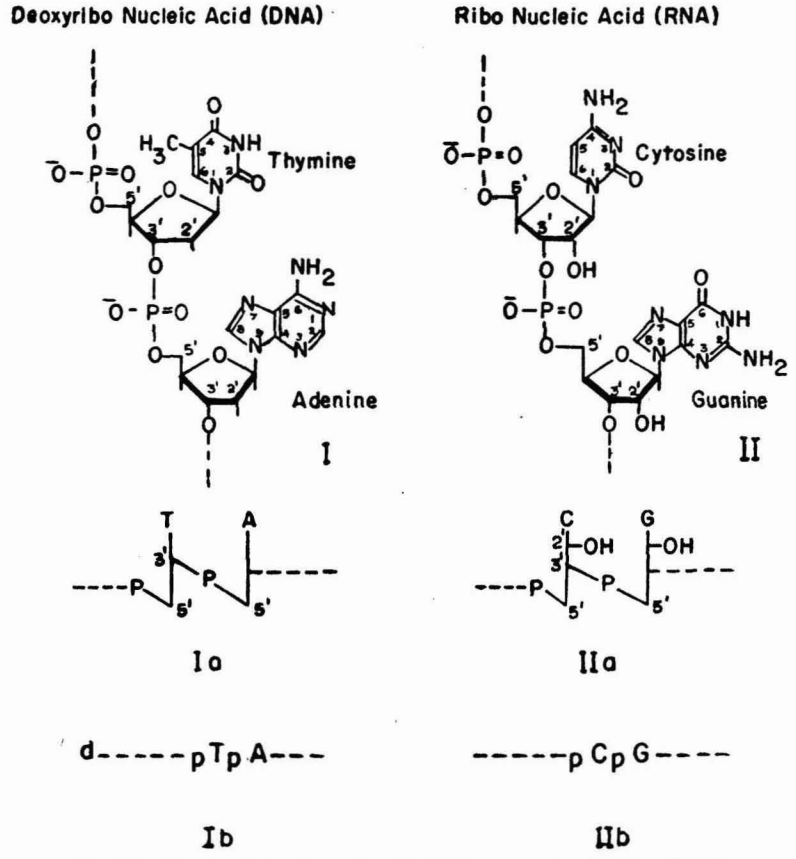

Chart 1 — Structural formulae and abbreviations for parts of DNA and RNA

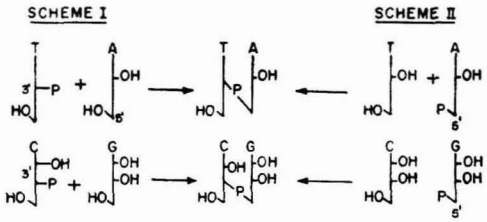

Chart 2 — Modes of synthesis of dinucleoside phosphate

Protected Nucleosides and Nucleotides

There are many considerations in choosing the protecting groups for the different functional groups. It should be possible to put them on readily and specifically in high yields. They should be stable enough to withstand the conditions of condensation, work up and isolation. The protecting groups chosen for different types of functional groups should vary in their stabilities, so that it should be possible to remove one type at a time, thereby providing freedom for manipulation. The conditions for the removal of the protecting groups should be such that no harm is done to the polynucleotide chain. In the ribo- as well as deoxyribopolynucleotide synthesis, Khorana and his collaborators[8,13-22] protected the 5'-hydroxyl function by forming an acid labile but alkali stable ether linkage with triphenyl methyl (trityl), *p*-monomethoxytrityl, *p*-dimethoxytrityl or *p*-trimethoxytrityl groups. The acid lability in this series increases with methoxy substitution. They used an alkali labile acyl group for the protection of 2'- and 3'-hydroxyl functions, and also for the protection of the amino groups. At *p*H above 13, the acyl group at the hydroxyl function can be removed preferentially without harming the acyl protecting group on the amino functions. β-Cyanoethyl group[33] can be split off with alkali, by a β-elimination mechanism, and has proved to be a useful protecting group for phosphomonoester functions in the deoxyribonucleotide synthesis. In the ribonucleotide synthesis more labile protecting groups are used for this purpose[34]. Phosphomonoester groups have also been protected by benzyl[35], benzhydryl[36], *t*-butyl[37], β-trichloroethyl[38] and ethyl thio[39] groups. The 2'- and 3'-hydroxyl functions of ribose can be protected by the formation of *p*-methoxybenzylidene[40], *p*-dimethylaminobenzyl-

idene[41] or ethoxymethylene[42-44] derivatives. During the acylation of ribonucleoside 3'-phosphates, cyclic phosphates are formed, unless special experimental procedures[45] are followed. The 2'-hydroxyl group can also be protected with dihydropyran[36,40] or ethyl vinyl ether[47].

Preparation of protected nucleosides —5'-O-*p*-monomethoxytrityl or 5'-O-*p*-dimethoxytrityl thymidines are prepared[48] by the action of the appropriate methoxytritylchloride[40] with thymidine in the presence of pyridine. In the case of deoxycytidine[48], deoxyadenosine[48] and deoxyguanosine[49], the amino functions are protected by the appropriate acyl groups like anisoyl, benzoyl, or acetyl, before the tritylation reaction. The tritylation is fairly specific for the primary 5'-hydroxyl group compared to the secondary 3'-hydroxyl group and high yields are obtained uniformly.

The classical approach to the synthesis of protected ribonucleosides bearing free 5'-hydroxyl groups involves the tritylation of the nucleosides, acetylation of the trityl derivatives and subsequent detritylation under acidic conditions. Essentially the same principle is used[50] for the preparation of $O^{2'},O^{3'}$-dibenzoyl uridine; $N^{2},O^{2'},O^{3'}$-triacetyl guanosine; and $N,O^{2'},O^{3'}$-tribenzoyl cytidine. The preparation of $N,N,O^{2'},O^{3'}$-tetrabenzoyl adenosine[40,50] involves the prior protection of the amino function before tritylation.

Preparation of protected nucleotides — For deoxyribopolynucleotide synthesis, the protected mononucleotides generally needed are of 3 types: nucleoside 5'-phosphates protected at the amino group alone, nucleoside 5'-phosphates protected both at the amino group and 3'-hydroxyl group, and nucleoside 5'-phosphates protected at the amino and phosphoryl functions.

3'-O-acetyl thymidine 5'-phosphate[51] and $N,O^{3'}$-diacetyl guanosine 5'-phosphate[52] are prepared by the simple acetylation of the nucleotides in pyridine using acetic anhydride. By controlled alkali treatment the O-acetyl group is removed preferentially. N-anisoyl-3'-O-acetyl deoxycytidine 5'-phosphate is prepared[53] from N-anisoyl deoxycytidine 5'-phosphate[54] by acetylation; N-anisoyl deoxycytidine 5'-phosphate is prepared[54] by the anisoylation of deoxycytidine 5-phosphate with anisylchloride and subsequent controlled alkali de-anisoylation. 3'-O-acetyl, N-benzoyl, deoxyadenosine 5'-phosphate and N-benzoyl deoxyadenosine 5'-phosphate are prepared in a similar manner[55]. To introduce a cyanoethyl group on the phosphate group of mononucleotides, the amino protected mononucleotide is treated with excess hydracrylonitrile and DCC in pyridine, the protection of the hydroxyl function being unnecessary[56-58].

The major problems in the preparation of suitably protected ribonucleoside 3'-phosphates are the isomerization and cyclic phosphate formation due to the presence of the 2'-hydroxyl group. These problems have been overcome by the acylation procedure[45,59] using carboxylic acid anhydrides in the presence of tetraethyl ammonium hydroxide. By this procedure, fully acylated ribonucleoside 3'-phosphates can be prepared starting from ribonucleoside 3'-phosphates. Pyridinium uridine 3'-phosphate, on reaction with mono- or dimethoxy tritylchloride, in pyridine, is converted in high yield to the 5'-O-methoxytrityl derivative[45,59,60]. Similarly, adenosine 3'-phosphate on tritylation in pyridine gives 5'-O-monomethoxytrityl derivative in satisfactory yield[22,61]. 5'-O-monomethoxytrityl and 5'-O-dimethoxytrityl derivatives of cytidine 3'-phosphate are prepared[22] by conducting the tritylation in dimethyl formamide with restricted amounts of pyridine, under which conditions the amino group is presumably protonated. The same method has been applied for the preparation of 5'-O-dimethoxytrityl adenosine 3'-phosphate[22], a simple extraction procedure being used for the isolation of the required product. This procedure when applied to guanosine 3'-phosphate gave N-tritylation[22] and so an alternate procedure was developed for the preparation of 5'-O-methoxytrityl-N-acetyl guanosine 3'-phosphate[22]. Starting from the 5'-O-protected derivatives of uridine, cytidine, adenosine and N-acetyl guanosine 3'-phosphates the acylation reaction using acetic anhydride or benzoic anhydride and tetraethyl ammonium hydroxide is quite general[22,59] and gives good yields of $O^{5'}$, $O^{2'}$ and N-protected nucleoside 3'-phosphates. When benzoic anhydride is used for the acylation, the benzoyl phosphate formed is converted to the acetylphosphate by treatment with excess acetic anhydride, because the acetyl phosphates are labile in aqueous pyridine and can be removed without affecting the O-benzoyl protecting group. An efficient procedure reported for the preparation of pyrimidine 3'-phosphates[22] involves the cyclization of commercially available mixtures of 2'- and 3'-phosphates of cytidine or uridine to the 2',3'-cyclic phosphates and subsequent tritylation followed by ring opening with pancreatic ribonuclease in aqueous dimethyl formamide, to the 3'-phosphates.

Special care has to be taken to remove the salts of the carboxylic acids from protected mononucleotides[14]; otherwise they will get activated by the condensing agents used for the internucleotide bond synthesis and function as acylating agents and thereby block the free hydroxyl functions and result in low yields.

Synthesis of Oligo- and Polynucleotides

Ribotrinucleotides — The synthesis of all the 64 possible ribotrinucleotides derived from the four common bases has been reported[22] (Chart 3).

It required 3 types of starting materials: (a) the four protected ribonucleosides of the type (III), with free 5'-hydroxyl groups; (b) the four protected nucleotides of the type (IV), which serve as the middle component and which bear acid labile protecting groups at the 5'-hydroxyl functions, that can be preferentially removed after the condensation to give products of the type (V); and (c) the four fully acylated ribonucleoside-3-phosphates of the type (VI). Following the scheme in Chart 3, all the 16 protected dinucleoside phosphates of type (V) were prepared[22] in yields varying from 28 to 82 per cent. Starting from the protected dinucleoside phosphates of type (V), the yields of the trinucleoside diphosphates of type (VII) were in the range 30-92 per cent. The condensing agents used were either DCC or tri-isopropylbenzene sulphonyl chloride (TPS).

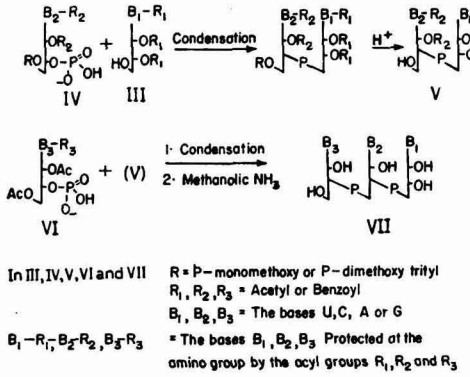

Chart 3 — Synthesis pathway for ribotrinucleotides

Synthesis of other oligo- and polyribonucleotides — The general principles employed in the synthesis of the 64 ribotrinucleotides, as shown in Chart 3, can be applied for the synthesis of oligo- and polyribonucleotides of specific sequences. Here the main aspects are that the chain elongation takes place from the 2′,3′-hydroxyl end to the 5′-hydroxyl end of the ribonucleotide chain and that the 2′,3′-hydroxyl functions and the amino functions are kept protected until the final product is obtained, whereas the 5′-hydroxyl function is exposed at intermediate steps by a controlled acid treatment. For the synthesis of dinucleoside phosphates, starting materials of types (III) and (VI) are needed (Chart 3). After condensation, methanolic ammonia treatment provides the required product, which is purified by chromatography. When further chain elongation is planned, the starting materials used are of the types (III) and (IV), because (IV) carries an acid labile group at the 5′-hydroxyl function, which can be selectively removed to expose the 5′-hydroxyl function, which is the site of chain elongation. The synthesis of the tetranucleoside triphosphate UpApUpU has been reported[59]. It will be interesting to explore the feasibility of this approach for the synthesis of long ribopolynucleotide chains.

Using 5′-O-acetyl, 2′-O-tetrahydropyranyl, nucleoside 3′-phosphates[62] as the building blocks, the hexanucleoside pentaphosphate, UpUpUpUpUpU was prepared[63] in rather low yield. In this synthesis, the protecting group used at the 5′-hydroxyl function is alkali labile. It is removed at intermediate steps, whereas the acid labile tetrahydropyranyl group at the 2′-hydroxyl function is removed on the completion of the synthesis. In the case of higher oligonucleotides, it is rather difficult to remove the tetrahydropyranyl group completely without damaging the internucleotide bond. Often, the acid conditions necessary for the removal of the tetrahydropyranyl group from synthetic oligonucleotides caused detectable[45] isomerization of the internucleotide bond ($C_{3'}-C_{5'} \rightleftharpoons C_{2'}-C_{5'}$). This disadvantage could be overcome by finding out a protecting group for the 2′-hydroxyl function, which is more acid labile than the tetrahydropyranyl group.

Several diribonucleotides containing free 3′-phosphate groups have been synthesized. Typical among these are[64]: UpAp, ApAp, CpAp, CpUp, ApUp, IpUp, UpUp and UpCp.

Homopolymers have been prepared by the polymerization[40,60] of suitably protected mononucleotides. Even though the ethyl polyphosphate method is claimed[28] to give high yields of ribonucleic acid polymers starting from unprotected mononucleotides, some workers[29] have found the method unsatisfactory. Michelson's procedure[66] provides a rather rapid way to prepare ribonucleotide polymers with random 3′-5′ and 2′-5′ linkages. They are useful for several physico-chemical studies.

Deoxyribopolynucleotides — The first synthesis[67] of the $C_{3'}-C_{5'}$ internucleotide bond was that of dithymidine dinucleotide and it involved the synthesis of a nucleoside phosphite, its conversion to a phosphorochloridate and the subsequent phosphorylation of a second nucleoside. Recently, there has been good progress in the synthesis of deoxyribopolynucleotides. Synthesis of specific sequences has been accomplished by the elongation of the nucleotide chain by the addition of mononucleotide units[13-15,19,49] or oligonucleotide blocks[16,20,21]. For the preparation of polymers with repeating di-[56-58], tri-[18] and tetra-[19] nucleotide sequences, polymerization of preformed oligonucleotides has proved to be a rapid procedure. The preferred synthetic procedure in the deoxyribopolynucleotide synthesis is the one utilizing free 5′-phosphates for condensation to a free 3′-hydroxyl function, in which case the chain elongation takes place from the 5′-hydroxyl end of the chain towards the 3′-hydroxyl end. This is the reverse of the one discussed in the case of oligoribonucleotide synthesis.

Addition of mononucleotide units — The elongation of the deoxyribonucleotide chain by one mononucleotide unit at a time requires two types of starting materials: (i) the four protected deoxyribonucleosides carrying free 3′-hydroxyl groups, and (ii) the four protected deoxyribomononucleotides carrying free 5′-phosphomonoester groups. The preparation of these compounds has been discussed earlier. The synthesis of the dodecanucleotide[14] d-T(pTpCpT)$_3$pTpC is illustrated in Chart 4.

The 5′-hydroxyl function at the beginning of the chain is protected by an acid labile but alkali stable trityl (Tr) group. *p*-Monomethoxy- or *p*-dimethoxytrityl protecting groups are to be used when purine nucleotides are present in the chain, because of the high acid lability of their glycosidic linkage. The trityl group remains intact during all stages of condensation and is removed only at the end of the synthesis, after the removal of the protecting groups on the amino functions also. At intermediate steps, the acetyl group at the 3′-hydroxyl function is removed by a mild alkali treatment, which is safe for the protecting groups at the amino functions and the products are isolated by suitable procedures like solvent extraction[13], DEAE-cellulose column chromatography[14] or gel filtration[19]. The isolation procedure can be further simplified[68] by carrying out the synthesis on an inert polymer support, if one is not very particular about the purity of the product at each step. A number of deoxyribo-, oligo-[13,49,53,69] and polynucleotides[14,15,19] have been synthesized by the above procedure.

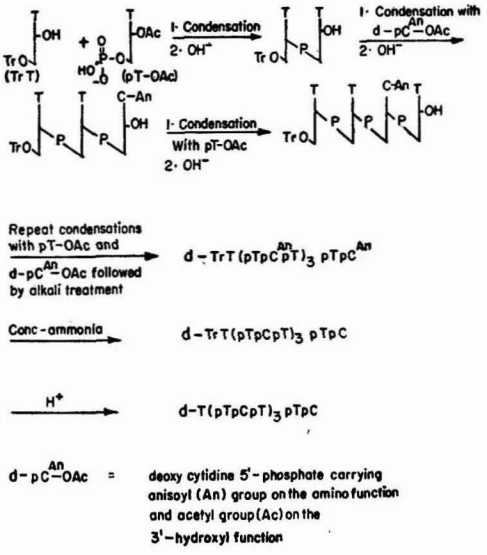

Chart 4 — Synthesis pathway for dodecanucleotide d-T(pTpCpT)$_3$pTpC

Addition of nucleotide blocks — The main advantage in using oligonucleotide blocks instead of mononucleotide units to build the polynucleotide chain is in the separation of the desired product from the unelongated chain, especially when the polynucleotide chains are of chain length longer than ten. Earlier attempts[70-72] to use this approach did not give encouraging results, but recently a number of oligo-[16] and polynucleotides[20,21] have been synthesized following this approach. As in the addition of mononucleotide units, the preferred direction of chain elongation in this approach is also from the 5′-hydroxyl end to the 3′-hydroxyl end of the polynucleotide chain. The 5′-hydroxyl group is protected by an acid labile group and is kept throughout the course of the synthesis. The 3′-hydroxyl group of the growing chain is selectively unblocked by a mild alkali treatment. The building blocks are protected at the 3′-hydroxyl group and at the amino functions, but carry free 5′-phosphomonoester groups. The synthesis of such oligonucleotide blocks is discussed in the following section.

Polymerization — For the preparation of polynucleotides containing repeating sequences, or repeating sequences ending in a different sequence, polymerization is the easier technique. Thus, by the polymerization of suitably protected mononucleotides, poly T[73], d-poly C[54] d-poly A[55], d-poly G[52] and poly T ending in d-C[72] were prepared. Polymerization of suitably protected dinucleotides[56-58], trinucleotides[17,18] and tetranucleotides[19] has been reported. Both for polymerization and block condensation, the appropriate oligonucleotide blocks are needed and their synthesis is discussed below.

The dinucleotide blocks are synthesized (Chart 5) by the condensation of two components of the types (VIII) and (IX), whose preparations have been discussed earlier. A mild alkali treatment removes both the cyanoethyl (CE) and the acetyl (Ac) protecting groups to give compounds of the type (X), which are isolated by DEAE-cellulose column chromatography. Satisfactory methods are not available in which the protecting groups on the phosphate could be removed keeping that on the 3′-hydroxyl group or vice versa. A simple acetylation of compound (X) using acetic anhydride and pyridine, followed by aqueous pyridine treatment to decompose the acetylphosphate, gives the protected dinucleotide (XI), which is isolated by precipitation from ether. Compounds of the type (XII) are obtained[17] from compound (X) by cyanoethylation using excess hydracrylonitrile and DCC.

As shown in Chart 6, a trinucleotide[17] of the type (XIII) can be prepared by the condensation of compound (VIII) with compound (XI) or compound (XII) with compound (IX), followed by controlled alkali treatment. Compounds of the type (XV) are prepared from (XIII) by cyanoethylation[19]. In the cyanoethylation reactions, some tertiary esters are also formed, but they can be removed under controlled conditions. Compounds of type (XIV) are prepared[17] from compound (XIII) by acetylation as shown in Chart 5.

Protected tetranucleotides[19,21] of the type (XVI) are prepared by condensing compounds of type (XV) with compound (IX) or (XII) with compound (XI), followed by mild alkali treatment. Compounds of the type (XVII) are obtained from compound (XVI) by acetylation[19].

The same general procedure can be used for the synthesis of longer chains of protected deoxyribopolynucleotides to serve as blocks in stepwise synthesis or polymerization, but has not been reported so far.

Enzymatic Synthesis

Deoxyribopolynucleotides — DNA polymerase[74] of *Esch. coli* has been used to synthesize several high molecular weight DNA-like molecules having known

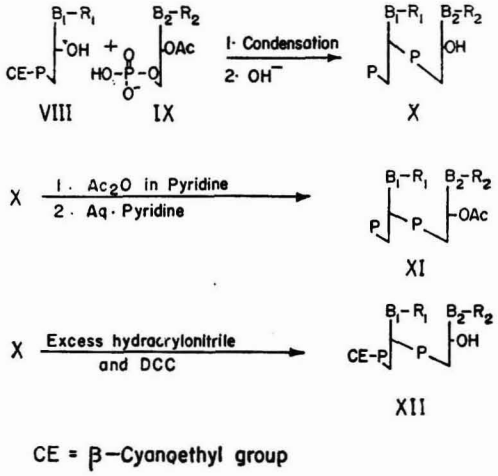

Chart 5 — Preparation of protected deoxyribodinucleotides

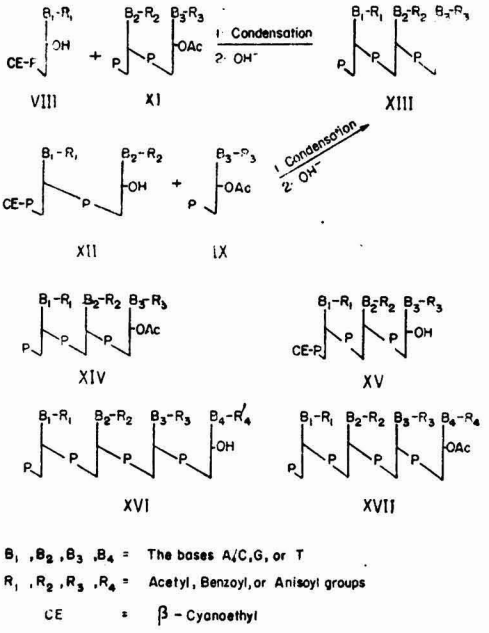

Chart 6 — Protected deoxyribotri- and tetranucleotides

repeating sequences[75-80], using chemically synthesized low molecular weight deoxyribopolynucleotides of known repeating sequences as templates. These polymers have provided[7,81,82] a precise system for studies of the transcription-translation processes (DNA → RNA → protein). The structures of the polymers have been established by nearest neighbour frequency analysis and were found to have the same repeating nucleotide sequences provided in the short chain template chemically synthesized. The polymers obtained were of molecular weights in the order of millions in most of the cases studied. In addition to the advantage of amplification of the short chain templates and of extensive net synthesis, the DNA polymerase is able to utilize the high molecular weight DNA-like polymers as templates for the multiplication of the same product. This makes the repetition of the time-consuming chemical synthesis unnecessary. A list of the deoxyribopolynucleotides prepared in this way is given in Table 1.

It will be interesting to investigate whether the same procedure could be used for the preparation of polymers with repeating penta-, hexa- and higher nucleotide sequences.

In the absence of a DNA template, the DNA polymerase of *Esch. coli* does not synthesize DNA in the presence of all the four deoxyribonucleoside triphosphates; but if only the two triphosphates, d-ATP and d-TTP, are provided, poly d-(AT) is synthesized[83-85] and if the other two triphosphates, d-GTP and d-CTP, only are provided, poly (d-C: d-G) are synthesized[83,86] even in the absence of DNA template. DNA polymerases have been isolated[87,88] from calf thymus, which are capable of building homopolynucleotide chains on to the end of oligonucleotide chains.

TABLE 1 — LONG POLYDEOXYRIBONUCLEOTIDES OBTAINED ENZYMATICALLY USING CHEMICALLY SYNTHESIZED SHORT POLYDEOXYRIBONUCLEOTIDES[6] AS TEMPLATES FOR DNA POLYMERASE

Template	Substrate	Product	Reference
$d\text{-}T_{11}$: $d\text{-}A_7$	d-ATP, d-TTP	Poly d-A: poly d-T 20S, M.Wt 6×10^6	76
$d\text{-}(AT)_{3\text{-}7}$	d-ATP, d-TTP	Poly d-AT	75
$d\text{-}(TC)_5$: $d\text{-}(AG)_5$	d-ATP, d-TTP, d-CTP, d-GTP	Poly (d-TC: d-AG) 16S, M.Wt $> 16^6$	76
$d\text{-}(TG)_5$: $d\text{-}(AC)_5$	do	Poly (d-TG: d-AC) M.Wt 1×10^6 0·5 μ length	77
$d\text{-}(TTC)_4$: $d\text{-}(AAG)_3$	do	Poly (d-TTC: d-GAA)	78, 79
$d\text{-}(TTG)_3$: $d\text{-}(AAC)_5$	do	Poly (d-TTG: d-CAA)	79
$d\text{-}(TAC)_5$: $d\text{-}(TAG)_4$	do	Poly (d-TAC: d-(TAG)	79
$d\text{-}(ATG)_3$: $d\text{-}(ATC)_3$	do	Poly (d-ATG: d-CAT)	79
$d\text{-}(TTAC)_4$: $d\text{-}(TTAG)_2$	do	Poly (d-TTAC: d-GTAA)	80
$d\text{-}(TATC)_3$: $d(TAGA)_2$	do	Poly (d-TATC: d-GATA)	80

Poly (d-TC : d-AG) indicates two long deoxyribopolynucleotide chains where one chain has the repeating sequence d-TpCpTpCpTpC . . . and the other chain has the repeating sequence d-ApGpApG . . . . The 'p' is omitted to save space. The same system is followed to designate homopolymers and polynucleotide chains containing repeating tri- and tetranucleotide chains.

$d\text{-}(TC)_5$: $d\text{-}(AG)_5$ indicates two deoxyribopolynucleotides d-TpCpTpCpTpCpTpCpTpC and d-ApGpApGpApGpApGpApG. The same system is followed in the case of other poymers.

Oligo- and Polyribonucleotides

DNA dependent RNA polymerase — The RNA polymerase[89-94] brings about the synthesis of RNA from the four ribonucleoside, 5′-triphosphates in the presence of a DNA template. The composition and sequence of the nucleotides in the RNA product are complementary to those of the DNA template, according to the Watson-Crick base pairing principle[95,96].

RNA polymerase has been shown to make use of short deoxyribo-, oligo- and polynucleotides as templates in special cases. Thus, $d\text{-}T_{6\text{-}11}$ in the presence of ATP gave[97] a high yield of poly A, which was much longer than the template[97]. $d\text{-}(TTC)_{3\text{-}4}$ in the presence of ATP and GTP gave[98] poly (AAG). This type of reaction, in which a short single stranded DNA serves as template for its reiterative copying by RNA polymerase, seems to be favoured only when the templates are constituted predominantly of pyrimidines. The potentials of this type of reaction have not been investigated so far.

The high molecular weight double stranded, enzymatically synthesized deoxyribopolynucleotides of known repeating base sequences described under DNA polymerase have been used as templates

to prepare single stranded polyribonucleotides of known repeating base sequences. Which strand of DNA is copied in this system depends on the nature of the nucleoside triphosphates offered in the reaction medium, because a maximum of only 3 types of nucleotides are found on any one strand in these cases. Thus, many ribopolynucleotides with repeating di-[99,100], tri-[78,101] and tetra-[102,103] nucleotide sequences have been prepared (Table 2).

Polynucleotide phosphorylase — Polynucleotide phosphorylase[104] polymerizes ribonucleoside diphosphates to polynucleotides with the liberation of inorganic phosphate. This enzyme is a depolymerizer too, which is its main function in the cells. It does not need a template and has been used extensively[105-107] for the preparation of homopolymers and copolymers of random sequences. From such random copolymers, using pancreatic ribonuclease or T_1-ribonuclease, homopolymers ending in pyrimidines[108] or guanosine[64] can be prepared. Even though the crude or partially purified enzyme does not need a primer, highly purified enzyme[109] needs a primer[110,111] and this property has been used for the synthesis of a number of homopolymers starting with definite triplet sequences at the 5′-end. The primer dependent enzyme from *M. lysodeicticus*, if used in the presence of the appropriate sodium chloride concentration[64,112,113] after a sufficiently long reaction time, gives products in which a dinucleoside phosphate primer is extended by a few units. With highly purified polynucleotide phosphorylase, when the ratio of dinucleoside phosphate primer to nucleoside diphosphate substrate is one or higher than one, trinucleoside diphosphates can be prepared[114] in reasonable yields; this method is in principle suitable for the preparation of all the 64 trinucleoside diphosphates and many of them have already been synthesized this way.

Ribonucleases —Bovine pancreatic ribonuclease A[115] splits pyrimidine nucleoside 3′-phosphodiester bonds through the intermediate formation of pyrimidine nucleoside 2′,3′-cyclic phosphodiesters; under suitable conditions the enzyme could be used for the preparation of oligonucleotides, starting from 2′,3′-cyclic pyrimidine mononucleotides[116]. The high hydrolytic activity of the enzyme has prevented the use of this method for the synthesis of oligonucleotides in reasonable yields, but some derivatives of ribonuclease, like RNase S-protein[117], 1-carboxymethylhistidine-119-RNase[118], and ε-dinitrophenylaminolysine-41-RNase[119], possess significant synthetic activity[120] but negligible hydrolytic activity and have been used[64,121] for the synthesis of oligonucleotides. T_1-ribonuclease[122] splits guanosine 3′-phosphodiester bonds through the intermediate formation of guanosine 2′,3′-cyclic phosphate and the reverse of this reaction can be made use of for the synthesis of oligonucleotides containing guanosine, by using guanosine 2′,3′-cyclic phosphate as the starting material.

TABLE 2 — RNA OF KNOWN SEQUENCES PREPARED USING DEOXYRIBOPOLYNUCLEOTIDES AS TEMPLATES FOR DNA DEPENDENT RNA POLYMERASE

Template	Substrates	Product	Reference
d-T_{6-11}	ATP	Poly A	97
d-$(TC)_5$	ATP, GTP	Poly (AG)	Khorana *et al.* (unpublished data)
d-$(TTC)_{3-4}$	ATP, GTP	Poly (AAG)	98
d-$(TTTC)_{2-3}$	ATP, GTP	Poly (AAAG)	Khorana *et al.* (unpublished data)
Poly d-(TC: AG)	ATP, GTP	Poly (AG)	99
Poly d-(TC: AG)	CTP, UTP	Poly (UC)	99
Poly d-(TG: AC)	ATP, CTP	Poly (AC)	99
Poly d-(TG: AC)	UTP, GTP	Poly (UG)	99
Poly d-(TTC: GAA)	ATP, GTP	Poly (GAA)	101
Poly d-(TTC: GAA)	UTP, CTP	Poly (UUC)	101
Poly d-(TTG:CAA)	ATP, CTP	Poly (CAA)	101
Poly d-(TTG:CAA)	UTP, GTP	Poly (UUG)	101
Poly d-(TAC:GTA)	ATP, UTP, GTP	Poly (GUA)	101
Poly d-(TAC:GTA)	CTP, ATP, UTP	Poly (UAC)	101
Poly d-(TAC:GTA)	CTP, ATP, UTP	Poly (UAC)	101
Poly d-(ATC:GAT)	UTP, ATP, GTP	Poly (GAU)	101
Poly d-(ATC:GAT)	CTP, UTP, ATP	Poly (AUC)	101
Poly d-(TATC: GATA)	ATP, UTP, GTP	Poly (GAUA)	102, 103
Poly d-(TATC: GATA)	CTP, UTP, ATP	Poly (UAUC)	102, 103
Poly d-(TTAC: GTAA)	ATP, UTP, GTP	Poly (GUAA)	102, 103
Poly d-(TTAC: GTAA)	CTP, ATP, UTP	Poly (UUAC)	102, 103

The various terms used have the same significance as in Table 1.

Biochemical Studies Using Oligo- and Polynucleotides of Defined Sequences

The genetic code — Early biochemical studies to relate various code words to their amino acids, made use of the *in vitro Esch. coli* amino acid incorporation system developed by Nirenberg and Matthaei[123], in which they showed that poly U directed the synthesis of polyphenylalanine. Nirenberg and coworkers[124] as well as Ochoa and coworkers[125] used ribopolynucleotides synthesized with polynucleotide phosphorylase and containing two, three or four bases in random sequence, to deduce the code words. These experiments furnished in many cases the type of bases present in a code word, but did not give information as to the order of the bases in the code words[126]. This information[1] and other particulars regarding the amino acid code were obtained by the use of oligo- and polynucleotides of defined sequences.

Nirenberg and Leder[2] found that a ribotrinucleotide, which represents the codon for an amino acid, can specifically direct the binding of the corresponding aminoacyl sRNA to ribosomes. This method has proved to be a rapid way for codon assignments[3,4]

and also for the determination of the specificities of different tRNA species[6,127-129] for different code words. The data obtained by both Khorana's group and Nirenberg's group, independently, have shown good agreement. But the results of the trinucleotide stimulated ribosomal binding of aminoacyl tRNA are not always unambiguous[4].

Khorana and coworkers used ribopolynucleotides containing completely defined nucleotide sequences to direct amino acid incorporation in cell-free systems. Alternating copolymers[99,100], such as poly UC, poly UG, poly AC and poly AG were found to direct the synthesis of co-polypeptides of ser and leu, val and cys, thr and his, arg and glu respectively in alternating sequence. Poly AAG[98] directed the synthesis of 3 homopolypeptides[5]: poly-lys, poly-glu and poly-arg. Similar studies were conducted using several ribopolynucleotides containing repeating tri-[101] and tetranucleotide[102,103] sequences. A ribonucleotide polymer with a repeating tetranucleotide sequence furnished a polypeptide with a repeating tetrapeptide sequence[103]. On the basis of these experiments many code words were proved and new ones established. These studies also provide good biochemical evidence as to the following aspects of the genetic code: (a) a group of three nucleotide units in a linear sequence specifies the incorporation of a particular amino acid (3-letter code), (b) each nucleotide unit in a polynucleotide chain is used only once in forming groups of 3 nucleotides (non-overlapping property of the code), and (c) the reading of a polynucleotide chain occurs in units of 3 letters (codons) without omission of a single nucleotide unit. Khorana and coworkers started from DNA of known sequences and prepared from it the RNA of complementary sequences and using this RNA as messenger prepared polypeptides. This is a very precise system for studying different aspects of transcription-translation. Their studies also provide a good biochemical proof for the thesis that " the linear sequence of deoxyribonucleotides in DNA specifies the linear sequence of amino acids in polypeptide chains and this information is expressed through the intermediate formation of RNA (messenger) under the direction of DNA ".

The direction of reading of the genetic code has been investigated in different laboratories. It was found that the amino acid incorporation directed by the oligonucleotides of type (A)nC, in a bacterial cell-free system produced[108] poly lys with an asn at the carboxy terminal end of the polypeptide (AAA and AAC are codons for lys and asn respectively). The oligonucleotide A_3U_3 directed[130] the synthesis of the dipeptide lys-phe, but not phe-lys. Poly (UUAC) provides[102,103] in the amino acid incorporation system, the repeating tetrapeptide (thr-tyr-leu-leu)n. Hence, because the polypeptide is assembled from the amino-terminal end[131], the message must be read from the 5'-end of the messenger RNA to the 3'-end.

Ribopolynucleotides containing repeating dinucleotide and trinucleotide sequences have given definite information from cell-free polypeptide synthesis experiments, as to the nature of codons which signal polypeptide chain initiation[132] and termination[101].

Genetic Suppression

Many examples are known where the effect of a mutation in a gene is reversed by a second mutation in another gene. Those genes which cause suppression of mutations in other genes are called suppressor genes. Suppressor genes do not act by changing the nucleotide sequences in the mutant DNA; instead they change the way in which the mRNA templates are read.

The involvement of tRNA in the suppression of amber and ochre mutants (nonsense suppression) has been established by the work on the coat protein of RNA bacteriophages[133,134]. The synthetic ribopolynucleotides with the repeating tetranucleotide sequences (AUAG)n and (GUAA)n are good messengers for the study of ochre and amber suppression (nonsense suppression). In one of these polymers, every fourth codon is the amber codon, UAG (nonsense), and in the other polymer every fourth codon is the ochre codon, UAA (nonsense). Using *in vitro* amino acid incorporation system prepared from bacteria having ochre or amber suppressor genes, amino acids can be expected to be incorporated corresponding to the nonsense codons (UAA, UAG) when the above polymers are used as messengers. Consequently, long polypeptide products can be expected to be formed. If the *in vitro* amino acid incorporation system is prepared from bacteria containing no ochre or amber suppressor genes, the peptides formed cannot be longer than a tripeptide when the above polymers are used as messages.

The alternating copolymer, poly AG, which usually stimulates the incorporation of arg and glu, was found[135] to incorporate gly instead of arg into polypeptides, in the presence of tRNA from a suppressor mutant. Similarly, poly UG, which normally directs the synthesis of val-cys co-polypeptides, directed[136] the synthesis of val-gly co-polypeptides when supplemented with tRNA from a suppressed strain. Thus, polyribonucleotides of defined sequences are useful in studying not only nonsense suppression but also mis-sense suppression.

By the use of ribonucleotides containing repeating dinucleotide sequences, the pattern of misreading induced by certain antibiotics like streptomycin has been studied[137]. This study could be extended by using other polymers of defined sequences.

Other Studies

Studies on the direct translation of single stranded deoxyribopolynucleotides in the presence of aminoglycoside antibiotics have been made[138] based on the finding[139] that denatured DNA can directly serve as messengers in the presence of certain antibiotics.

Using synthetic oligonucleotides as substrates, the modes of action of a number of enzymes like snake venom phosphodiestrase[140], spleen phosphodiestrase[141], *L. acidophilus* phosphodiestrase[142], which cause degradation of the nucleic acids, have been established.

In the present study, only a few examples of the different biochemical studies that have been conducted in recent years using oligo- and polynucleotides have been cited. The recent availability of synthetic oligo- and polynucleotides of well-defined

sequences has opened up opportunities for further studies on the different aspects of protein biosynthesis and genetic suppression and also the chemistry and enzymology of RNA and DNA.

Summary

The methods used for the chemical and enzymatic synthesis of oligo- and polynucleotides of defined sequences are reviewed. Some of the important biochemical studies carried out using oligo- and polynucleotides are discussed. Under chemical synthesis, the condensing agents, the preparation of protected nucleosides and nucleotides and the synthesis of the 64 ribotrinucleotides, oligo- and polyribonucleotides and of deoxyribopolynucleotides are dealt with. Under enzymatic synthesis, the use of enzymes, DNA polymerase DNA dependent RNA polymerase, pancreatic ribonuclease and T_1-ribonuclease, for the synthesis of oligo- and polynucleotides of defined sequences are discussed. The biochemical studies discussed are those related to the genetic code, genetic suppression, misreading of the genetic code and the specificity of nucleases.

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REVIEWS

STATIC ELECTRIFICATION: 1967 CONFERENCE PROCEEDINGS edited by A. C. Stickland (Institute of Physics and Physical Society, London), 1967. Pp. vi+173. Price £10 0s. 0d.

The book under review is a collection of invited and contributed papers on the subject of static electrification of (i) solids, (ii) liquids and (iii) its industrial implication.

In Chapter I, W. R. Harper has given a very stimulating review of contact (frictional) electrification, which can claim to be the oldest branch of electrical studies. The problem is to find an explanation of the charging of solids in the light of our knowledge about the movement of ions in conduction and valency bands. When two insulators are rubbed vigorously, the heated spots become temporarily semiconducting leading to transfer of electrons from one body to the other; subsequent cooling leaves the charge frozen in the insulators. The dissociation of ice into protons and hydroxyl ions is strongly temperature dependent; any asymmetric rubbing induces a temperature gradient leading to diffusion of protons from less cold to the colder regions. That explains the generation of high voltage in thunderstorms. Some of the treatments are sketchy, which is natural in a short article like this. The details of these ideas will be available in the forthcoming book entitled *Contact and frictional electrification* by the author. Helene Bertein has given an account of the mechanism of discharge of electric insulators due to ionization. By powdering the insulator with a mixture of sulphur and red lead, the author has developed an ingenious method for the study of charge distribution on the surface of the insulator. J. C. Cross has given a nice review of the properties of thermoelectrets which are produced by allowing a molten mass to solidify under an electric field. When the electric field is removed, the solid body retains surface charge of opposite polarity to the applied field. This charge slowly decays to zero and a charge of reversed polarity appears. The second charge persists for many years. D. K. Davies has given a clear picture of the static charge generation and the dissipation of charge from dielectric surfaces in a vacuum. 'Electrification by friction in a 3×10^{-7} torr. vacuum' by I. I. Inculet and F. P. Wituschek confirms some of the findings of D. K. Davies. J. Latham gives detailed account of atmospheric electricity.

Chapter II begins with the invited paper by A. Klinkenberg entitled 'Static electricity in liquids'. The importance of the subject stems from the movement of petroleum and allied things leading to vast accumulation of charge on account of low conductivity of petroleum and formation of explosive air-vapour mixture on account of its high volatility. The author has analysed the mechanism of charging and discharging with the help of charge carriers. M. D. Foster in his paper has given a mathematical treatment to explain the variation of conductivity of petroleum with the degree of charge accumulation in it. The distribution of petroleum from refinery to customer and refuelling of aircraft constitute potential hazards; a method to dissipate static charges as quickly as possible is of paramount importance in avoiding mishaps. N. Gibson and F. C. Lloyd give their results about the electrification of toluene in pipeline flow. The study of J. T. Leonard and H. W. Larhart about electrical discharges from a charged fuel leads one to devise means for making the fuel-less incendiary.

Chapter III encompasses the industrial implications of static electricity. The invited paper by N. J. Felici gives an elegant review of electrostatic precipitation with allied techniques and studies on electrostatic power converters. The papers by H. W. Cleveland and E. Heidelberg deal respectively with problems of sweeping away ions from a corona-stream and safety problem of fuel carried in tanks coated with non-conducting plastics to prevent corrosion. R. W. Hubbard discusses build-up of electrostatic charge in a belt of dielectric material running over earthed metal rollers. M. Point gives an excellent review about the latest developments in electrostatic spraying of paints and plastic powders.

All the papers in the book carry the latest information in their respective fields and will be equally useful to specialists of different branches and to general readers for a glimpse of the overall progress made in electrostatics.

D. BASU

MANY-BODY PROBLEMS AND OTHER SELECTED TOPICS IN THEORETICAL PHYSICS edited by M. Moshinsky, T. A. Brody & G. Jacob (Gordon & Breach Science Publishers, New York), 1966. Pp. vii+955. Price $ 49.00 (cloth), $ 15.75 (paper)

This volume incorporates notes on courses of lectures at the advanced graduate level delivered at the 1965 session of the Latin American School of Physics held in Mexico. Five series of lectures on the main subject of the school, namely many-body problems, and four courses on other topics are included.

The first lecture of the many-body series is 'Basic many-body concepts from a unified collective point of view' by G. Carmi. The emphasis here is on the distinction between 'collective' and 'individual' modes of a many-body system and the role of quantum mechanics in permitting the collective modes to be 'frozen out' at low temperatures, causing thereby long-range correlations which have an important bearing on the occurrence of phase transitions. An analysis of the electron gas in metals, from this point of view, leading to a discussion of superconductivity and a novel view of the phenomenon of melting of metals is presented.

In the next section on 'A short introduction to superconductivity theory', R. A. Ferrel introduces the superconducting transition as a singularity in the response of the superconductor to the formation of Cooper pairs, and then proceeds to discuss

many of the old and recent phenomena of superconductivity. The treatment is often heuristic and not really systematic.

The account of 'Collective excitations in nuclei' by S. T. Belyaev begins with a brief but lucid discussion of elementary excitations in some many-body systems including correlated pair-excitations of the type familiar in superconductivity. The treatment of the nuclear problem itself is through a self-consistent field method which leads to the possibility (in principle) of determining uniquely a collective Hamiltonian for nuclear excitations. The next section on 'Group theory and the many-body problem' consists of three reprints of articles on this subject by M. Moshinsky and his collaborators, giving a very clear presentation of group theory methods as applied especially to nuclear structure problems. Finally, B. F. Bayman discusses the 'Recent experimental tests of nuclear models' beginning with a brief discussion of representations of the rotation group and going on to a detailed comparison of various types of shell model calculations with experimental results, particularly on direct nuclear reactions. This concludes the treatment of many-body problems.

The remainder of the volume consists of four articles on diverse topics. 'The group SU_3 and elementary particles' by A. J. Macfarlane is a systematic and pedagogically very satisfying review. This is followed by 'Symbol manipulation techniques for physics' by T. A. Brody, a set of lectures dealing with computer languages, in particular LISP, aimed at labour-saving in the programming of routine algebraic calculations. Though intended for 'physicists with no previous computer experience', the material contains quite a bit of computer jargon which would deter those without any previous experience. The next section is a very lucid summary of both theoretical and experimental aspects of 'The Mössbauer effect and its applications' by J. Danon. A brief qualitative discussion of 'Critical phenomena' by M. S. Green forms the concluding section of the volume.

Presenting a summary of recent developments in a rather wide range of areas in theoretical physics, this volume has a great deal to offer to students and research workers in theoretical physics and would form a valuable addition to any library of physics at an advanced level.

P. M. MATHEWS

POLYMERS-MACROMOLECULES VISCOSITY-MOLECULAR WEIGHT STUDIES by A. R. Kemp (A. R. Kemp, 231 Prospect Avenue, Long Beach, California 90803, USA), 1967. Pp. ix+168

Dr Kemp, a consulting chemist in California who expired recently, had carried out extensive studies on osmotic and viscometric behaviour of solutions of high polymers. He came out with some unusual results which are in wide disagreement with existing results and viewpoints. Editors of journals naturally refused to publish such results and hence this book. Dr Kemp's osmotic experimental techniques were also quite different from those of others and his osmotic molecular weight values were unusually low. He finally proposed a new equation correlating viscosity with molecular weight, which he claimed to be a universal one, i.e. it holds for all polymer-solvent systems, provided the solvent is a 'maximum viscosity' solvent. There is no doubt that the accuracy and reproducibility of osmotic pressure measurements are not yet as high as one would desire it to be. Nevertheless, it is difficult to believe that the existing data are really as much in error as made out by Dr Kemp. Dr Kemp's thesis does not, therefore, appear convincing enough to be accepted, particularly as it is in variance with the accumulated experience of almost all workers in this field and so it appears that Dr Kemp would remain a lone crusader in this field.

SANTI R. PALIT

PHYSIOLOGICAL CLOCK by Erwin Bünning (Longmans, Green & Co. Ltd, London), 1967. Pp. 167. Price 24s.

It is only recently that the phenomenon of time measurement in living organism has attracted the attention of various workers. The publication of Cold Spring Harbor Symposium on Quantitative Biology, *Biological clock* in 1960, may be said to be an important publication on this subject in which information has been covered in a comprehensive manner. Although there have been a number of reviews and articles on the subject of biological clock, there has been a need for a book containing information on all the aspects in a simplified manner. This monograph is limited to the considerations of the biological measurements of time in which unicellular organisms as well as cells of higher plants, animals and man utilize period of approximately 24 hr. The subject matter of this book could very well be entitled as *Biological clock*; however, the author prefers the title *Physiological clock*, since he has emphasized the physiological aspects more in this publication. He has attempted to discuss the present state of our knowledge about the physiological nature of the biological clock and the manifold means by which the living organisms use this clock. The book is not designed for specialists who may be working in the area of biological clock. The author has done away with complicated terminology which might have made reading difficult for a non-specialist. The reader will find it very informative to go through this slender monograph.

The book contains some fourteen chapters. Various aspects of the biological clock such as the principle of endodiurnal oscillations, periodicity, fade out and its reinitiation by external factors, and effects of temperature and light have been discussed. A chapter on the models to explain the nature of the clock is also included. Biochemical and biophysiological aspects including enzymes and the effect of different chemicals on the rhythms have been dealt with in a separate chapter. There are other chapters which have discussed the use of the clock in direction finding and its response to external factors. In another chapter the author has included interesting information concerning the disturbances associated with the endogenous rhythms.

Although the book is very informative and the author has attempted to condense a great deal of material in this small volume, a reader not interested

in this field may find it straining to go through, unless he has developed a special liking for the subject.

G. S. SIROHI

THERMAL PERFORMANCE OF BUILDINGS by J. F. Van Straaten (Elsevier Publishing Co., Amsterdam), 1967. Pp. xiv+604

The main purpose of the book has been to outline the relevant principles of thermal and ventilation design of buildings. A large section of the data and design principles are for the conditions that exist under warm weather. Very little data on the functional design of tropical buildings are available in literature. In this context, therefore, the contents of this book will, by and large, satisfy the needs of the architects, engineers and builders.

Chapter 7, which deals with 'Heat transfer through opaque elements under periodically fluctuating conditions', is very useful for design of buildings in the tropics.

Chapter 9 on 'Thermal insulation' also is very interesting and useful. Thermal data on a large variety of materials needed by architects and designers are given. There is, however, no chapter which deals with measurement and computation of solar radiation. As this is a factor that causes most of the heat flow in tropical buildings, it could have been a valuable addition.

Chapter 11, on 'Moisture in buildings and its control', deals with different relevant problems very simply, both at medium and low temperatures.

Chapter 13 contains a very crisp description of the method of calculating cooling loads, but Eq. (7.7) on page 100 could perhaps be modified as

$$q = AU\left[(0_{sam} - 0_i) + f\frac{h_i}{U}(0_{sa} - 0_{sam})\right]$$

The description of natural ventilation in Chapter 14 will help the reader in understanding the mechanism of air flow inside and around the buildings. On page 238, Eq. (14.2), there appears to be a printing error. The words 'ft per min.' should read ft^3 per min. In the procedure for ventilation design, there is a simple equation [Eq. (14.7)] with which the minimum window area for a given amount of fresh air can be computed. In order to follow the 'Design procedure for summer air movement' (Art. 14.3.2, p. 255), knowledge of outdoor and indoor wind velocities is necessary. An empirical relationship, if provided, between the available wind velocity and window size, will greatly help the architects and designers.

Concluding, it may be stated that the book is very useful and valuable to the designers and architects of buildings in tropical regions.

N. K. D. CHOUDHURY

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

The handbook is based on the recommendations of IS 456-1964 for working stress method and covers the design of members in flexure, members subjected to axial load and bending, long columns, etc. Provisions for shear, bond, anchorage and torsion are also included. The material is presented in a very simple manner while retaining the necessary logic and argument. Various charts and tables are included which will be very much helpful in reducing the routine calculations in the design of simple reinforced concrete sections, after the forces and moments at the sections are known.

The term 'Design handbook' can be interpreted differently by each individual. However, in general, a design handbook would be expected to give the relevant information for the calculation of forces also in special structures, such as flat slabs, etc. It should also suggest the economic proportions, guidelines for the final choice from the alternatives and give typical details for the arrangement of reinforcement, etc. In the absence of such desirable additional information the present handbook should have been preferably titled as *Handbook for design of RC sections*. Even then, the handbook in its present form can serve as a very handy companion for students taking an elementary course in RC design. It will be of use to the practising engineers as well, as this is the only book giving charts and tables, based on the latest IS code. An example using the combination of bent up bars together with vertical stirrups for resisting shear should be added to the last chapter which will be of great practical importance.

The IS code does not insist on deflection calculations, if general requirement of span: depth ratio is satisfied. However, the handbook can incorporate some material on deflection calculations which may be required in some cases. An alphabetical index at the end will be quite useful for easy reference.

On the whole the book is very useful and can be recommended to all concerned. Its utility can be further increased by incorporating additional information as suggested above.

C. K. RAMESH

PUBLICATIONS RECEIVED

ADVANCED QUANTUM MECHANICS by J. J. Sakurai (Addison-Wesley Publishing Co. Inc., London), 1968. Pp. xii+336. Price 140s.

LECTURES ON THE THEORY OF NORMAL METALS by A. A. Abrikosov (Hindustan Publishing Corporation, New Delhi), 1968. Pp. xi+216. Price Rs 35.00

MEDICINAL PLANTS by S. K. Jain (National Book Trust, New Delhi), 1968. Pp. xii+176. Price Rs 5.75

DIFFUSION KINETICS FOR ATOMS IN CRYSTALS by John R. Manning (D. Van Nostrand Co. Inc., Princeton), 1968. Pp. xv+257. Price $ 9.75

EUROPIUM by Shyama P. Sinha (Springer-Verlag, Berlin), 1967. Pp. vii+164

NOTES & NEWS

Pulsers

The recent discovery of rapidly pulsating radio sources, tentatively named pulsers, in the constellation Vulpecula [*Nature, Lond.*, **217** (1968), 709] has revealed the existence of a new class of objects whose signals are repeated in short and remarkably precise intervals. In fact, the precision with which radio pulses are radiated from these pulsers was not witnessed till now in the case of any other known astronomical object. Each of the four pulsers detected so far has a characteristic interval between pulses; the longest is 1·3373 sec. for pulser 1 and the shortest is 0·2508 sec. for pulser 3. The pulse durations are also characteristic, 30 msec. for pulser 1 and 18 msec. for pulser 3.

The pulsers apparently emit signals at all frequencies. The signals take 32 sec. to reach the earth — the lower the frequency, the greater the time taken. This reveals that the pulsers are roughly 300 light years away from the earth and they appear to be within our galaxy. The power output is estimated at 10^{22} W.

An important feature of the pulsers is that the pulses of three of them are composed of two or three subpulses. The first subpulse is nearly identical for each of the three pulsers and closely matches the single pulse emitted by the fourth. These similar pulse structures, which lower the upper limit on the size of the objects to about 3000 km., indicate that similar physical mechanisms are responsible in all the known pulsers.

At first the pulsers were thought to be signals sent by an intelligent extraterrestrial civilization. This now seems very unlikely, specially when one considers the enormous power required for signalling over an entire spectrum, concentrating much power at frequencies masked by galactic noise and many such formidable arguments. A number of explanations, e.g. pulsations of a neutron star, rotation of a neutron star, a binary pair of neutron or white dwarf stars, etc., are offered, but none seems particularly satisfactory.

A new mounting for concave grating spectrometers

A mounting for an X-ray spectrometer which requires no movement of the grating or the electronic counter has been developed at the Osaka Electro Communication University, Neyagawa City, Osaka, Japan. At present, spectrometers use a fixed source and a movable counter and the development of sensitive electronic counters with delicate and complex cooling systems makes the movement of the counter undesirable. The new mounting makes it possible to use a fixed counter and the spectra can be obtained using a multiple channel scaler or displayed on a Braun tube directly.

The sketch of the new mounting is shown in Fig. 1. The direction of diffracted beams is controlled by the following two conjugate equations:

$$(\sin^2 \phi/\rho) - (\sin \phi/R) = -1/D$$
$$(\sin^2 \theta/r) - (\sin \theta/R) = 1/D$$

where D is a parameter to be chosen, and the others are as shown in the figure. When D is infinite, the equations reduce to the well-known Rowland circle. But for other values of D, the (R, θ) curve as well as its conjugate (ρ, ϕ) curve have different shapes. The shortest distance from the mid-point of grating G to a point on the $(\rho\text{-}\phi)$ curve lies in direction given by $\sin \phi = 2R/D$, where $D > 2R$. The same point also lies on the circle $\rho = -2R \sin \phi$, the centre of which is at C and which has a radius equal to that of the grating itself. This circle is called the 'Sawada circle'. When the source is put on the circle and moved back and forth along a straight line, which is a tangent at F to the curve (1-1′) in Fig. 1, so that the scanning direction is perpendicular to the vector GF, then diffracted spectra are always at the slit S of the counter. This point lies on curve (2-2′) in Fig. 1.

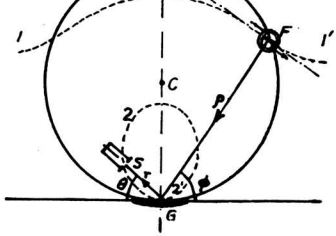


Fig. 1—Principle of Sawada mounting for concave grating spectrometer [C, centre of Sawada circle; G, grating; F, X-ray tube provided with a cathode ray deflector; S, slit of an electronic counter]

Analysis of the astigmatic and spherical aberrations shows that the aberrations are smaller in the Sawada mounting. Another merit of the Sawada mounting is that the total optical path-lengths are practically constant compared with the Rowland mounting, so that cumbersome intensity corrections can be avoided [*Nature, Lond.*, **217** (1968), 1247].

Zero-point bubbles in liquids

A theory attributing the long lifetime of positronium atoms in liquid helium to the fact that the positronium atoms are trapped in a 'zero-point bubble' was proposed by Ferrel [*Phys. Rev.*, **108** (1957), 167]. A direct measurement of the bubble size in helium and other liquids, when the entrapped particle is a positronium atom, has now been carried out [*Phys. Rev. Lett.*, **20** (1968), 493]. The principle involves the measurement of the small departure from 180° of the angle between the annihilating γ-rays. The width of the momentum distribution of annihilating photons (and hence of positronium atoms) is inversely proportional to the size of the bubble.

The curve obtained by plotting the angle between annihilation photons (milliradians) against coincidence counting rate showed a broad and a narrow peak, implying two momentum distributions. The narrow peak is attributed to singlet positronium atoms annihilating in the bubble state.

The bubble appears to have been formed by the repulsive interaction between a positronium atom and the atoms of the liquid. The size of the bubble has been determined by minimizing the Gibbs' free energy of the system with respect to the radius R following the equation:

$$G = 4\pi(R - Z_0)^2\sigma + P\,(4/3)\,\pi R^3 + E_{ZP} + G_{SP} + G_{BT}$$

where σ is the surface tension; $R-Z_0$, the radius of the surface of tension; and P, the pressure of the bulk liquid; E_{ZP}, G_{SP} and G_{BT} are the zero-point energy of the centre-of-mass motion of the positronium atom, the contributions to the free energy from the surface phonons of the bubble and the translational motion of the whole bubble with the positronium atom respectively.

The positronium atoms have been observed in the singlet state, $^1S_0$, decaying by two-photon emission with a lifetime $\sim 10^{-10}$ sec. The simple theoretical model fits bubble sizes fairly well over a wide range of liquid densities from argon to helium.

Novel apparatus for countercurrent distribution and gas chromatography

An apparatus for countercurrent distribution (c.c.d.), as simple as the absorption chromatography apparatus, has been reported from Syntex Research, Palo Alto, Calif., USA. The apparatus is likely to encourage wider use of c.c.d., which at present is used only when other separation methods fail to resolve the mixture in hand.

The apparatus (Fig. 1) consists of a glass tube (A) divided into cells by tightly fitting teflon discs (B), each having a hole at the centre. Alternatively, tube A may also be divided into cells by constrictions at short intervals made by a simple glass blowing operation. A large volume of the lower phase, e.g. formamide, is introduced into the apparatus at a position near C with a hypodermic syringe, each cell retaining a small portion of the phase while excess drains out of the tube. A solution of the mixture to be separated is placed in one or more cells at D and, with the tube A rotating, an upper phase, e.g. heptane, is slowly added at E from the funnel F. The components of the mixture pass through the system with the upper phase, while equilibrating in each cell with the lower phase and are finally eluted separately in the order of their partition coefficients between the phases in the same way as in ordinary c.c.d.

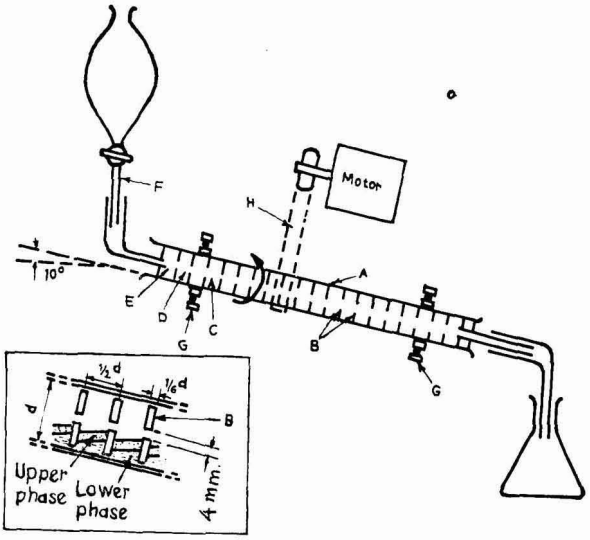


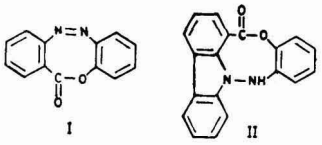
Fig. 1 — Apparatus for countercurrent distribution [A, glass tube; B, teflon discs; F, funnel; G, ball races; H, drive belt (rubber band); inset at bottom is an enlarged section showing cells and the two liquid phases; clamps and stands are omitted]

To adjust the partition coefficients to the useful range (0·01-0·5), water can be added to the lower phase or benzene to the upper phase. For efficient separation, the edges of the cells must be free from imperfections. 25 to 50 cells give sufficient separating power for normal organic syntheses.

Using alumina as the lower phase, the same apparatus can be used for absorption chromatography. Using a stream of nitrogen as the upper phase and a low viscosity liquid as the lower phase, gas chromatography can be performed for bulk samples [*Chemy Ind.*, (1967), 399].

1,6-Cycloaddition in dehydrobenzene

1,6-Dipolar cycloaddition in dehydrobenzene has been reported for the first time [*Tetrahedron Lett.*, (No. 23) (1968), 2743]. Benzenediazonium *o*-carboxylate, prepared from anthranilic acid, was warmed in acetone till gas evolution and left undisturbed for 1 hr when all the gas evolution ceased. The reaction mixture contained, besides benzoic acid and *o*-biphenylene, two new compounds, a yellow crystalline product, I, $C_{13}H_8O_2N_2$, mol. wt 220, m.p. 195-96°C. and colourless needles, II, $C_{19}H_{12}N_2O_2$, mol. wt $\sim$300, m.p. 200-200·5°C. By physical and chemical evidence the following structures have been assigned:



I II

Molecular models show that I can exist only in the boat form. Though the mechanism of formation of compound II is not yet certain, it could be imagined to have been formed by the 1,6-dipolar addition of dehydrobenzene with compound I.

Synthesis of perbromates

Perbromates, salts of heptavalent bromine, hitherto thought to be non-existent, have been synthesized at the Argonne National Laboratory, USA, by both chemical and electrolytic oxidation of bromates [*J. Am. chem. Soc.*, **90** (1968), 1900].

An electrolytic cell was set up with a platinum cathode immersed in 3 ml. of 2·8*M* $HClO_4$ in a porous porcelain cup and a rotating platinum microanode in a similar cup immersed in a slurry of Li_2CO_3 in 3 ml. of 2·8*M* $LiBrO_3$ tagged with 36 hr ^{82}Br. The two cups were placed in a container of 2·8*M* $LiClO_4$ and thermostated at −15°C. A total of 1 amp. hr of electricity was passed through the cell at a current density of 10 amp. $cm.^{-2}$. At the end of electrolysis 2 per cent of the bromine activity coprecipitated with $RbClO_4$ but did not coprecipitate with $Ba(BrO_3)_2$. The solution containing both bromate and perbromate was treated with HBr up to 1·5*M* to reduce bromate. Argon was bubbled through until all the bromine colour disappeared and did not return on standing. The solution was diluted with saturated HBr and titrated iodimetrically using NaI and thiosulphate. The titre agreed with the tracer results, each mole of perbromate consuming 8 equivalents of thiosulphate.

In the chemical oxidation method several hundred mg. of XeF_2 was stirred with 0·4*M* $NaBrO_3$; the resulting solution analysed 0·07*M* in perbromate. Bromate was precipitated with excess AgF at 0°C. and the ice-cold supernatant solution was made 0·5*M* in RbF, when $RbBrO_4$ was precipitated. The precipitate, after washing, was analysed for total bromine, bromate and perbromate. It analysed to $<2\times10^{-4}M$ in bromate; 0·0302*M* in total bromine and 0·242*N* in total oxidizing power. The ratio of oxidizing power to total bromine being 8·00 agrees well with the value expected for heptavalent bromine.

The mass spectrum of perbromic acid was also taken and compared with those of perchloric and periodic acids. The spectral intensities for perbromic acid peaks are intermediate between those of the other analogues. Perbromic acid was also found to be less volatile than perchloric acid as expected.

Central Drug Research Institute, Lucknow

The annual report of the institute for 1966 presents its main research activities during the year. Out of 232 medicinal plants collected during the year, 200 were screened pharmacologically. Over 520 new compounds were synthesized.

Experimental study of the problem of bleeding, occasionally occurring after the insertion of an intrauterine contraceptive device (IUCD), has indicated that it is traumatic in nature and is caused by increased vascularity of endometrium due to direct stimulation by the IUCD. No long-term effect on the physiology and metabolism of the uterus and fallopian tube has been observed in experimental animals. Hundred per cent reduction in fertility of female rats and mice has been observed on administering 2-phenyl-3-[*p*-(β-pyrrolidinoethoxyphenyl)] - 6 - methoxybenzofuran hydrochloride orally on days 1 to 5, or once on days 1, 2 or 3, post-intercourse. The minimum effective dose on a 5-day schedule was 4 mg./kg., and on a single dose regime it was 20 mg./kg. The antifertility effect was reversible. Progesterone, 16α-hydroxypregnenolone, Δ^{11}-pregnene-3,20-dione and 16α,17α-dihydroxyprogesterone acetophenide (Deladroxone) have shown effective antispermatogenic activity.

Several dichloroacetyl derivatives have been synthesized as possible antispermatogenic agents; of these, *p*-dichloroacetamidoacetophenone thiosemicarbazone has proved to be a strong inhibitor of spermatogenesis in rats. Podophyllotoxin obtained from *Podophyllum peltatum* has been shown to possess antimitotic and antitumour activities, while hydrazide of podophyllic acid has pronounced antispermatogenic activity.

Experimental hepatic amoebiasis has been produced in hamsters by a technique which ensures cent per cent infection and eliminates the chances of secondary infection. Boeck and Drabohlav medium for the cultivation of amoebae has been modified to use bovine serum in place of the expensive horse serum. Amoebic cysts have been found to become permeable to emetine on prior treatment with certain surfactants like sodium deoxycholate and sodium lauryl sulphate. It has been found that hartmannellid amoebae are pathogenic only in the presence of living *Esch. coli*. The pathogenicity of myxamoebae was established to be due to a heat-labile endotoxin.

Vaccinia virus (Bangalore strain) has been adapted to give tissue culture and encephalitis-producing strains; these two host virus systems are used for screening of antiviral agents. Immunological studies on crow filariasis indicated that eosinophils play a role in the removal of microfilariae. Investigation of the antigenic mosaic of the crow filarial worm suggests that protective immunity is produced by metabolic antigens rather than by somatic antigens.

Of the several compounds of 2,3-disubstituted benzo-6,7-quinazol-4-ones series synthesized, 3-amino-benzo-6,7-quinazol-4-one has been found to have tranquillo-sedative action comparable to that of chlordiazepoxide. A detailed study has been undertaken of structure-acivity relationship in a series of 1-pyridyl-4-substituted piperazines; one compound in this group showed good anticonvulsant activity in animal tests.

A method for the purification of mitochondrial monoamine oxidase (MAO) isolated from rat liver has been developed; the enzyme is obtained in crystalline form. About 150 N-substituted 1-aryl-, aryloxy- and thioaryloxy-3-amino-2-propanols in which the amino group either forms part of a ring system or is substituted by a 2-pyridyl, 2-piperidyl, ω-phenylalkyl or ω-phenylalkanol residue have been synthesized and screened for cardiovascular activity. Tetrahydro-2-amino-2-naphthoic acids have been prepared as possible hypotensive agents. Other polypeptides based on bacterial cell wall glycopeptides and ribonuclease have also been prepared.

In a study of the factors responsible for atherosclerosis it has been found possible to induce atherosclerosis and myocardial necrosis by a low protein diet with choline (50 mg./rat/day) in 3 months. Glucagon was found to stimulate mobilization of free fatty acids in rats. Nicotine induced hypercoagulability of blood in rats, but glucagon did not have any effect.

An attenuated, non-pathogenic mutant of *V. cholera* has been produced with the aid of a

chemical mutagen, N-methyl-N'-nitro-N-nitrosoguanidine.

Several new compounds have been isolated from indigenous medicinal plants. These include hayatidin from *Cissampelos pareira*, ankorine and allangimarckine from *Alangium lamarckii*, brahmic acid from *Centella asiatica* and crotsparine from *Croton sparciflorus*.

Among the medicinal plants screened pharmacologically, *Cocculus laurifolius* showed neuromuscular blocking activity. *In vitro* anticancer activity has been shown by five plants: *Semecarpus anacardium*, *Quercus semecarpifolia*, *Melia azedrach*, *Polygonum recumbens* and *Lyonia ovolifolia*.

FORTHCOMING INTERNATIONAL SCIENTIFIC CONFERENCES, 1968

Date	*Conference*	*Place*
7-11 Oct.	International Congress on Rheology	Kyoto
7-11 Oct.	International Foundry Congress	Kyoto
7-11 Oct.	International Conference on Modern Trends in Activation Analysis	Gaithersburg, USA
9-15 Oct.	International Congress and Exhibition for Instrumentation and Automation	Dusseldorf
13-19 Oct.	International Astronautical Congress	New York
15-19 Oct.	World Fertilizer Congress	Lisbon
19-27 Oct.	International Building Congress	Essen
21-25 Oct.	International Symposium on the Physico-chemical Mechanisms of Carcinogenesis	Jerusalem
7-9 Nov.	International Conference on Sodium Technology	Argonne
10-15 Nov.	International Conference on the Constructive Uses of Atomic Energy	Washington, DC
10 Nov. to 23 Dec.	International Geographical Congress	New Delhi
11-13 Nov.	International Symposium on Microelectronics	Munich
20-26 Nov.	International Automation and Instrumentation Conference and Exhibition	Milan

Journal of the Patent Office Technical Society

This journal is intended to serve as a medium for the dissemination of information relating to patent and design applications and patent cases. It also forms a forum for discussion of patent law and practice. Being the first publication of its type in India, it is expected to span the gap between the inventors and the industry. An annual publication (Price Rs 5.00) at present, the journal would be transformed into a quarterly, or even a monthly, during the coming years.

Ocean Engineering

This bimonthly journal, started in May 1968, by the Pergamon Press Ltd, Oxford, concerns itself mainly with the design, construction and operation of submersible vehicles and laboratories. It also covers the properties and fabrication of materials; stress analysis and hydrodynamic properties of shells; underwater instrumentation; and power and propulsion systems. The second, third and fourth issues of the journal are devoted to papers presented at the International Symposium on Materials — Key to Effective Use of the Sea held in New York during 12-14 September 1967. Annual subscription of the journal is £ 16 5s. 0*d*.

Announcements

▪ *The Second International Conference on Organolead and Organozinc Chemistry* will be held during 8 and 9 May 1969 at Utrecht, The Netherlands. The conference is being organized jointly by the International Lead Zinc Research Organization Inc., New York, and the Institute of Organic Chemistry, TNO, Utrecht. The conference will cover many fundamental as well as diverse applied aspects of either subject. Further information can be obtained from the Organizing Committee of the Conference, c/o Jaarbeurs, Utrecht, The Netherlands, or from the Indian Lead Zinc Information Centre, 5A Lord Sinha Road, Calcutta 16.

▪ *The Society of Nuclear Medicine, India*, has recently been formed and registered. Membership is open to all medical and non-medical graduates interested in various aspects of nuclear medicine. Membership fee is Rs 10.00 per annum and Rs 100.00 for life. Application forms for membership can be obtained from Prof. R. S. Satoskar, Radioisotope Laboratory, Seth G.S. Medical College, Parel, Bombay 12.

▪ *The Sixth Annual Convention of the Indian Chemical Society* will be held at Ahmedabad during 27-30 October 1968. Further particulars can be obtained from the Honorary Secretary, Indian Chemical Society, 92 Acharya Prafulla Chandra Road, Calcutta 9.

▪ *The 22nd Annual Technical Meeting of the Indian Institute of Metals* will be held in Calcutta during January 1969. A symposium on aluminium will also be held during the meeting. All papers presented at the meeting will be considered by the editorial board for publication in the *Transactions of the Indian Institute of Metals*. The papers published in the *Transactions* will be considered for the Kamani gold medal, the Binani gold medal and the Pandya memorial medal. Further details can be obtained from the Honorary Secretary, Indian Institute of Metals, 31 Chowringhee Road, Calcutta 16.

▪ *The Second International Conference on Medical Physics* will be held at Boston during 10-14 August 1969. The conference has been jointly sponsored by the US National Committee for Medical Physics and the International Organization for Medical Physics. Further details are available from Dr Edward W. Webster, Secretary-General, Massachusetts General Hospital, Boston, Mass. 92114, USA.

▪ *A Nuclear Physics and Solid State Physics Symposium*, organized by the Physics Committee of the Department of Atomic Energy, will be held at the Indian Institute of Technology, Powai, Bombay, during 28-31 December 1968. Further details can be obtained from Shri M. K. Mehta, Converer, Nuclear Physics and Solid State Physics Symposium Committee, Bhabha Atomic Research Centre, Nuclear Physics Division, Trombay, Bombay 74.

New Publications

FISH & FISHERIES

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by

DR G. KRISHNAN

Director, Zoological Research Laboratory, Madras University

This well-illustrated memoir provides information on 10 species of *Thyropygus*. *T. poseidon* Attems is described in detail, the account covering the following aspects: (i) external features; (ii) integument; (iii) skeleto-muscular system; (iv) alimentary canal; (v) blood-vascular system; (vi) excretory organs; (vii) fat body; (viii) repugnatorial glands; (ix) nervous system; (x) sense organs; (xi) neuro-secretory system; (xii) reproductive system; (xiii) larval development; (xiv) water relations; (xv) habit and habitat; and (xvi) affinities. The memoir includes a selected annotated bibliography and an exhaustive index. Instructions for practical work are given, which make the publication more useful for students.

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