Journal of Scientific & Industrial Research

J. sci. industr. Res. Vol. 25 No. 6 Pp. 231-282
June 1966
Published by the Council of Scientific & Industrial Research, New Delhi

DELCON

AIR and GAS LEAKS are LEAKS in PROFIT

LOCATE GAS LOSSES QUICKLY

MODEL 48
ULTRASONIC
TRANSLATOR DETECTOR

Hewlett-Packard Delcon Ultrasonic Translator Detectors bring new simplicity to the detection of air and gas leaks in either pressure or vacuum systems. As easy to operate as a flashlight, so even untrained personnel can detect leaks at a distance from the equipment under inspection. The directional probe quickly pinpoints the exact leak location more accurately than with old-fashioned bubble solutions. Great saving in cost and man hours has already been proven in hundreds of applications throughout the industrial world.

Positive detection in seconds — Hewlett-Packard Detectors locate leaks immediately in pressure or vacuum systems without costly set-up time, and are more accurate than many tape-burnt or sniffing techniques because their directional probe homes on the exact leak source. Indoors or out, even in high-noise or explosive environments the portable, battery operated HP detector can find leaks in bulk heads or inflange fittings.

Economy through efficiency — Bearings, flow in hydraulic lines and other friction producing apparatus also create ultrasonic energy detectable by the contact probe. In noisy environments where bearing noise is masked, the contact probe detects wear by converting ultrasonic energy to audible sound.

Corona discharging from high voltage electrical equipment generates ultrasonic energy. Therefore, the Ultrasonic Translator Detector is an ideal device to locate arcing in power stations, genera. rs, bushings, insulators and other associated equipment.

DELCON — a division of HEWLETT-PACKARD CO.

For details, please write to :

SOLE DISTRIBUTORS

THE SCIENTIFIC INSTRUMENT COMPANY LIMITED

ALLAHABAD BOMBAY CALCUTTA MADRAS NEW DELHI

Head Office: 6 Tej Bahadur Sapru Road, Allahabad

SICO 1911–1966

The Journal of Scientific & Industrial Research is issued monthly.

Communications regarding contributions for publication in the Journal, books for review, subscriptions and advertisements should be addressed to the Editor, Journal of Scientific & Industrial Research, Publications & Information Directorate, Hillside Road, New Delhi 12.

Annual Subscription

A : For Libraries, Government Departments and Industry Rs 15.00 (inland); £ 3.10.0 or $ 10.00 (foreign)

B : For individuals Rs 11.25 (inland); £ 2.5.0 or $ 8.50 (foreign)

Single Copy

Rs 2.00 (inland); 6s. or $ 1.50 (foreign)

Payments in respect of subscriptions and advertisements may be sent by cheque, bank draft, money order or postal order marked payable to Publications & Information Directorate, Hillside Road, New Delhi 12.

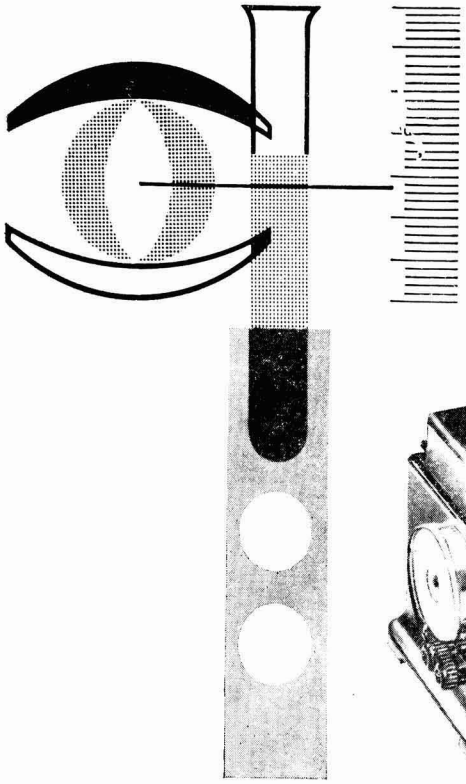
Sole Distributors Outside India
PERGAMON PRESS
Oxford London Paris Frankfurt New York

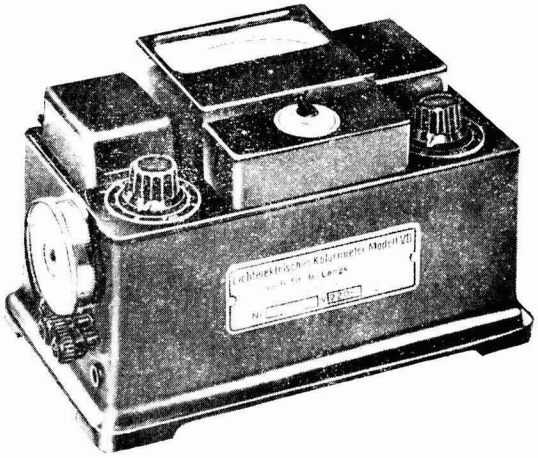
Journal of Scientific & Industrial Research

VOLUME 25 NUMBER 6 JUNE 1966

CONTENTS

For Index to Advertisers, see page A17





Indispensable in Research and in Practice . . .

Photoelectric Colorimeter UK VII S

according to Dr. B. Lange

It operates on the principle of deflection and compensation

- for individual, mass and continuous measurements
- for colorimetric, turbidity and fluorescence measurements
- for photoelectric titrations
- for amounts of liquid from 0.1 ml to 100 ml

Direct Reading and Recording

DR·B·LANGE BERLIN

ELEKTROPHYSIKALISCHE WERKSTÄTTEN
117 Berlin-Köpenick, Grünauer Strasse 101a
German Democratic Republic

AGENCY

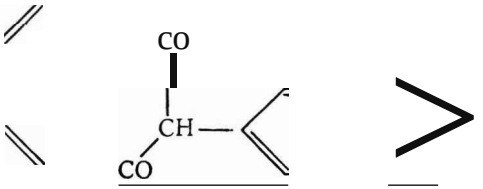
MESSRS K. LAL BHAKRI
P.O. Box 487, New Delhi, India

Coronary Thrombosis

A development in the treatment of coronary thrombosis and deep venous thrombosis has been the introduction of

BINDAN

(2-Phenyl-1: 3-indanedione)



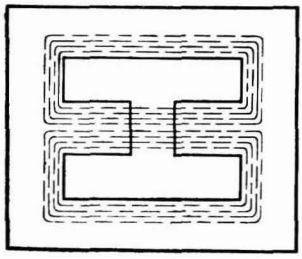
In oral anticoagulant therapy

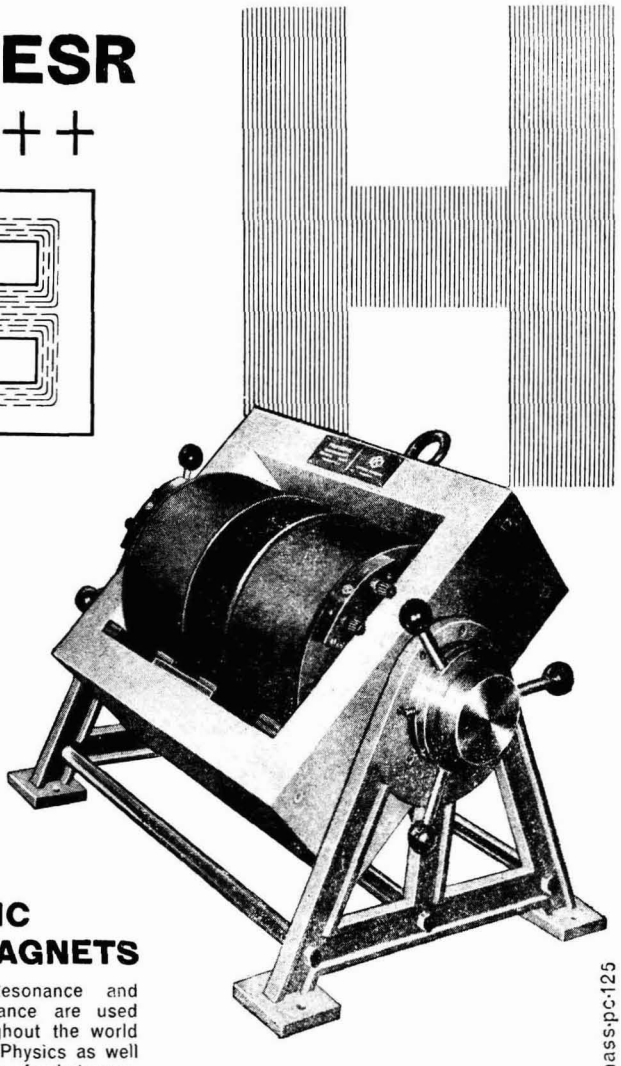
BINDAN provides
* more rapid onset of action
* easy management
* uniformity in action

Manufactured by

BENGAL IMMUNITY
COMPANY **LIMITED**
CALCUTTA 13

NMR . ESR
μWS++





mass-pc-125

POLYTRONIC ELECTROMAGNETS

Nuclear Magnetic Resonance and Electron Spin Resonance are used more and more throughout the world for basic research in Physics as well as in chemical analysis of substances. Both **NMR** and **ESR** experiments require reliable Electromagnets capable of producing High Fluxdensity with continously variable air-gap facility. Polytronic 'H' Type Electromagnets fulfil these exacting requirements.

Polytronic 'H' Type Electromagnets are available from 75 mm to 150 mm Pole sizes with **Constant Current Regulators.**

for electronics, science and technology

POLYTRONIC CORPORATION
20A, Shakti Sadan, Junction of Tardeo Bridge & Lamington Road, Bombay-7 WB.

INDEX TO ADVERTISERS

Current Topics

Afro-Asian Collaboration in Science & Technology

THE Symposium on Collaboration between Afro-Asian Countires for Promotion and Utilization of Science and Technology (CAAUST), organized by the Association of Scientific Workers of India and held in New Delhi during 25 April to 2 May 1966, marks an important step in creating the necessary climate and machinery for generating self-confidence and self-reliance in the Afro-Asian countries to meet the complex challenges posed by their development plans and programmes. Conceived at the time of the symposium on 'Science and the Nation' [This Journal, **23** (1964), 359] held earlier (July 1964) in New Delhi, the broad objectives of the CAAUST symposium were : to provide opportunities for exchange of information, discuss common problems, work out a machinery for frequent contacts, promote the commitment to science and technology in developing countries, exchange information on patterns of international assistance, and finally prepare joint programmes for seeking international assistance in the field of science and technology. In the words of Dr S. Husain Zaheer, Director-General, Scientific & Industrial Research, and Convener of the Preparatory Committee for the CAAUST Symposium, "the holding of the symposium was based on the belief that each country and nation has its own path of development. It seeks to break the isolation of scientists from neighbouring countries from each other. It hopes to build fresh and continuing contacts between scientists of different countries and initiate a process of collaboration in all spheres of science and technology on the basis of concrete proposals. The symposium is organized around the belief that apart from indigenous effort in each country, mutual aid and assistance for development of science and technology is possible. But perhaps more important than that, the symposium might initiate steps towards exchange of information to enable the preparation of an Afro-Asian plan for receiving international assistance." The decisions and recommendations that emerged from the symposium should pave the way for a greater measure of co-operation and collaboration between these countries in the fields of science and technology.

Attended by about 270 delegates from 40 countries, besides observers from Unesco, FAO, OECD and the World Federation of Scientific Workers, the symposium was addressed among others by Shrímati Indira Gandhi, Prime Minister of India, Dr Zakir Husain, Vice-President of India, Shri M. C. Chagla, Union Minister for Education and Scientific Research, Prof. V. K. R. V. Rao and Prof. P. C. Mahalanobis. Over 200 papers were presented at various sessions devoted to (1) National Science Policy, Planning, Organization and Financing of Scientific Research, Order of Priortity in Scientific Development, Coordination of Scientific Activities and Integration of Science with the Needs of the Country; (2) Communication of Scientific Information and Utilization of Results of Research; (3) Education and Training of Scientific and Technological Personnel; (4) History of Science, Development of Scientific Societies, Creation of Scientific Outlook and Popularization of Science; (5) Equipment and Text-books; (6) Medical; (7) Agriculture; (8) Engineering; (9) Power and Atomic Energy; (10) Basic Sciences; (11) Housing; and (12) System of Aid from International Agencies and Their Critical Evaluation, Suggestions for Better Methods, etc. Reports from a dozen countries on the state of science and technology, particularly research, teaching and training facilities were presented.

While deliberations at the technical sessions were more concerned with generalities, the eleven commissions which met on the penultimate day dealt mostly with practical aspects of collaboration between the participating countries. There were separate commissions on Science policy; Scientific and technical information; Education and training; History and popularization of science; Technical aid from international agencies; Medicine and health; Agriculture, irrigation and food; Natural resources; Power and atomic energy; Energy and industrial research; and Housing and town planning. The commissions covered a wide ground and their recommendations were aimed at laying the foundations for joint activity. Several recommendations dealt with organizational matters. The main recommendations related to the need for establishing (i) Joint Scientific Boards on a bilateral or multilateral basis for the purpose of scientific collaboration; and (ii) an Afro-Asian Association for the Advancement of Science and an Afro-Asian Centre for the Coordination of activities. The Commission on Medicine and Health recommended the establishment of Asian and African Health Organizations in place of the existing WHO regional offices. The need for creating suitable machinery to ensure effective and speedy exchange of information in science and technology between the various countries in the region was stressed by almost all the commissions. Realizing the diversity of conditions prevailing in different countries, the Commission on Science Policy felt that no single model in respect of science policy was applicable to all the countries and that each country should work out a model suited to its needs.

Formulation of plans and policies in respect of international aid received by the participating countries from various agencies and countries was

one of the important items that came up for consideration. It was recommended that foreign aid received should have an intimate bearing on the requirements of the country and should not conflict with its socio-economic policies. While stressing the need for coordinating all international aid, it was pointed out that the foreign aid should be primarily concerned with the training of technologists in each country and procurement of equipment.

Other major recommendations worthy of notice are : (1) establishment of national science publication agencies charged with the responsibility of producing standard, well-produced and low-cost scientific and technical books and other literature for different levels of education; (2) compilation of national registers giving information on scientific and technological institutions, facilities available for scientific and technical education and training in research, scientific and technical manpower available, industrial-cum-training centres or organizations imparting in-plant and in-service training; (3) drawing up of common programmes of research on disease patterns, screening of natural products, infestation control, survey and exploitation of natural resources; and (4) exchange of scientists, postgraduate students, medical experts and teachers so as to build up a cadre of men and women who understand the problems of the whole region. It was also decided to take necessary steps towards the implementation of the decisions and recommendations of the symposium. A permanent committee, with one member from each of the participating nations and provision to opt members' from other countries which did not send representatives to the CAAUST symposium was set up to continue the work of organizing and strengthening Afro-Asian collaboration in science and technology and to continuously indicate the areas of collaboration to all official and non-official agencies in Afro-Asian countries. A 16-member Executive Committee was also formed to look after the implementation of the programmes formulated.

In spite of the organizational difficulties and the short time at the disposal of the organizers, the symposium achieved a good measure of success. Such conferences should be planned more meticulously and a good deal in advance to achieve significant results. In multi-nation conferences of this type, the active interest and encouragement of the governments of the participating nations is essential for the implementation of the recommendations of the conferences. This calls for closer association of representatives of the governments concerned in future conferences. The discussions must be based on more specific and concrete material available to the delegates in the form of key papers prepared sufficiently in advance about the state of science and technology in each country, what each country requires in terms of technical expertise and equipment and what it can offer to others. This would ensure objective and critical discussion of the problems to be tackled and help in arriving at workable solutions.

Indo-Yugoslavian Scientific Cooperation Agreement

The Council of Scientific & Industrial Research and the Federal Council for the Coordination of Scientific Activities of the Socialist Federal Republic of Yugoslavia signed, in New Delhi on 1 March 1966, a five-year agreement for scientific cooperation, and promotion and expansion of relations pertaining to science and scientific promotion of technology. The salient features of the agreement are: (i) cooperation in scientific researches by scientific research institutions which are collateral according to their scientific profile and corresponding best to their possibilities and to the experience of scientists to the technical outfit and other conditions necessary for solving definite scientific problems; (ii) exchange of scientists so as to ensure scientific researches, consultations, exchange of experience, scientific improvement, participation in scientific meetings, delivery of lectures, assistance in organizational and scientific matters; (iii) training of junior scientists and providing the trainees with opportunities for participating in scientific investigations and similar activities; (iv) mutually inviting delegates for attending national scientific meetings, congresses, conferences, advisory meetings, symposia and seminars; (v) inviting research scientists and university professors for holding lectures, consultations and carrying out surveys for short periods; (vi) exchange of information on the organization of scientific research work, data about the scientific institutions and scientists as well as information and material of interest; (vii) mutual assistance in publication of scientific papers, and the results and development of scientific work of the other countries in their countries' scientific periodicals; (viii) mutual support for acquisition of scientific equipment, literature, microfilms, photo copies and other necessary materials for scientific research and also, whenever feasible, organizing of science exhibitions; (ix) encouraging the cooperation among scientific libraries and scientific institutions in the exchange of books, periodicals and bibliographies; and (x) encouraging direct cooperation among those Yugoslav and Indian institutions whose activities include publishing of scientific literature.

Symposium on Radioactivity & Metrology of Radionuclides

K. G. VOHRA
Atomic Energy Establishment, Trombay, Bombay 74

THE All India Symposium on Radioactivity and Metrology of Radionuclides organized by the Atomic Energy Establishment, Trombay, was held at Trombay, Bombay, during 14-18 March 1966. The papers and invited lectures included in the symposium covered diverse topics, including radiation dosimetry and standards, environmental radioactivity, life sciences and techniques of measurement and their applications in prospecting for uranium and thorium. Forty-six papers were presented in eight sessions including three invited lectures. Twenty-two of these papers presented were from institutions outside Bombay. Of the 74 delegates who participated in the symposium, 24 were from 16 institutions outside Bombay. Many staff members in addition to the 50 delegates from the Trombay Establishment also attended the sessions of their interest.

Techniques and Instrumentation

The papers on techniques and instrumentation dealt with a variety of methods successfully adopted in the country. These included the use of proportional counters for absolute measurements, potentiality of liquid scintillation counting, fast neutron monitoring, gamma-ray spectrometry, alpha spectrometry and isotope scanning instruments and techniques applied to measurements on gaseous, liquid and solid samples. Stimulating discussions were held on gamma-ray spectrometry techniques, liquid scintillation counting and absolute measurement methods.

Environmental Radioactivity

The session on environmental radioactivity included papers on natural radiation environment and environmental contamination from nuclear weapon tests, both of which enable us to put into a proper perspective different aspects of radiation and radioactive substances in their widespread applications. A vivid example of this was provided by Prof. C. A. Mawson in his opening lecture of this session when he gave a comparison of the activities on the Canadian NPD reactor air inlet filters and the stack filters. Radioactive aerosols from the normal outside air gave higher activity on the inlet filters as compared to the activity on the stack filters due to the discharge of effluent air from the reactor.

Radioactivity in Life Sciences

The papers on radioactivity in life sciences gave an idea of the progress made in the country in the use of isotope techniques in biological and medical sciences using a variety of isotopes. Papers in this session gave an account of the studies carried out with different isotopes such as I-131, P-32, Au-198, Co-60, Fe-59, Po-210 and Pb-210. There were also papers presenting interesting observations on the mechanism of exchange of I-131 with certain enzyme systems which are important in the biosynthesis of thyroxine, effect of ^{32}P injection in inducing certain biological reactions in the bone marrow in rats, medical applications of ^{198}Au in malignant diseases, studies on the changes in the fish liver as a result of internal irradiation with ^{60}Co, studies on the mechanism of uptake of radioisotopes by plants with ^{210}Po and ^{210}Pb, and kinetics of radioisotopes in biological systems.

Prospecting Techniques

The session on techniques in prospecting included papers on identification of uranium and thorium, age determination, aerial prospecting and radon measurements. The symposium revealed the commendable activity in this field of development of new techniques of prospecting in the country.

Invited Lectures

The invited lectures by Prof. P. B. Price from General Electric, USA, and Prof. C. A. Mawson from the AECL, Canada, provided an international touch to the symposium. The new technique of detection of nuclear charged particles by means of tracks in insulating solids described by Prof. Price is yet another milestone in the history of methods of detection of charged particles. This technique holds a great promise of finding useful applications in the metrology of radionuclides and in the analysis of trace quantities of materials. The lecture by Prof. Mawson on environmental radioactivity illuminated the possibilities in the use of simple inexpensive techniques in environmental monitoring which should be of particular interest in this country.

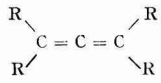
The symposium was unique in that it brought together diverse disciplines involving the participation of physicists, chemists, biologists, members of the medical profession, geologists and hydrologists.

Although the papers presented at this symposium represented only a small cross-section of the overall effort made in the country, they provided an indication of the transformation which has taken place since the days when handling of radioactive materials was dreaded. This has been to a large extent due to the widespread educational programmes and the health physics assistance provided by the Atomic Energy Establishment, Trombay, to different institutions in the country, as well as due to the availability of isotopes and electronic instruments from the Trombay Establishment.

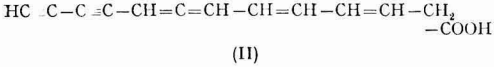
Recent Progress in the Chemistry of Allenes

D. DEVAPRABHAKARA & P. S. SAMPATHKUMAR
Department of Chemistry, Indian Institute of Technology, Kanpur

ALLENES are a class of dienes containing double bonds in 1,2-positions (I). Thus they contain a highly reactive unsaturated system present in a chain or a cyclic system. They are of interest because they are not only monomers for polymerization but also unique chemical intermediates. Though a great deal is known about the isomeric acetylenes, relatively little is known about allenes. Many of the reactions are still to be investigated. The stereochemistry is another interesting feature in this unusual unsaturated system. Only limited numbers of natural products containing allenic system are known. One of them is the antibiotic mycomycin (II).



(I)

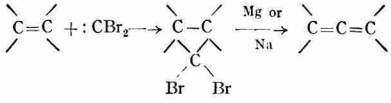


(II)

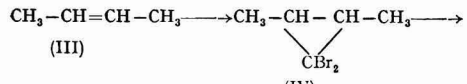
Synthesis of Allenes

Various unambiguous methods of synthesizing allenes have been reported in the literature prior to 1958. There are a number of disadvantages and uncertainties in these methods. Further, they do not lend themselves to the synthesis of both acyclic and cyclic allenes.

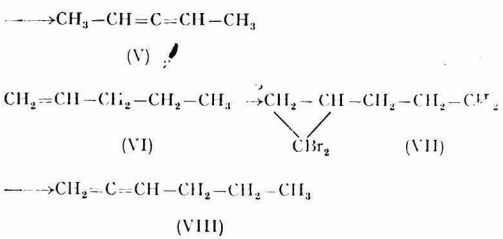
In 1958, Doering and LaFlamme[1] reported a novel two-step synthesis of allenes from olefins. The first step involves the addition of dibromocarbene to an olefin and the second involves the reaction of the resulting substituted 1,1-dibromocyclopropane with magnesium or sodium to give the allene. Thus the overall structural change involves the insertion of a single carbon atom between the two of the original double bond.



1,1-Dibromocyclopropanes were prepared by these workers conveniently in one step through the reaction in which an olefin is treated with bromoform and potassium *tert*-butoxide. Thus they were able to synthesize 2,3-pentadiene (V) and 1,2-hexadiene (VIII) from 2-butene (III) and 1-pentene (VI) respectively in 40-65 per cent yield.

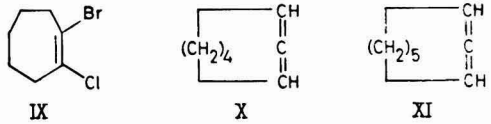


(IV)

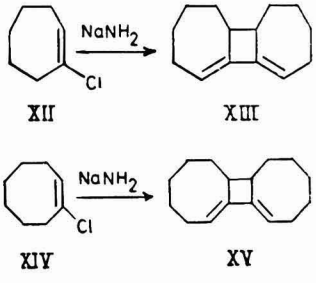


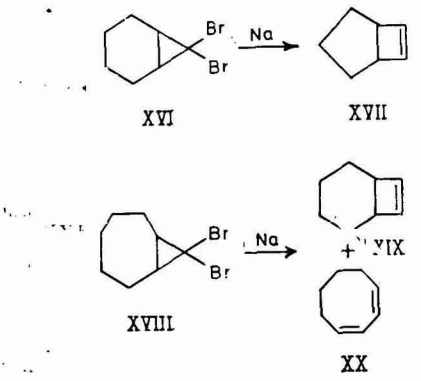
Yields were found to be better with sodium than magnesium. This two-step method has the advantage of being adaptable to the synthesis of both acyclic and cyclic allenes since the starting material is an olefin which is readily available. The only disadvantage is the formation of a small amount of isomeric acetylene when magnesium metal is used.

Cyclic allenes were first described by Favorskii[2] and Dominin[3] who claimed to have prepared 1,2-cycloheptadiene (X) by the action of sodium on 1-bromo-2-chlorocycloheptene (IX). Attempts by other workers, using more general methods, to prepare 1,2-cycloheptadiene (X) and 1,2-cyclooctadiene (XI) were not successful.



Ball and Landor[4,5] were able to obtain only dimers (XIII) and (XV) by dropwise addition of 1-chlorocycloheptene (XII) and 1-chlorocyclooctene (XIV) respectively to sodamide in liquid ammonia. They also attempted to prepare 1,2-cycloheptadiene (X) and 1,2-cyclooctadiene (XI) using the procedure of Doering and LaFlamme[1]. The addition of 7,7-dibromobicyclo[4.1.0]heptane (XVI) to sodium dispersion on alumina gave bicyclo[3.2.0]hept-2-ene (XVII) while the products from 8,8-dibromobicyclo-[5.1.0]octane (XVIII) were bicyclo[4.2.0]octa-2-ene





(XIX) and 1,3-cyclooctadiene (XX). These results suggest that seven- and eight-membered cyclic allenes dimerize or rearrange internally when adsorbed on alumina.

By a similar procedure, Ball and Landor[4,5] were able to synthesize 1,2-cyclononadiene (XXII) and 1,2-cyclodecadiene (XXIV) from 1-chlorocyclononene (XXI) and 1-chlorocyclodecene (XXIII) and also by the addition of 9,9-dibromobicyclo[6.1.0]-nonane (XXV) and 10,10-dibromobicyclo[7.1.0]-decane (XXVI) respectively, to sodium dispersion on alumina (Chart 1).

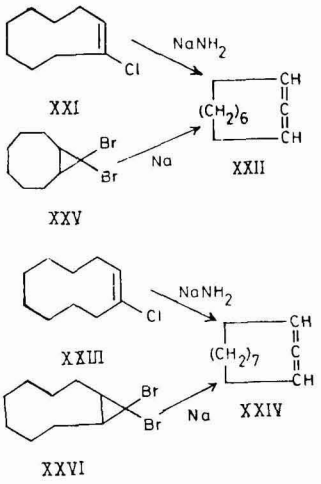


Chart 1 — Synthesis of 1,2-cyclononadiene (XXII) and 1,2-cyclodecadiene (XXIV)

Molecular models show little, if any, overlap of one pair of *p*-orbitals in 1,2-cycloheptadiene (X) and only little overlap in 1,2-cyclooctadiene (XI). Models of 1,2-cyclononadiene (XXII) and 1,2-cyclodecadine (XXIV) can be constructed with little difficulty.

Quite recently Moore and Ward[6] reported an elgant conversion of 1,1-dibromocyclopropanes to allenes using methyllithium or butyllithium. They were able to synthesize 2,3-heptadiene, 1,2-undecadiene and 1,2-cyclodecadiene in 70-90 per cent yields.

The generality of the reaction of Moore and Ward[6,7] was later extended by Skattebol[8,9] who prepared 2-methyl-2,3-pentadiene, 2,4-dimethyl-2,3-pentadiene, 1,2,6-heptatriene, 1,1-diphenyl-1,2-propadiene, 1-phenyl-1,2-propadiene, 1,2-cyclononadiene and 1,2,6-cyclononatriene starting from a suitable olefin in each case. Of particular interest is the synthesis (Chart 2) of two cyclic diallenes (XXIX) and (XXXII) from 1,5-cyclooctadiene (XXVII) and 1,8-cyclotetradecadiene (XXX) via 9,9,10,10-tetratricyclo[7.1.0.0]decane (XXVIII) and 15,15,16,16-tetrabromotricyclo[13.1.0.0]hexadecane (XXXI) respectively.

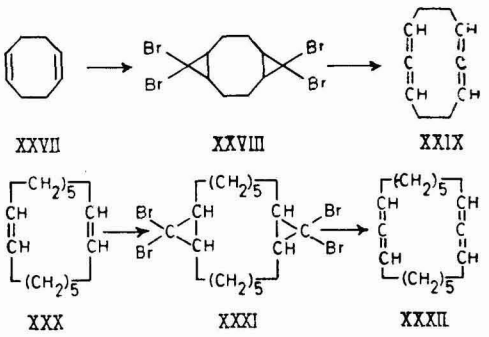


Chart 2 — Synthesis of cyclic diallenes (XXIX) and (XXXII)

Logan[10] found that 1,1-dichlorocyclopropane derivatives react with butyllithium to give only low yields of allenes accompanied by a multiplicity of other products.

Recently, Craig and Moyle[11] have described a new method of preparing allenes from the enol phosphates. Enol phosphate R′—CH=CROPO $(OEt)_2$ is readily converted into an allene on treatment with sodamide in liquid ammonia when the enol phosphate contains a methylene group adjacent to the central carbon-carbon double bond, and the elimination requires the presence of a double bond in the group R′ in conjugation with the olefinic linkage in the enol or, when R′ is equal to H in the group R.

Although the last two reported synthesis of allenes are the new methods of making allenes, the methods are not promising in view of the better ones already described.

Mechanism of Formation

Various possible mechanistic pathways (Chart 3) have been proposed by Doering and LaFlamme[1] in the conversion of 1,1-dibromocyclopropane derivative (XXXIII) to an allene (I) by metals like magnesium and sodium. Though a particular path cannot be decided with certainty, two of the paths shown in Chart 3 seem to be unattractive.

The first scheme depicts the opening of an intermediate cyclopropyl radical (XXXIV) to the corresponding allyl radical (XXXV). Although this kind of cleavage occurs at high temperature, it does not occur in the low temperature photochemical chlorination of cyclopropane to cyclopropyl chloride[12].

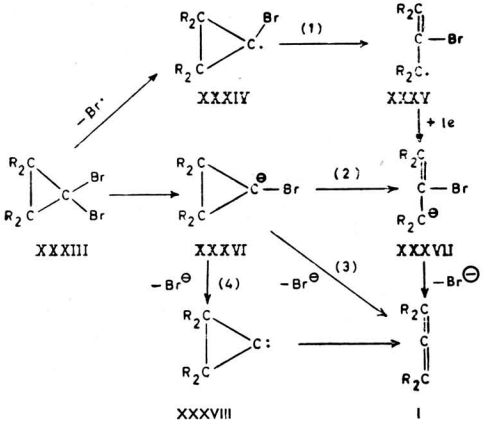


Chart 3 — Various pathways (1-4) for the conversion of 1,1-dibromocyclopropane to allene

Hence, the radical scheme does not seem to be attractive. The second scheme, involving cleavage of the cyclopropyl carbanion (XXXVI) to allyl carbanion (XXXVII), also seems unlikely, in view of the failure of cyclopropyl carbanion itself to isomerize[13]. The third scheme involving the simultaneous loss of halide ion with ring opening from carbanion seems to be a possibility. The last scheme, where the loss of the halide ion leads to carbene intermediate (XXXVIII) and finally to allene (I), seems to be far more attractive.

In the reaction of 1,1-dibromocyclopropane derivative (XXXIII) with methyllithium (Chart 4), the first step in the reaction can be depicted as a halogen-lithium interconversion with the formation of 1-lithio-1-bromocyclopropane derivative (XXXIX). The exact mechanism of the reaction is not fully understood. It is expected that this intermediate would readily eliminate lithium bromide to form allene (I). This may occur in two different mechanisms: (a) concerted elimination and ring opening to allene (I) and (b) α-elimination to the carbene

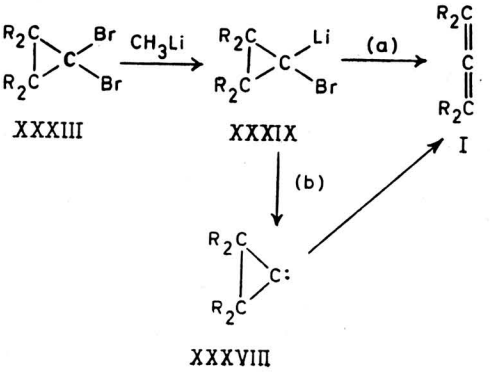


Chart 4 — Mechanism of the reaction between 1,1-dibromocyclopropane (XXXIII) and methyllithium leading to the formation of allene

intermediate (XXXVIII). Path (a) may well be the way by which the allenic product is formed. However, the formation of a carbene intermediate (XXXVIII) can be tested through addition and insertion reactions of carbenes.

Accordingly, a number of 1,1-dibromocyclopropanes were treated with methyllithium in the presence of an olefin like isobutylene, but no spiro pentanes were detected. But in 1961 Moore *et al.*[14] have been able to trap the carbene intermediate (XL) in the reaction of 7,7-dibromobicyclo[4.1.0]-heptane (XVI) with methyllithium employing cyclohexene as a trapping agent (Chart 5). The treatment of 7,7-dibromobicyclo[4.1.0]heptane (XVI) with methyllithium in ether-cyclohexene at −80° gave a mixture of products which contained a white solid 7,7′-{bicyclo(4.1.0) heptylidene} (XLI) and a liquid 7,7′-spirobi{bicyclo(4.1.0)heptane} (XLII). In the absence of cyclohexene, only (XLI) was obtained. This suggests that the formation of spiro pentane may involve a carbene intermediate (XL). Further investigation by the same workers gave evidence that the reaction of 7,7-dibromobicyclo[4.1.0]heptane (XVI) with methyllithium gives rise to carbene (XL). Using diethyl ether as the solvent they were able to isolate three insertion products (XLIII), (XLIV) and (XLV). Of greater interest is the fact that the carbene (XL) can undergo intramolecular insertion to produce highly strained compounds (XLIV) and (XLV).

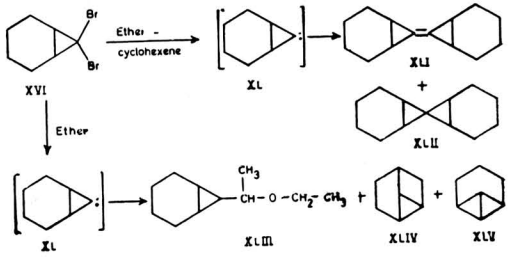


Chart 5 — Reaction of Moore *et al.*[14], providing evidence for the carbene intermediate

Later Skattebol[15] also came with an evidence of a carbene intermediate in the reaction of 1,1-dibromo-2-(but-3-enyl) cyclopropane (XLVI) with methyllithium. When this reaction was carried out at −78°, he obtained two isomeric products. The major component was identified as the allene 1,2,6-heptatriene (XLIX) while the other has been assigned the structure tricyclo[4.1.0.0^{4,6}]heptane (XLVIII) on the basis of physical and chemical data. The formation of these isomeric products may be visualized through the carbene intermediate (XLVII) as shown in Chart 6.

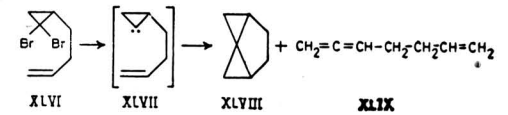


Chart 6 — Reaction of Skattebol[15], providing evidence for the carbene intermediate

At present it is impossible to choose between the two reaction paths of Moore *et al.* and Skattebol, both of which might lead to the formation of allenes. It is indeed reasonable to assume that both mechanisms are operating. It is hoped that future work will shed some light on the mechanism of this reaction.

Spectral Data

Wolf and Mancuso[16] have concluded after the infrared study of 58 allenic compounds that bands at 1950 and 850 cm.$^{-1}$ are characteristic of allene bond system with certain variations due to substitution. The antisymmetrical stretching frequency at 1950 cm.$^{-1}$ appears as a doublet when the allene group is terminal and is substituted by an electron attracting group (—COOH, —COOR, —$CONH_2$, —COCl, —COR, —CF_3, —CN). The band at 850 cm.$^{-1}$, which is due to torsional motion of an allene terminal methylene group, is good evidence for the presence of a terminal allene. This has an overtone at 1700 cm.$^{-1}$ of low intensity.

When the double bonds are adjacent as in allenes the ultraviolet absorption is greatly intensified in the longer wavelengths but is still far less than that of a conjugated system[17]. For example, 1,2-pentadiene shows $\lambda_{max.}^{hexane}$ 2270 (ϵ, 630) and $\lambda_{max.}^{vapour}$ 1700 (ϵ, 4000).

In NMR the allenic protons appear to absorb at higher frequency by 0·3 ppm than those of the analogous olefins. The region for non-terminal and terminal allenes are 5·2 τ and 5·6 τ respectively[18]. Snyder and Roberts[19] have shown recently the existence of long-range nuclear spin-spin coupling in some allenic systems.

Reactions of Allenes

The more important reactions of allenes studied so far, viz. addition, reduction, isomerization and polymerization, are discussed in the following sections.

Addition Reactions

Fedorova[20] has studied the bromination of 2,3-nonadiene (L) and 6-methyl-2,3-heptadiene (LII) in chloroform at —40°. The allenes give predominantly 3,4-dibromo-2-nonene (LI) and 3,4-dibromo-6-methyl-2-heptene (LIII) respectively.

$$\underset{\text{(L)}}{CH_3-(CH_2)_4-CH=C=CH-CH_3} \xrightarrow{Br_2} CH_3-(CH_2)_4-\underset{\text{(LI)}}{CHBr-CBr=CH-CH_3}$$

$$\underset{\text{(LII)}}{CH_3-CH(CH_3)-CH_2-CH=C=CH-CH_3} \xrightarrow{Br_2} CH_3-CH(CH_3)-CH_2-\underset{\text{(LIII)}}{CHBr-CBr=CH-CH_3}$$

Jacobs and Dankner[21] have carried out the addition of hydrogen chloride to allenes. The addition of hydrogen chloride to 1,2-butadiene (LIV) involves attack at the terminal methylene group of the allenic system. 1,2-Butadiene (LIV) gives a mixture of *cis*- and *trans*-2-chloro-2-butenes (LV) in the ratio 1:6·7. The orientation of addition of hydrogen chloride to the allenic bond is reversed when one end of the system is disubstituted, attack occurring at the middle carbon. 3-Methyl-1,2-butadiene (LVI) gives a mixture of 3-chloro-3-methyl-1-butene (LVII) and 1-chloro-3-methyl-2-butene (LVIII) in the ratio 1·8:1.

$$\underset{\text{(LIV)}}{CH_2=C=CH-CH_3} \longrightarrow \underset{\text{(LV)}}{CH_3-CCl=CH-CH_3}$$

$$\underset{\text{(LVI)}}{CH_2=C=C(CH_3)_2} \longrightarrow \underset{\text{(LVII)}}{CH_2=CH-CCl(CH_3)_2} + \underset{\text{(LVIII)}}{CH_2Cl-CH=C(CH_3)_2}$$

Fedorova[20] has also studied the addition of dry hydrogen bromide to 2,3-nonadiene (L) and 6-methyl-2,3-heptadiene (LII) at —40°. 2,3-Nonadiene (L) gives a product consisting of 3-bromo-3-nonene (LIX) and 2-bromo-3-nonene (LX) while 6-methyl-2,3-heptadiene (LII) gives a product consisting of 3-bromo-6-methyl-3-heptane (LXI) and 2-bromo-6-methyl-3-heptene (LXII).

$$\underset{\text{(L)}}{CH_3-(CH_2)_4-CH=C=CH-CH_3} \longrightarrow CH_3-(CH_2)_4-\underset{\text{(LIX)}}{CH=CBr-CH_2-CH_3} + CH_3-(CH_2)_4-\underset{\text{(LX)}}{CH=CH-CHBr-CH_3}$$

$$\underset{\text{(LII)}}{CH_3-CH(CH_3)-CH_2-CH=C=CH-CH_3} \longrightarrow CH_3-CH(CH_3)-CH_2-\underset{\text{(LXI)}}{CH=CBr-CH_2-CH_3} + CH_3-CH(CH_3)-CH_2-\underset{\text{(LXII)}}{CH=CH-CHBr-CH_3}$$

Free radical addition of hydrogen bromide to allene (LXIII) has been investigated by Griesbaum *et al.*[22]. Allene yields 2-bromopropene (LXIV) as the major product.

$$\underset{\text{(LXIII)}}{CH_2=C=CH_2} \xrightarrow[\text{UV light}]{HBr} \underset{\text{(LXIV)}}{CH_3-CBr=CH_2}$$

It has been shown that allene (LXIII) adds to *n*-propyl thiol (LXV) in the presence of a radical initiator to form mainly *n*-propylallyl sulphide (LXVI)[23]. Jacobs and Illingworth[24] have extended this work with unsymmetrical allenes using ethyl and phenyl thiols.

$$\underset{\text{(LXIII)}}{CH_2=C=CH_2} + \underset{\text{(LXV)}}{CH_3-CH_2-CH_2SH} \longrightarrow \underset{\text{(LXVI)}}{CH_3-CH_2-CH_2-S-CH_2-CH=CH_2}$$

Fedorova *et al.*[25] have shown that the addition of methyldichlorosilane (LXVIII) to 1,2-hexadiene (LXVII) in the presence of hexachloroplatinic acid catalyst gives mainly 2-hexenyltrimethylsilane (LXIX) after the treatment with methyl magnesium bromide.

$$\underset{\text{(LXVII)}}{CH_3-CH_2-CH_2-CH=C=CH_2} + \underset{\text{(LXVIII)}}{CH_2SiCl_2H} \longrightarrow CH_3-CH_2-CH_2-CH=CH-\underset{\text{(LXIX)}}{CH_2Si(CH_3)_3}$$

Moore and Bertelson[26] have studied the addition of 2,4-dinitrobenzenesulphinyl chloride (LXX) to cyclic allenes. For example, cyclononadiene (XXII) gives an adduct which is believed to be 3-(2,4-dinitrobenzenethio)-2-chloro-1-cyclononene (LXXI) (Chart 7).

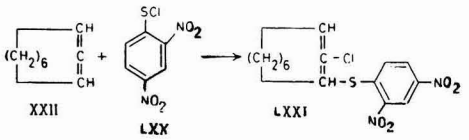


Chart 7 — Addition of 2,4-dinitrobenzenesulphinyl chloride (LXX) to cyclononadiene (XXII)

Quite a few studies on the Diels-Alder reaction between allenes and activated double bonds have been reported. Alder and Ackermann[27,28] obtained 9,10-octahydro-naphthalene-2,3,6,7-tetracarboxylic acid dianhydride (LXXIII) from the reaction between propadiene (LXIII) and maleic anhydride (LXXII) (Chart 8).

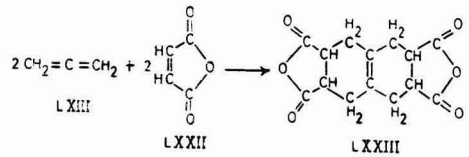


Chart 8 — Diels-Alder reaction between an allene (LXIII) and a compound (LXXII) having activated double bond

The Diels-Alder reaction between allene (LXIII) and a conjugated diene has been studied by Pledger[29]. Hexachloropentadiene (LXXIV) reacts with propadiene (LXIII) yielding only 1,2,3,4,7,7-hexachloro-5-methylene[2.2.1]hept-2-ene (LXXV) (Chart 9).

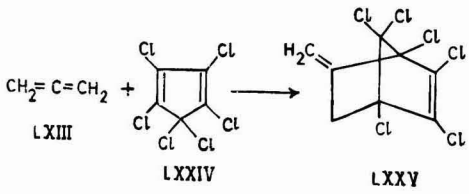


Chart 9 — Diels-Alder reaction between an allene (LXIII) and a conjugated diene (LXXIV)

The cycloaddition of allenes[30] to appropriately substituted olefins affords a general route to —CN, —COOR, —COOH, —CHO and —Ar substituted 3-alkylidenecyclobutanes. For example, propadiene (LXIII) reacts with a large excess of acrylonitrile (LXXVI) under proper conditions of temperature and pressure to yield 3-methylenecyclobutane carbonitrile (LXXVII) in 60 per cent yield (Chart 10).

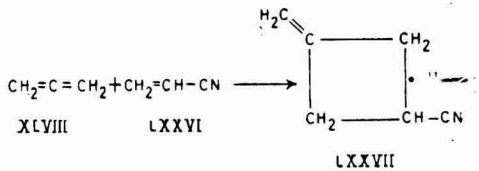
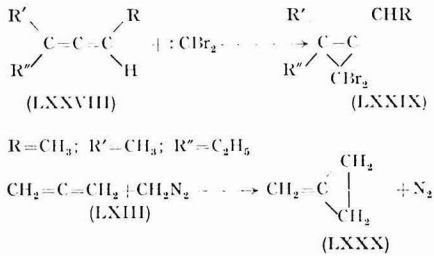


Chart 10 — General route for the synthesis of substituted 3-alkylidenecyclobutane

Ball and Landor[31] have found that dibromocarbene adds to allenes (LXXVIII) to give methylenecyclopropane derivatives (LXXIX) in 40-60 per cent yield, addition always being at the more substituted double bond. Thus the addition of dibromocarbene to allenes opens a new route to the synthesis of methylenecyclopropane systems which are not readily accessible by other methods. A later report of Blomquist and Connoly[32] is quite interesting. The report describes the synthesis of methylenecyclopropane (LXXX) by the photochemical reaction of propadiene (LXIII) with diazomethane. No spiro pentane or insertion product was obtained in this reaction.



Hydroboration of allenes has been studied[33,34]. Monohydroboration (MHB) of 1,2-cyclononadiene (XXII) followed by oxidation of the intermediate organoborane (LXXXI) gives mainly cyclononanone (LXXXII) (Chart 11).

1,2-Cyclodecadiene and 1,2,6-cyclononatriene behave similarly. This suggests that the attack of diborane on the allene linkage occurs primarily at the central carbon atom to form tris-1-cyclononenyl borane (LXXXI).

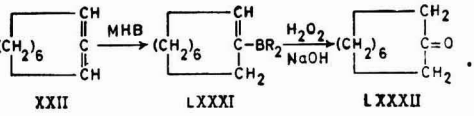


Chart 11 — Monohydroboration of 1,2-cyclononadiene (XXII)

It has been shown that dihydroboration of propadiene (LXIII) and subsequent oxidation yields predominantly 1,3-propanediol (LXXXIII). Similar results have been obtained with 1,2-heptadiene and phenylallene.

$$CH_2=C=CH_2 \xrightarrow[(2)\ H_2O_2,\ NaOH]{(1)\ DHB} CH_2(OH)-CH_2-CH_2(OH)$$

(LXIII) (LXXXIII)

The carbonylation of propadiene (LXIII) in water in the presence of ruthenium carbonyl catalyst under suitable conditions of temperature and pressure has been reported to yield methacrylic acid (LXXXIV)[35].

$$CH_2=C=CH_2 + CO \xrightarrow{H_2O} CH_2=C(CH_3)-COOH$$

(LXIII) (LXXXIV)

It has been shown that 2,3-heptadiene (LXXXV) and 6-methyl-2,3-heptadiene (LII) undergo hydration on shaking with 80 per cent sulphuric acid for 20 min. to yield ethyl *n*-butylketone (LXXXVI) and ethyl isoamylketone (LXXXVII) respectively[36].

$$CH_3-(CH_2)_2-CH=C=CH-CH_3 \longrightarrow CH_3-CH_2-CH_2-CH_2-C(=O)-CH_2-CH_3$$

(LXXXV) (LXXXVI)

$$CH_3-CH(CH_3)-CH_2-CH=C=CH-CH_3 \longrightarrow CH_3-CH(CH_3)-(CH_2)_2-C(=O)-CH_2-CH_3$$

(LII) (LXXXVII)

Reduction

Devaprabhakara and Gardner[37] have shown that sodium-ammonia reduction (Chart 12) of allenes gives olefins in good yield. 1,2-Cyclononadiene (XXII) and 1,2-cyclodecadiene (XXIV) give *cis*-cyclononene (LXXXVIII) and *cis*-cyclodecene (LXXXIX) respectively. Hence the reaction can be utilized in the stereospecific synthesis of these interesting olefins. The reduction of 1,2-cyclotridecadiene (XC), on the other hand, gives 48 per cent *cis*-cyclotridecene (XCI) and 52 per cent *trans*-cyclotridecene (XCII). The synthetic utility of this method is illustrated in the reduction of 1,2,6-cyclononatriene (XCIII) which gives only *cis,cis*-1,5-cyclononadiene (XCIV). These results do not allow confident prediction of the mechanistic course of the reaction.

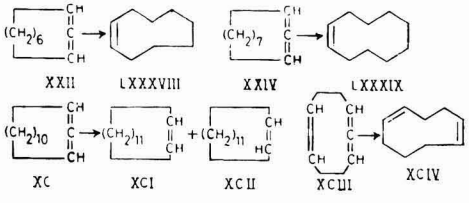


Chart 12 — Reduction of allenes to olefins

Moore[38] has studied the partial hydrogenation of 1,2-cyclononadiene (XXII) and 1,2-cyclodecadiene (XXIV) under one atmosphere of hydrogen in the methanol at about 25° over palladium. 1,2-Cyclononadiene (XXII) gives exclusively *cis*-cyclononene (LXXXVIII) and 1,2-cyclodecadiene (XXIV) gives predominantly *cis*-cyclodecene (LXXXIX). The mechanism of the formation of these thermodynamically stable isomers is discussed.

Isomerization

Although allenes to acetylenes and dienes interconversions have been known for many years, most of these observations have not been of much value in establishing relative thermodynamic stabilities[39]. The enthalpies data[40] of acyclic isomeric acetylenes indicate that a terminal allene is of slightly lower energy than an isomeric terminal acetylene, but that an internal acetylene should be significantly lower in energy than an isomeric internal allene. All allenes and acetylenes possess much higher energies than the conjugated dienes. If the acetylenic and allenic linkages are incorporated into cyclic systems, provided that the rings are sufficiently large, it seems that the stability order should be same as already described. However, when the ring size is reduced sufficiently to cause classical strain, the order may be changed. The following results indicate the relative stabilities of some of these compounds. The naturally occurring allene, mycomycin (II), is known to undergo isomerization to isomycomycin (XCV) in the presence of a base[41].

$$CH\equiv C-C\equiv C-CH=C=CH-CH=CH-CH=CH-CH_2-COOH$$

(II)

$$\xrightarrow{OH^-}$$

$$CH_3-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH=CH-CH_2-COOH$$

(XCV)

Diethylallene (XCVI) has been shown to undergo isomerization[42] to 3-ethyl-1,3-pentadiene (XCVII) by heating it with potassium *tert*-butoxide in *tert*-butyl alcohol.

$$(C_2H_5)_2C=C=CH_2 \longrightarrow CH_3-CH=C(C_2H_5)-CH=CH_2$$

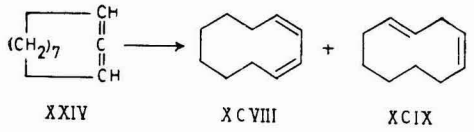
(XCVI) (XCVII)

Moore and Ward[43] have been able to establish the allene-acetylene equilibria for C_9-, C_{10}- and C_{11}-cyclic systems in *tert*-butyl alcohol employing potassium *tert*-butoxide as a catalyst. The following data (Table 1) show that the ring-strain is more pronounced in cyclic acetylenes than in cyclic allenes.

It has also been shown that prolonged heating of 1,2-cyclodecadiene (XXIV) gives mainly *cis, cis*-1,3-cyclodecadiene (XCVIII) and a 1,4-cyclo-

TABLE 1 — ALLENE-ACETYLENE RATIOS AT EQUILIBRIUM AT 100-3°

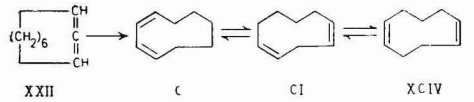
Starting material	Ratio
1,2-Cyclononadiene	13·23
Cyclononyne	13·10
1,2-Cyclodecadiene	1·82
Cyclodecyne	1·82
1,2-Cycloundecadiene	0·34
Cycloundecyne	0·34



XXIV XCVIII XCIX

decadiene (XCIX) which probably has *cis,trans*-configuration[26].

Devaprabhakara *et al.*[44] have found that 1,2-cyclononadiene (XXII) undergoes facile isomerization (Chart 13) via 1,3-cyclononadiene (C) and 1,4-cyclononadiene (CI) to *cis,cis*-1,5-cyclononadiene (XCIV) by potassium *tert*-butoxide base in dimethylsulphoxide solvent at 70° for 144 hr. It thus would appear that *cis,cis*-1,5-cyclononadiene (XCIV) is, by a substantial margin, the most stable of the isomeric cyclononadienes.

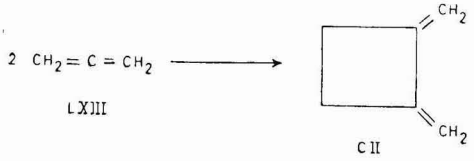


XXII C CI XCIV

Chart 13 — Isomerization of 1,2-cyclononadiene (XXII)

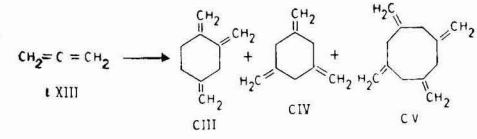
Polymerization

A number of polymers have been prepared by the thermal polymerization of allenes. Blomquist and Verdol[45] have been able to obtain allene dimer, 1,2-dimethylene cyclobutane (CII) in 50 per cent yield by passing propadiene (LXIII) over glass beads heated to 500-10°.



LXIII CII

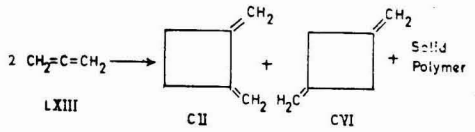
Benson and Lindsey[46] have shown that cyclopolymerization (Chart 14) of propadiene (LXIII) at 110° under 112 atm. with a phosphorus modified catalyst gives two trimers, identified as 1,2,4-trimethylenecyclohexane (CIII) and 1,3,5-trimethylenecyclohexane (CIV) and a remarkable stable tetramer identified as 1,3,5,7-tetramethylenecyclooctane (CV).



LXIII CIII CIV CV

Chart 14 — Cyclopolymerization of propadiene (LXIII)

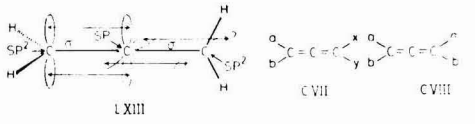
Recently, Slobodin and Khitrov[47] have obtained mainly solid polymer plus 3 per cent dimer by heating propadiene (LXIII) under nitrogen for 80 hr at 110-15°. The dimer has been proven to be a mixture of 1,2-dimethylenecyclobutane (CII) and 1,3-dimethylenecyclobutane (CVI) in the ratio 7·2 : 1.



LXIII CII CVI

Stereochemistry

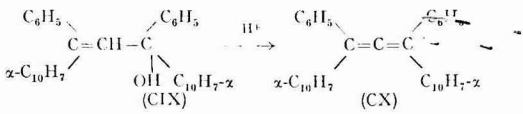
The atomic orbital model of an allene (LXIII) is shown in Chart 15. In this formulation, the central carbon atom is taken to form two collinear sp-σ bonds to the terminal sp^2 hybridized carbon atoms. The two remaining electrons of the central carbon atom occupy p-orbitals directed at right angles to each other. The π-bonds are formed by overlap of the p-orbitals of the central carbon atom and the p-orbitals of the terminal carbons. In allene (CVII) the p-orbitals of the double bond lie in planes perpendicular to each other and the groups a and b lie in a plane perpendicular to that occupied by groups x and y; hence the molecule is asymmetric and should be capable of existing in non-superimposable mirror image forms. Even an allene of the type (CVIII) should be resolvable into a pair of enantiomers.



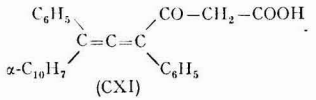
LXIII CVII CVIII

Chart 15 — Stereochemical configuration of allenes

Verification of the predicted stereochemistry of allenes came true when Maitland and Mills[48] effected asymmetric dehydration of alcohol (CIX) with (+)-camphorsulphonic acid and obtained an optically active form of hydrocarbon (CX), while dehydration with (−)-camphorsulphonic acid gave the other enantiomer. Later Kohler *et al.*[49] synthesized an allenic acid (CXI) and resolved it by fractional crystallization of the brucine salt.

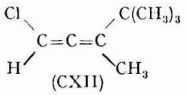


(CIX) (CX)



(CXI)

Landor and Smith[50] have been able to synthesize an optically active allenic chloride (+)-3-*tert*-butyl-1-chloro-3-methylallene (CXII) by a stereospecific method. The absolute configuration of the allene (CXII) has also been established by Eliel[51].



(CXII)

Summary

The advances made in the chemistry of allenes during the last 10 years (1954-64) have been critically reviewed with special reference to the various available methods of synthesis, mechanism of formation, spectral data and reactions of allenes including addition, reduction, isomerization and polymerization. Stereochemical configuration and the isolation of a few optically active allenes are discussed. Scope for farther research in allene chemistry specially in the mechanism of formation is indicated.

References

1. Doering, W. Von E. & LaFlamme, P. N., *Tetrahedron*, **2** (1958), 75.
2. Favorskii, A. E., *J. gen. Chem., Moscow*, **6** (1936), 720.
3. Dominin, N. A., *J. gen. Chem., Moscow*, **8** (1938), 851.
4. Ball, W. J. & Landor, S. R., *Proc. chem. Soc.*, (1961), 143.
5. Ball, W. J. & Landor, S. R., *J. chem. Soc.*, (1962), 2298.
6. Moore, W. R. & Ward, H. R., *J. org. Chem.*, **25** (1960), 2073.
7. Moore, W. R. & Ward, H. R., *J. org. Chem.*, **27** (1962), 4179.
8. Skattebol, L. R., *Tetrahedron Lett.*, (No. 5) (1961), 167.
9. Skattebol, L. R., *Acta chem. scand.*, **17** (1963), 1683.
10. Logan, T. J., *Tetrahedron Lett.*, (No. 5) (1961), 173.
11. Craig, J. C. & Moyle, M., *J. chem. Soc.*, (1963), 3712.
12. Roberts, J. D. & Dirstine, P. H., *J. Amer. chem. Soc.*, **67** (1945), 1281.
13. Slabey, V. A., *J. Amer. chem. Soc.*, **74** (1952), 4928.
14. Moore, W. R., Ward, H. R. & Merrit, R. F., *J. Amer. chem. Soc.*, **83** (1961), 2019.
15. Skattebol, L. R., *Chem. & Ind. (Rev.)*, (1962), 2146.
16. Wotiz, J. H. & Mancuso, D. E., *J. org. Chem.*, **22** (1957), 207.
17. Burr, G. O. & Miller, E. S., *Chem. Rev.*, **29** (1941), 419.
18. Chamberlain, N. F., *Anal. chem.*, **31** (1959), 56.
19. Snyder, E. I. & Roberts, J. D., *J. Amer. chem. Soc.*, **84** (1962), 1582.
20. Fedorova, A., *J. gen. Chem., Moscow*, **33** (1963), 3575.
21. Jacobs, T. L. & Dankner, D., *J. org. Chem.*, **22** (1957), 1424.
22. Griesbaum, K., Oswald, A. A. & Hall, D. N., *J. org. Chem.*, **29** (1964), 2404.
23. Van der ploeg, H. J., Knotnerus, J. & Bickel, A. F., *Rec. Trav. chim. Pays-Bas*, **81** (1962), 775.
24. Jacobs, T. L. & Illingworth, G. E., *J. org. Chem.*, **28** (1963), 2692.
25. Fedorova, A. V., Stadnichuk, M. D. & Potrov, A. A., *C.R. Acad. Sci., U.R.S.S.*, **145** (1962), 837.
26. Moore, W. R. & Bertelson, R. C., *J. org. Chem.*, **27** (1962), 4182.
27. Alder, K. & Ackermann, O., *Ber. dtsch. chem. Ges.*, **87** (1954), 1567.
28. Alder, K. & Ackermann, O., *Ber. dtsch. chem. Ges.*, **90** (1957), 1697.
29. Pledger, H., *J. org. Chem.*, **25** (1960), 278.
30. Cripps, H. N., Williams, J. K. & Sharkey, W. H., *J. Amer. chem. Soc.*, **80** (1958), 751.
31. Ball, W. J. & Landor, S. R., *Proc. chem. Soc.*, (1961), 143.
32. Blomquist, A. T. & Connoly, D. J., *Chem. & Ind. (Rev.)*, (1962), 310.
33. Devaprabhakara, D. & Gardner, P. D., *J. Amer. chem. Soc.*, **85** (1963), 1458.
34. Corsano, S., *Chem. Abstr.*, **60** (1964), 3993.
35. Kealy, T. J. & Benson, R. E., *J. org. Chem.*, **26** (1961), 3126.
36. Fedorova, A. V. & Petrov, A. A., *J. gen. Chem., Moscow*, **32** (1962), 1755.
37. Devaprabhakara, D. & Gardner, P. D., *J. Amer. chem. Soc.*, **85** (1963), 648.
38. Moore, W. R., *J. Amer. chem. Soc.*, **84** (1962), 3788.
39. Jacobs, T. L., in *Organic reactions*, Vol. 5, edited by R. Adams (John Wiley & Sons, New York), 1949, 1.
40. Rossini, F. D., Wagman, D. D., Evans, W. H., Levine, S. & Jaffe, I., *Selected values of physical and thermodynamic properties of hydrocarbons and related compounds* (Carnegie Press, Pittsburgh), 1953.
41. Jones, E. R. H., Leeming, P. R., Thomson, J. M. & Bulock, J. D., *J. chem. Soc.*, (1956), 3767.
42. Smadja, W., *C.R. Acad. Sci., Paris*, **257** (1963), 3950.
43. Moore, W. R. & Ward, H. R., *J. Amer. chem. Soc.*, **85** (1963), 86.
44. Devaprabhakara, D., Cardenas, C. G. & Gardner, P. D., *J. Amer. chem. Soc.*, **85** (1963), 1553.
45. Blomquist, A. T. & Verdol, J. A., *J. Amer. chem. Soc.*, **78** (1956), 109.
46. Benson, R. E. & Lindsey, R. V., *J. Amer. chem. Soc.*, **81** (1959), 4247.
47. Slobodin, Ya. M. & Khitrov, A. P., *J. gen. Chem., Moscow*, **33** (1963), 2822.
48. Maitland, P. & Mills, W. H., *Nature, Lond.*, **135** (1935), 994.
49. Kohler, E. P., Walker, J. T. & Tishler, M., *J. Amer. chem. Soc.*, **57** (1935), 1743.
50. Landor, S. R. & Smith, R. T., *Proc. chem. Soc.*, (1959), 154.
51. Eliel, E. E., *Tetrahedron Lett.*, (No. 8) (1960), 16.

Aspects of Primary Structure of Proteins

L. K. RAMACHANDRAN
Department of Chemistry, Osmania University, Hyderabad 7
&
K. S. V. SAMPATHKUMAR
Biochemistry & Food Technology Division, Atomic Energy Establishment, Trombay, Bombay 8

THE valuable and exhaustive reviews in the continually expanding series of *Advances in protein chemistry*[1] and *Annual reviews of biochemistry*[2] deal with detailed up-to-date information on the subject of protein structure. Enormous strides in the understanding of the primary structure of many naturally occurring peptides and proteins have been made since 1950, and all this can ultimately be traced to the fundamental achievements in improved analytical techniques, separation methods, and methods for the degradation of polypeptides. The following review is only intended to highlight some of the achievements in the elucidation of the primary structure of naturally occurring peptides and proteins within the past decade. The coverage is intended to be neither comprehensive nor detailed. In many cases to avoid an excessively lengthy bibliography resort has been made to citing reviews rather than the original papers.

The origins of the developments, during the past decade, in the elucidation of the primary structure of proteins can clearly be traced back to Sanger's classical studies on the hormone insulin (mol. wt 6000) leading to the postulation of a unique structure for bovine insulin, and to the pinpointing of variations in the primary structure for insulins derived from diverse species[3]. Techniques used today to obtain fragments from peptide chains and for determining amino acid sequence differ from the techniques used by Sanger, but the skill, tenacity and courage which aided him in both undertaking and solving the structure of insulin have prompted others to take up the problem of structure of proteins of even much higher molecular weight.

Thus, today the primary structure of several proteins are known. These include ribonuclease, tobacco mosaic virus protein, haemoglobin, myoglobin, cytochrome *c*, lysozyme, chymotrypsin, trypsin, papain, azurin, and others. Equally rewarding have been the numerous studies conducted on relatively smaller molecular weight peptides. Outstanding among these was the elucidation of the structures of the hormones oxytocin and vasopressin, and their subsequent synthesis by du Vigneaud[4-8]. To this class belong various peptide antibiotics, including gramicidin S whose structure was the first among these to be worked out[9], the angiotensins (hypertensins)[10], α- and β-melanophore stimulating hormones (MSH)[11], adrenocorticotrophichormone[12], glucagon[13] and others.

All those features of the molecular organization of proteins, including amino acid sequence and cross-linking of chains, which can be discussed within the traditional framework of organic chemistry, come under the descriptive term primary structure. While advances in techniques for the study of the primary structure of proteins have enabled the protein chemist to decipher the structures of many peptides and proteins, the potentiality of a notable alternative technique, namely X-ray diffraction, has been examined with two proteins, haemoglobin and myoglobin[14-16]. For both proteins it has been possible to reconstruct the three-dimensional model of the polypeptide chains and the configuration of the heme groups. The 2 A. Fourier of myoglobin showed resolution of a great majority of the side chains, and a unique electron density distribution was frequently found which would allow only one kind of side chain to be fitted at most positions along the polypeptide chains. A 1·5 A. Fourier of myoglobin will provide even better detail of the sequence. It is indeed a tribute to X-ray crystallographers to note that today an actual mapping of amino acid sequences of proteins has become possible by a physical technique. Indeed, it is possible that in the future for good proof for the structure of a protein the requirement that evidence from both physical and chemical techniques agree will be there. The main requirement for success with the technique is that isomorphous heavy atom derivatives of the proteins be available for study, where the locations of the heavy atoms are known. Uniform success does not attend all efforts to form such derivatives of proteins, and, as Perutz[17] once remarked, "it appears that some fundamental organic chemistry is needed to get heavy atom compounds". A thorough investigation of metal-protein interactions and of methods for preparation of well-characterized metal-protein compounds is much needed. What surprises there may be in store is evident from one recent finding that tryptophan residues, most unlikely candidates as judged from earlier literature, are sites (pyrrole ring) for mercury binding in proteins[18]. Another illustration of the impact of X-ray diffraction methods in the protein structure field is available in the advances made in the structure of collagen. The idea of a triple helix had been postulated in 1951 by Pauling and Corey[19], but to account for many of the experimental findings Ramachandran and Kartha[20] introduced new ideas involving a structure in which the configuration of the chains was related to that for polyproline with a repeat of 9·5 A. containing three residues related by a threefold screw axis. A revised model of this structure containing the chains forming gentle-coiled coils with a repeat period of 28·6 A. and which can accommodate the known -Gly-Pro-HyPro- sequence has been proposed and accounts for most of the experimental data[21-23]. The collagen triple helix structure (collagen II model of diam. 13·6 A.) has an overall length of 3000 A. and a mol. wt of 345,000[24].

While considerable success has attended the X-ray studies of myoglobin and haemoglobin, in cases such as chymotrypsinogen[25] at 10 A. resolution not even a unique path for the polypeptide backbone chain could be traced, nor anything like an α-helix of more than one turn. While the backbone chain could be seen, the disulphide bridges could not be seen. Substantial progress has been made also in studies on carbonic anhydrase[26] and on carboxypeptidase[27].

Knowledge of the purely chemical aspects of the structure of proteins is by no means adequate to explain many of their properties. The determination of the spatial organization of proteins is a separate and important part of the analysis of their structure. Here it is that X-ray diffraction comes in handy, supplementing other tools such as the hydrodynamic methods, infrared and ultraviolet absorption, optical rotation and optical rotatory dispersion, deuterium exchange studies, etc.

The knowledge of the primary structure of a protein is helpful in several regards. Firstly, it provides a description of the molecule in terms of the framework of classical organic chemistry. Secondly, in the elucidation of the active sites of hormones and enzymes, progression from the enumeration of essential functional groups to their allocation within the unique linear array of amino acid residues in the polypeptide chain becomes possible by studies of sequence on the fragments from derivatives of the active protein in which a substrate or an analogue has been tagged on to the active groups in the catalytic site. This provides a more detailed description of the active site region — a description which will become complete only when the three-dimensional structure of the chain is known and the important groups at the active site can be allocated a unique disposition in the three-dimensional model. The primary structure of a protein can perhaps also explain why it is more or less helical[28,29]. When a sequence of β- or random-forming amino acids is contiguous to an α-helical section of a protein chain, this may be the site where a loop or reversal of direction can occur, and the presence of large amounts of β- or random-forming amino acids may explain the relatively low helix content postulated for certain globular proteins, viz. TMV protein.

How important a role the primary structure has on the final steps in the biogenesis of a folded native enzyme is illustrated by the studies where reduced ribonuclease A (4 disulphide bonds) regains through reoxidation by air both the proper —S—S— bridges as well as the physical and enzymatic properties of native ribonuclease[30]. These experiments on the rapid conversion of an extended polypeptide chain to a specific, active three-dimensional structure can only mean that the multitude of interactions between functional groups along the chain, including the interactions that orient the half-cystine residues, almost instantaneously determine a prescribed conformation in solution, containing a three-dimensional geometry characteristic of that most probable of configurations — the native enzyme.

Finally, numerous studies have pinpointed the possibility of discovering principles governing the relations between variations of the primary structure of any selected protein and the evolution of species. The variations are a function of the degree of phylogenetic kinship between the species carrying the proteins. By comparison with suitable palaeontological standards, the times elapsed since the divergence of any two lines of evolution may be calculated, and the number of variant amino acids, that may be expected, predicted[31]. This approach is being exploited with the haemoglobins, cytochrome *c* and fibrinopeptides. Indeed, in future years one may look forward to a good understanding of relations between morphological evolution of species and evolution of primary structures of many proteins.

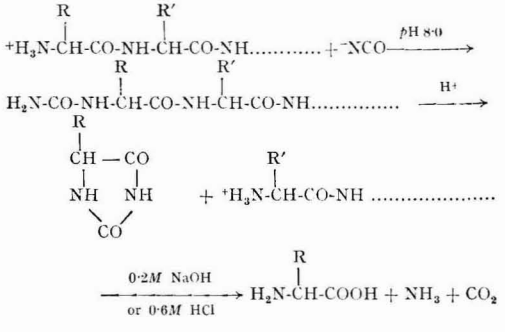
Methodology

The fascinating developments during the last two decades in the field of primary structure of the proteins can most be appreciated by one who is aware of the status of the field in the forties[32]. All the improvements can be traced principally to newer techniques of isolation and analysis. Highly effective procedures for separation and isolation were developed based on the various forms of chromatography, sedimentation, gel filtration, dialysis, ultrafiltration, countercurrent distribution, various forms of electrophoresis, and fingerprinting; and all these have now become common tools[33]. The older, tedious, and occasionally unreliable, gravimetric procedures for analysis of amino acids have been replaced by the elegant manual and automatic procedures for the determination of amino acids, by the ninhydrin colour reaction, after separation on ion-exchange columns[34]. This shift was the result of the painstaking and meticulous investigations of Moore and Stein in the field of amino acid analysis.

The specificities of a wide array of proteolytic enzymes have been established[35] and the recent advent of water-insoluble enzymes (proteolytic enzymes covalently linked to amino acid polymers) hold considerable promise for the future[36]. To supplement these tools there are available today several selective and preferential non-enzymatic techniques for the degradation of proteins[37,38]. Of all the chemical methods developed so far, which have been discussed at length in recent reviews[37,38], the selective cleavage of peptide bonds adjacent to methionine residues in proteins by the use of cyanogen bromide has been very effective in structural studies as exemplified by investigations on a number of proteins, e.g. ribonuclease, myoglobin, trypsinogen and carboxypeptidase A. Specific chemical cleavages find greater use now, and newer techniques continue to be developed, viz. the recent investigations on the cleavage of bonds adjacent to proline[39,40]. Even techniques such as the preferential release of aspartic acid from peptide chains by dilute acid may be put to good use in structural studies[41-43].

Older methods of peptide analysis have been refined, newer analytical tools have been developed and chemical reagents causing restricted cleavage at specific loci in the protein moiety are widely used. While Sanger's classical method[44] of determining NH_2-terminal residues in peptides and proteins by 1-fluoro-2,4-dinitrobenzene continues to be used in structural analyses, alternative procedures are available. Thus, the use of 2,4,6-trinitrobenzenesulphonic acid for acylating and determining primary

amino groups has been advocated[45-47], and the absence of undesirable side reactions with this reagent under the conditions of coupling should prompt its wide use. The advantages of 2-chloro-3,5-dinitropyridine as a reagent for NH_2-terminal group determination is detailed in a recent paper[48]. 3,5-Dinitropyridyl amino acids are liberated almost quantitatively on brief hydrolysis (less than 3 hr) of the 3,5-dinitropyridyl peptides in 6*N* HCl at 100°C. Stark and Smyth[49] have suggested the use of cyanate for coupling with the NH_2-terminal groups. Treatment of the carbamyl protein with acid results in the formation of hydantoins that correspond to the NH_2-terminal residue. After isolation, most of the hydantoins can be hydrolysed to the free amino acids. The reaction sequence is as follows:



Another method that shows considerable promise for the determination of NH_2-terminal groups has been suggested by Gray and Hartley[50]. The method utilizes 1-dimethylaminonapthalene-5-sulphonyl chloride which reacts with free amino and phenolic groups. The derivatives formed are resistant to acid hydrolysis and have an intense yellow fluorescence making the method applicable even to 10^{-4}-10^{-3} μmoles of peptide. These authors have developed a system to resolve all the substituted amino acids.

Eriksson and Sjoquist[51] have modified the sequential phenyl isothiocyanate method of Edman[52] for the determination of NH_2-terminal sequence of peptides and proteins. Isle and Edman[53] have recently defined the set of conditions which yield a quantitative formation of phenylthiohydantoins. These conditions together with separation methods for the phenylthiohydantoins of amino acids[54] should aid considerably in establishing the sequence of the NH_2-terminal residues. A subtractive procedure of sequence determination of small peptides has been adopted by Hirs *et al.*[55], and Konigsberg and Hill[56]. In this method, the NH_2-terminal residue is identified by the difference in amino acid composition of the peptide before and after application of the degradation procedure. This method is being widely used in several laboratories.

There is a paucity of effective chemical methods for the determination of COOH-terminal residues. Digestion with carboxy-peptidase A or B continues to be a popular method of identifying the amino acids at the COOH-terminus. The hydrazinolysis approach of Akabori *et al.*[57] is also in use.

The limited degradation of proteins to a few large peptides is carried out not only by chemical means but also by directing the specificity of tryptic hydrolysis only to bonds adjacent to arginine[58-62]. This is achieved by selective acylation of the ε-amino groups of lysine, thereby making the adjacent bonds refractory to the action of trypsin with the result tryptic digestion of the acylated protein yields fewer peptides and all with COOH-terminal arginine. The procedures involving the reaction of lysine side chains with carbon disulphide[61] or amidination with methylacetimidate[62] would prove of considerable value in that these reactions are easily reversible, so that hydrolysis at lysine bonds could be carried out later. These methods would be most usefully complemented by a method involving modifications of arginine residues which would permit the action of trypsin only on bonds adjacent to lysine. Itano and Gotlieb[63] have suggested that treatment of proteins with benzil in a strongly alkaline medium results in the quantitative modification of arginine residues leaving other amino acids intact, thereby restricting the action of trypsin to bonds adjacent to lysine. The method is still to be tested.

New points of cleavage in the protein have been introduced by modification of the cysteinyl side chains with 2-bromoethylamine to form S-(β-aminoethyl)-cysteine and a subsequent cleavage of the corresponding bonds by trypsin[64]. This method has been successfully used in determining peptide sequences in trypsinogen[65]. The disulphide bonds of trypsinogen were reduced by mercaptoethanol prior to treatment with 2-bromoethylamine. The direct use of ethylenimine which is the active intermediate in the acylation reaction with 2-bromoethylamine has been suggested for the quantitative conversion of cysteinyl side chains[66].

The disulphide bridges of proteins can be determined by the simple and elegant technique of Brown and Hartley[67]. An enzymic digest of the protein is subjected to high voltage electrophoresis on Whatman 3 mm. paper and is then exposed to performic acid vapour which converts each cystine-bridged peptide to a pair of cysteic acid peptides. The strip is dried *in vacuo* over sodium hydroxide, and ionophoresis is now done again at right angles to the original direction of migration. All the peptides will lie on a 45° diagonal, but each cystine peptide gives a pair of cysteic acid peptides lying off the diagonal. The authors were able to determine the disulphide bridges of chymotrypsin in this manner.

Thin layer chromatography of peptides is of recent origin[68]. The high resolving power of thin layer chromatography should induce developments in defining systems for resolution of peptide mixtures. Some attempts have already been made in this regard, and future reports would perhaps deal with the routine applicability of the system in sequence determination studies.

Gas chromatography of amino acids is still at an infant stage. The problems in the refinement of this technique[69,70] are many and a recent report[71] has claimed that it is possible to effect the quantitative separation of 16 amino acids in 10 min. provided they are converted to *n*-butyl-(N-trifluoroacetyl) esters. The latter process takes 5 hr but many samples of amino acids can be esterified at the same time. It holds considerable promise for the

future from the viewpoint of speed, accuracy, and sensitivity.

Specific Proteins

This section will deal with certain considerations of the primary structure of some well-characterized proteins. No attempt will be made to discuss all the methodology used in the elucidation of the structure, and the reader is referred to the original literature on the subject. Salient features of the structure determination along with certain aspects of the relation of the structure to function of the protein will be discussed.

Ribonuclease A

The covalent structure of pancreatic ribonuclease A has been elucidated by Hirs *et al.*[72], and Spackman *et al.*[73] determined the position of the disulphide linkages in the molecule. The procedure followed involved the scission of the performic acid-oxidized molecule with trypsin into 13 major peptides, the determination of sequence in each peptide by degradative and conventional methods, and the alignment of the peptides obtained by digestion with chymotrypsin and pepsin.

The position of the disulphide bridges was located through an analysis of the cystine containing peptides obtained by sequential peptic, tryptic and chymotryptic digestion of the protein taking care to ensure that no disulphide interchange occurs during the isolation procedure. Some of the sequences have been modified later by Smyth *et al.*[74] and the sequence is given in Chart 1. The application of cyanogen bromide cleavage at the methionine residues by the method of Gross and Witkop[75] has helped in the establishment of the correct sequence in one of the methionine containing peptides in ribonuclease.

Smyth *et al.*[71], while establishing the structure of this protein, bring out a few problems of methodology which interfere with the assignment of the correct sequence in certain places. Thus, when the cyclization in the Edman degradation step is carried out in glacial acetic acid-anhydrous HCl, a newly liberated NH_2-terminal glutamine from the penultimate position cyclizes to a pyrrolidone carboxylic acid residue, which, not having an α-amino group, does not allow further use of the Edman degradation. An internal asparagine or aspartic acid residue may form an imide in glacial acetic acid-HCl at 100°C. Since a β-aspartyl peptide is formed predominantly by the rupture of the imide, the step-wise degradation cannot proceed in good yield. Also, the acetylation of serine or threonine hydroxyl group which takes place in acetic acid-HCl interferes at the stage where these amino acids become NH_2-terminal because the acetyl group migrates from the —OH to —NH_2 group under alkaline conditions, thereby preventing subsequent degradation. All these problems are overcome by following the procedure of Konigsberg and Hill[56] using trifluoroacetic acid at room temperature for cyclization, and purifying the residual peptide after cleavage.

The determination of the linear structure of ribonuclease provided a tremendous stimulus to investigations on the relationship of structure to function of the enzyme, and in understanding the mechanism of catalysis in molecular terms. The knowledge accrued on these aspects is extensive, and its detailed discussion is beyond the scope of the article. A review by Scheraga and Rupley[76] deals with these aspects exhaustively.

The technique of fingerprinting has been used to define structural differences between ribonucleases obtained from various sources. Thus, Anfinsen *et al.*[77] reported that in ovine ribonuclease serine replaced threonine at position 3, glutamic acid replaced lysine at position 37, and a third unidentified difference existed between positions 99 and 104.

Ribonuclease T_1, isolated as a pure protein from takadiastase by Takahashi[78,79], has a molecular

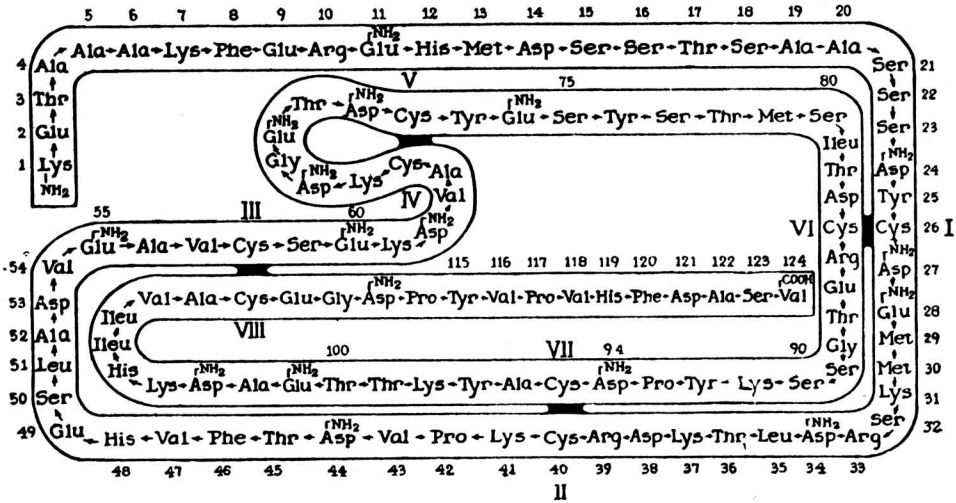


Chart 1 — Amino acid sequence for bovine pancreatic ribonuclease A

weight of 11,000 and consists of 109 residues. Its NH_2-terminal residue is alanine, followed by a cystine involved in intra-chain cross-link. The sequence at the COOH-terminus of the molecule was Val-Thr-. The complete structure of the molecule of T_1 ribonuclease is now available[80].

Bovine pancreatic ribonuclease B, a glycoprotein containing two residues of (acetyl) glucosamine and five of mannose, has otherwise the same amino acid composition as ribonuclease A. Digestion with trypsin yielded a single glycopeptide which on further chymotryptic breakdown also gave rise to a single glycopeptide containing the Asp and Leu residues of the Try-11 peptide found in tryptic hydrolysates of ribonuclease A. The carbohydrate residues are attached to the β-carboxyl of the Asp residue[81].

Proteolytic Enzymes of the Pancreas

Since the proteolytic enzymes were the first proteins to be purified and characterized by the methods of protein chemistry, these served as models in studies on the elucidation of the relationship of structure to function. Many of these enzymes occur as inactive precursors, and studies on the mechanism of activation of these precursors have thrown light on the subtle intramolecular changes which produce the active enzyme. All the current work on their sequence, X-ray diffraction measurements, and behaviour of model compounds is providing vital information on the mechanism of action of these enzymes.

Trypsinogen

The activation of trypsinogen to the active enzyme is catalysed by trypsin which directs and limits its action to the most susceptible bond between lysine and isoleucine near the NH_2-terminus of the zymogen[82]. While the same bond is split in both bovine and procine trypsinogens during activation, the composition of the peptide liberated is different in each case[83]. Thus, a hexapeptide, Val-$(Asp)_4$-Lys, is liberated from the bovine zymogen, while Phe-Pro-Thr-$(Asp)_4$-Lys is the product of porcine zymogen activation. It is significant that the same characteristic structure $(Asp)_4$-Lys precedes the strategic bond and Ile-Val- becomes the new NH_2-terminal sequence in both the enzymes.

Conventional approaches to the determination of the amino acid sequence in proteins were not adequate for the long and heavily cross-linked polypeptide chain of bovine trypsinogen. Enzymatic degradation of di-isopropylphosphoryltrypsin or S-sulphotrypsinogen by trypsin[84–87], chymotrypsin[85] or pepsin[88] have given rise to many peptides whose sequences have been determined. However, it has not been possible to recover all the amino acids of the parent protein in soluble peptides from any single digest. Recourse was had to two additional methods which were evolved for determining overlaps and for positioning of peptides derived by enzymatic hydrolysis[65]. One of these is the redirection of the action of trypsin from peptide bonds adjacent to lysine residues to those adjacent to half-cystine residues, by masking the ε-amino groups of lysine by the method of Goldberger and Anfinsen[60] and converting the cystine residues to lysine analogues after reductive cleavage, with 2-bromoethylamine[64]. The other method involved the use of cyanogen bromide to cleave at methionine residues which causes fragmentation of the polypeptide chain into three fragments of comparable length after reduction of the disulphide bonds[89]. A tentative structure of bovine trypsinogen has been recently assigned by Walsh and Neurath[90] and is given in Chart 2. The position of all the disulphide bridges have to be worked out, but it has been reported[65,91] that two of the histidines in the molecule are present in one of the cystinyl peptides isolated from a peptic digest and having the following sequence:

```
25                                          35
Asn-Ser-Gly-Tyr-His-Phe-Cys-Gly-Gly-Ser-Leu
                           |
                           S
                           |
                           S
                           |
41                         |                53
Val-Val-Ser-Ala-Ala-His-Cys-Tyr-Lys-Ser-Gly-Ile-Gln
```

The significance of this finding will be discussed later under chymotrypsin.

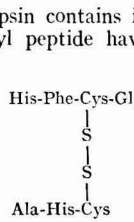
Chymotrypsinogen

The activation of chymotrypsinogen involves the tryptic cleavage of an Arg-Ile bond which may be followed by subsequent chymotryptic cleavages which liberate the dipeptides Ser-Arg and Thr-Asn to form the well-characterized α-chymotrypsin[82,83]. The initial cleavage occurring near the NH_2-terminus is not followed by liberation of any peptide because of the NH_2-terminal cystine residue holding the molecule together. Chymotrypsin has three peptide chains: A chain (13 residues), B chain (131 residues) and C chain (98 residues). The sequence of bovine chymotrypsinogen[92] has been worked out by Hartley and is given in Chart 2 along with trypsinogen for comparison. The method involved the initial conversion of cystines in α-chymotrypsin to cysteic acid, S-sulphocysteine or S-carboxymethylcysteine derivatives, followed by separation of the three modified chains on columns of Dowex-50 or DEAE-cellulose. The sequences in these chains were determined by conventional methods employing different types of cleavage. The dansylation technique[50] for determination of NH_2-terminal residues has been employed in the analysis of several peptides. The disulphide bridges which are determined by the diagonal technique[67] are between residues 1 and 122, 42 and 58, 136 and 201, 168 and 182, and 191 and 221. This sequence differs from that proposed by Keil *et al.*[93], but the latter work is not yet complete. The work is to be completed to permit comparison of the areas of disagreement.

Chymotrypsin and trypsin resemble each other in functional and compositional characteristics. These include their formation in the acinar cells of the pancreas at similar rates[94,95] as the inactive precursors. Both are formed from their zymogens by trypsin which cleaves a susceptible bond near their respective NH_2-termini. They behave in a similar fashion in their capacity to cleave peptide, amide and ester linkages, and the specificity is dictated by the amino acids donating the COOH groups[96–98]. These carboxyl groups appear to be acylated as an intermediate step during catalysis[99–101].

Certain organophosphates like DFP (di-isopropyl-fluorophosphoridate) inhibit these enzymes in a specific fashion, and the site of action of this inhibitor has been established as a serine hydroxyl group in a unique tetrapeptide sequence, Gly-Asp-Ser-Gly[84,102,103]. The same sequence is present in elastase, another pancreatic enzyme, which is also inhibited by DFP in a similar fashion[104]. An imidazole has been implicated in the catalysis of both the enzymes on the basis of pH data, photooxidation, and by affinity-labelling studies[105–108]. Both the zymogens after activation yield a new identical NH_2-terminal tripeptide sequence, Ile-Val-Gly . . . (Chart 2).

Chymotrypsin contains its two histidine residues in a cystinyl peptide having the following structure[92,93]:



The determination of an identical sequence in the same disulphide bridged structure in trypsinogen[65] proves another area of similarity and raises the

```
                                1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18
Chymotrypsinogen                cys-gly-val-pro-ala-ile-gln-pro-val-leu-ser-gly-leu-ser-arg-ILE-VAL-GLY-
Trypsinogen                                                        val-asp-asp-asp-asp-lys-ILE-VAL-GLY-
                                                                    1  2  3  4  5  6  7  8  9

19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41
asp-glu-glu-ala-val-pro-gly-ser-trp-PRO-trp-GLN-VAL-SER-LEU-gln-asp-lys-thr-GLY-phe-HIS-PHE-
gly-tyr-thr-cys-gly-ala-asn-thr-val-PRO-tyr-GLN-VAL-SER-LEU-asn-        -ser-GLY-tyr-HIS-PHE-
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25          26 27 28 29 30

42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64
CYS-GLY-GLY-SER-LEU-ILE-ASN-glu-asn-TRP-VAL-VAL-thr-ALA-ALA-HIS-CYS-gly-val-thr-thr-ser-asp-
CYS-GLY-GLY-SER-LEU-ILE-ASN-ser-gln-TRP-VAL-VAL-ser-ALA-ALA-HIS-CYS-tyr-lys-ser-gly-ile-gln-
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53

65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87
VAL-val-val-ala-gly-glu-phe-asp-gln-gly-ser-ser-ser-glu-lys-ile-gln-lys-leu-lys-ile-ala-lys-
VAL-arg-leu-gly-glu-asp-asn-ile-asn-val-val-glu-gly-asp-glu-gln-phe-ile-ser-ala-ser-lys-ser-
54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76

88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110
val-phe-lys-asn-SER-lys-TYR-ASN-ser-leu-thr-ile-ASN-ASN-asn-ILE-thr-LEU-leu-LYS-LEU-ser-thr-
ile-val-his-pro-SER-    -TYR-ASN(pro,leu,thr,asn)ASN-ASN-asp-ILE-met-LEU-ile-LYS-LEU-lys-ser-
77 78 79 80 81          82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98

111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133
ALA-ALA-SER-phe-ser-gln-thr-VAL-ser-ala-val-cys-LEU-PRO-ser-ala-ser-asp-asp-phe-ala-ALA-GLY-
ALA-ALA-SER-leu-asn-ser-arg-VAL-ala-ser-ile-ser-LEU-PRO-thr-ser-cys-        -ala-ser-ALA-GLY-
99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115          116 117 118 119

134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156
THR-thr-CYS-val-thr-thr-GLY-TRP-GLY-leu-THR-arg-tyr-thr-asn-ala-asn-thr-PRO-ASP-arg-LEU-gln-
THR-gln-CYS-leu-ile-ser-GLY-TRP-GLY-asn-THR-lys-ser-ser-gly-thr-ser-tyr-PRO-ASP-val-LEU-lys-
120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142

157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179
gln-ala-ser-leu-PRO-leu-LEU-SER-asn-thr-asn-CYS-LYS-lys-tyr-trp-gly-thr-lys-ILE-lys-asp-ala-
cys-leu-lys-ala-PRO-ile-LEU-SER-asp-ser-ser-CYS-LYS-ser-ala-tyr-pro-gly-gln-ILE-thr-ser-asn-
143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165

180 181 182 183 184 185 186 187 188 189          190 191 192 193 194 195 196 197 198 199 200
MET-ile-CYS-ALA-GLY-ala-ser-gly-val-ser-        -SER-CYS-met-GLY-ASP-SER-GLY-GLY-PRO-leu-VAL-
MET-phe-CYS-ALA-GLY-tyr-leu-glu-gly-gly-lys-asn-SER-CYS-gln-GLY-ASP-SER-GLY-GLY-PRO-val-VAL-
166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188

201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223
CYS-lys-lys-asn-gly-ala-trp-thr-leu-val-GLY-ILE-VAL-ser-SER-TRP-GLY-SER-ser-thr-cys-ser-thr-
CYS-ser-gly-lys-leu-gln-                -GLY-ILE-VAL-   -SER-TRP-GLY-SER-gly-cys-ala-gln-lys-
189 190 191 192 193 194                 195 196 197     198 199 200 201 202 203 204 205 206

224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246
ser-thr-PRO-GLY-VAL-TYR-ala-arg-VAL-thr-ala-leu-VAL-asn-TRP-val-gln-GLN-THR-leu-ALA-ala-ASN
asn-lys-PRO-GLY-VAL-TYR-thr-lys-VAL-cys-asn-tyr-VAL-ser-TRP-ile-lys-GLN-THR-ile-ALA-ser-ASN
207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229
```

Chart 2 — Amino acid sequences for bovine chymotrypsinogen and trypsinogen [The comparison is designed to draw attention to the homologous areas which are shown with capitalized abbreviations]

question of its possible functional significance. Both bovine chymotrypsinogen B and porcine elastase also possess two histidines in a similar cystinyl peptide sequence[109]. It has been postulated[84,110] on the basis of the common -Gly-Asp-Ser-Gly- sequence that the active sites of these enzymes might have evolved from a common precursor. The demonstration of even a larger area of identity containing the histidines supports the postulate that the similarities are found only in areas of functional importance. The discovery that two histidines in trypsin are found in a decapeptide sequence strikingly similar to that present in chymotrypsin, in which one of the two histidines is an integral element of the active site, led Walsh *et al.*[65,91] to postulate that a functional role exists for this unique structure and that not one but two histidines are involved in the mechanism of catalysis. A similar postulate has been made by Hartley[92], and Bender and Kezdy[111] postulated a general catalytic mechanism based on the presence of two imidazoles. It is of interest to mention here that two imidazole groupings have been implicated in other unrelated enzymes[112,113]. Walsh and Neurath[90] made a comparison of the molecular structures of the two enzymes. The two sequences, given in Chart 2, are started with the point of activation as the common starting point, i.e. with Ile-Val-Gly, the new NH_2-terminal sequence formed on activation of the zymogens. As in the haemoglobin-myoglobin comparison, the alignment makes allowance for occasional deletions. Striking homology is evident and approximately 40 per cent of amino acid residues occur at identical positions. The homology can be extended further if one equates certain amino acid residues having analogous chemical properties. These studies give credence to the postulate that the degree of conservation of character is reflected in areas, which have persisted in the course of evolution of the two proteins from a common ancestral precursor, vital for function.

Porcine chymotrypsinogen A has a half-cystine NH_2-terminal residue and its amino acid composition indicates considerable differences from that of its bovine counterpart. In chymotrypsinogen B, the serine at position No. 14 is replaced by alanine near the NH_2-terminus[83].

Carboxypeptidase A

Carboxypeptidase A, like other enzymes of the pancreas, is formed essentially by tryptic activation of its zymogen, procarboxypeptidase A[114]. But the activation mechanism differs considerably from the other zymogens in molecular details. This is necessitated by the complex structure of procarboxypeptidase A which is a trimer of three subunits, subunit I giving rise to carboxypeptidase A, subunit II giving rise to an endopeptidase, and subunit III whose ultimate identity is not known[115-117]. The major chemical species in preparations of carboxypeptidase A, isolated from autolysing tissues of bovine pancreas[118], is carboxypeptidase A_γ whose NH_2-terminal residue is asparagine[119,120] and whose NH_2-terminal sequence has been shown to be Asn-Tyr-Ala . . .[121]. Carboxypeptidase A_α is the major chemical species in preparations of the enzyme isolated after activation of purified procarboxypeptidase A[122] and it contains NH_2-terminal alanine[120]. The chemical relationship of these two enzymes derived from the same source was clarified by Sampathkumar *et al.*[123] by subjecting chymotryptic digests of the two proteins, after performic acid oxidation, to the technique of fingerprinting. An additional pentapeptide, Ala-Arg-Ser-Thr-Asn, was found in the digests of carboxypeptidase A_α, and it was inferred that this sequence precedes the NH_2-terminal sequence Asn-Tyr-Ala . . . of carboxypeptidase A_γ and hence alternative pathways of activation of the zymogen yield different chemical species of the enzyme.

By the action of cyanogen bromide on carboxypeptidase A_γ a pentadecapeptide was isolated which appeared to be derived from the NH_2-terminus of the molecule[124]. A parallel study on carboxypeptidase A_α was expected to liberate an eicosapeptide containing the additional pentapeptide sequence at its NH_2-terminus. This fragment not only contained the expected pentapeptide sequence, but in addition a threonine and phenylalanine residue in a Thr-Phe sequence, which has not been identified in the fingerprints. The sequence of the NH_2-terminal fragment from cyanogen bromide cleavage is given in Chart 3, and a mechanism depicting the origin of the various chemical species of carboxypeptidase A from its precursor subunit I, has been postulated. Only precise chemical techniques have been able to detect subtle but significant differences between seemingly identical proteins derived from the same source but by different procedures. The existence of the different forms of carboxypeptidase A differing in their NH_2-terminal sequence has to be reckoned with the studies of Coombs *et al.*[121] who have shown that, in carboxypeptidase A_γ, the zinc atom which is essential for activity is bound by not only a —SH group but also by the single NH_2- group in the polypeptide chain.

The single cysteine side chain of carboxypeptidase A is bound to the zinc atom[125,126]. The protein also contains another amino acid residue which upon reduction gave rise to cysteine and upon oxidation to cysteic acid[127]. The active site cysteine has been

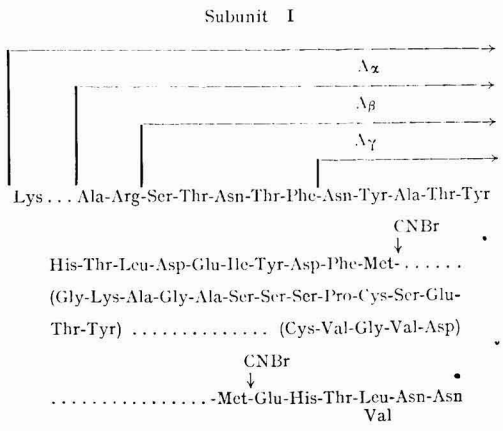


Chart 3 — Summary of the sequences known in bovine pancreatic carboxypeptidase A

labelled selectively and the sequence of amino acids around this sulphur determined[127,128] and it is given in Chart 3 (Also, Sampathkumar, K. S. V., Ericsson, L., Walsh, K. A. & Neurath, H., unpublished results).

The use of cyanogen bromide has facilitated determination of the COOH-terminal sequence of carboxypeptidase A. Since methionine is converted to homoserine during the cleavage with CNBr[75], the only fragment devoid of homoserine is the COOH-terminal fragment. The use of this reagent has been of value in determining the sequence of the COOH-terminal hexapeptide and in detecting an unusual heterogeneity in that region where a leucine is replaced by a valine residue[119]. Perhaps, it is of interest to mention here that in a single cow that was investigated the carboxypeptidase A_α consisted of two chains which differed from each other in the mutual replacement of leucine and valine near its COOH-terminus[129]. All the sequence knowledge about this protein is summarized in Chart 3.

Pancreatic Trypsin Inhibitor

The linear sequence of the bovine pancreatic inhibitor containing 58 residues (Chart 4) has been recently worked out using conventional techniques of sequence determination[130]. Contrary to the classical views of Kunitz, the inhibition of bovine trypsin by soybean trypsin inhibitor involves inactivation by an enzymatic event, rather than by a mere electrostatic interaction between the two proteins, and an arginyl bond in the inhibitor is cleaved in the process[131]. However, the product of the reaction is not liberated from the surface of the enzyme thereby preventing the turnover of the enzyme.

```
                                                    10
Arg-Pro-Asp-Phe-Cys-Leu-Glu-Pro-Pro-Tyr-
                                                    20
Thr-Gly-Pro-Cys-Lys-Ala-Arg-Ile-Ile-Arg-
                                                    30
Tyr-Phe-Tyr-Asn-Ala-Lys-Ala-Gly-Leu-Cys-
                                                    40
Gln-Thr-Phe-Val-Tyr-Gly-Gly-Cys-Arg-Ala-
                                                    50
Lys-Arg-Asn-Asn-Phe-Lys-Ser-Ala-Glu-Asp-

Cys-Met-Arg-Thr-Cys-Gly-Gly-Ala
```

Chart 4 — Amino acid sequence for bovine pancreatic trypsin inhibitor

Haemoglobins and Myoglobin

The structural studies on human haemoglobins have been prosecuted by three groups of investigators[132–134] and has led to the elucidation of structure of the α- and β-chains from Hb-A, of the δ-chains from Hb-A_2, and of that of the γ-chains from Hb-F. Hb-A contains 574 amino acid residues distributed in the four peptide chains it contains. There are two α-chains each containing 141 amino acid residues, while the two β-chains contain 146 each. The molecular weight for this globin portion works out to 61,992, while in association with heme the molecular weight would work out to 64,458. The γ-chains of foetal haemoglobin each contain 146 amino acids, like the β-chains of Hb-A, with 39 points of difference in the amino acid sequence when a homologous representation of structure is made. Numerous studies[135] on abnormal human haemoglobins, mammalian haemoglobin, fish and cyclostome haemoglobins, leghaemoglobin, and erythrocruorins have been made, but notable amongst these was an early study on Hb-S (from sickle cell anaemia cases) in which it was shown that the grossly different physical and physiological properties of the molecule could be accounted for by a defect residing solely in the β-chains which contain at position 6 in the chains a valine residue in lieu of lysine which occurs at that position in the β-chains of Hb-A.

Several recent reviews[136–139] cover the advances made in the past years on the haemoglobins and myoglobin, and deal extensively with species difference, the role of hybridization in accounting for some of the naturally occurring haemoglobins, and the physiological role of the molecule. The known primary structure of the haemoglobin molecule coupled with the three-dimensional model available from crystallographic data together constitute the most detailed concept yet of the structure of a protein molecule. A comparison of the structure of the γ-chain of Hb-F and the β-chain of Hb-A is illustrated in Chart 5.

Cytochromes c

Cytochromes *c* from several species have been investigated in regard to their structure and much of the work has been amply surveyed in several papers[140–146]. The detailed structure of the proteins from species such as horse, man, dog, baker's yeast,

```
      Val     Leu       Pro     Glu     Ser-Ala-Val     Ala                          Asp-Glu-Val
Gly-His-Phe-Thr-Glu-Glu-Asp-Lys-Ala-Thr-Ile-Thr-Ser-Leu-Try-Gly-Lys-Val-Asn-Val-Glu-Asp-Ala-Gly-Gly-

      Ala                                                          Glu          Asp       Thr-
Glu-Thr-Leu-Gly-Arg-Leu-Leu-Val-Val-Tyr-Pro-Try-Thr-Gln-Arg-Phe-Phe-Asp-Ser-Phe-Gly-Asn-Leu-Ser-Ser-

Pro-Asp   Val                                                    Gly-Ala-Phe-Ser   Gly-Leu-Ala-
Ala-Ser-Ala-Ile-Met-Gly-Asn-Pro-Lys-Val-Lys-Ala-His-Gly-Lys-Lys-Val-Leu-Thr-Ser-Leu-Gly-Asp-Ala-Ile-Lys-

          Asn                 Thr
His-Leu-Asp-Asp-Leu-Lys-Gly-Thr-Phe-Ala-Gln-Leu-Ser-Glu-Leu-His-Cys-Asp-Lys-Leu-His-Val-Asp-Pro-Glu-Asn-

      Arg                           Cys             His                                  Pro
Phe-Lys-Leu-Leu-Gly-Asn-Val-Leu-Val-Thr-Val-Leu-Ala-Ile-His-Phe-Gly-Lys-Glu-Phe-Thr-Pro-Glu-Val-Gln-Ala-

                Ala-Tyr     Val     Ala          Asn       Ala-His-Lys
Ser-Try-Gln-Lys-Met-Val-Thr-Gly-Val-Ala-Ser-Ala-Leu-Ser-Ser-Arg-Tyr-His
```

Chart 5 — Amino acid sequence of the gamma chain of foetal haemoglobin [The beta chain of adult haemoglobin differs from the above at 39 locations, and the amino acid replacements are indicated]

Acetyl.Gly.*Asp*.Val.Glu.**Lys**.Gly.*Lys*.*Lys*.Ileu.**Phe**.Val.GluNH$_2$.*Lys*.CyS.Ala.*GluNH$_2$*.CyS.**His**.**Thr**.
10 ⌊—HEME—⌋
Val.**Glu**.**Lys**.**Gly**.**Gly**.*Lys*.**His**.**Lys**.**Thr**.**Gly**.**Pro**.**Asp**NH$_2$.**Leu**.**His**.**Gly**.*Leu*.**Phe**.**Gly**.**Arg**.*Lys*.*Thr*.
20 30 40
Gly.**Glu**NH$_2$.**Ala**.**Pro**.**Gly**.**Phe**.**Thr**.**Tyr**.**Thr**.**Asp**.**Ala**.**Asp**NH$_2$.*Lys*.**Asp**NH$_2$.**Lys**.*Gly*.*Ileu*.**Thr**.**Try**.**Lys**.
50 60
Glu.Glu.*Thr*.*Leu*.*Met*.Glu.Tyr.Leu.*Glu*.**Asp**NH$_2$.**Pro**.**Lys**.**Lys**.**Tyr**.Ileu.**Pro**.**Gly**.**Thr**.**Lys**.*Met*.*Ileu*.
70 80
Phe **Ala**.**Gly**.*Ileu*.**Lys**.**Lys**.*Lys*.**Thr**.*Glu*.**Arg**.Glu.**Asp**.**Leu**.Ileu.*Ala*.**Tyr**.**Leu**.**Lys**.**Lys**.Ala.**Thr**.
90 100
AspNH$_2$.GluCOOH
104

Chart 6 — Amino acid sequence for horse heart cytochrome *c* [The residues in bold-face type are those that are identical in the cytochromes *c* from horse, man, pig, rabbit, chicken, tuna and Baker's yeast. The residues in italics are those that are identical in the vertebrate proteins only]

Species comparison	Number of variant residues	Divergence of in millions of years
Horse — — Man	12	130
Horse — — Pig	3	33
Horse — — Chicken	12	
Pig — — Chicken	10	108-150
Rabbit — — Chicken	11	
Man — — Chicken	14	
Horse — — Tuna	19	
Pig — — Tuna	17	
Rabbit — — Tuna	19	184-228
Man — — Tuna	21	
Chicken — — Tuna	18	
Horse — — Yeast	44	
Pig — — Yeast	43	
Rabbit — — Yeast	45	465-520
Man — — Yeast	43	
Chicken — — Yeast	43	
Tuna — — Yeast	48	

Chart 7 — Evolution of cytochrome *c*

and others are available. The work of Paleus and Tuppy[147], and Tuppy's earlier work[148] had pointed to limited amino acid exchanges occurring in the heme region of proteins derived from ox, horse, pig, chicken, salmon, silkworm, baker's yeast. *Rhodospirillum rubrum* (c_2) and further data enable comparisons of structure with the proteins from *Chromatium* ' RHP ', *Pseudomonas fluorescens* (*c*-551), and others. Acetylation of the NH_2-terminus is found in some of the cytochromes *c* as in TMV protein and ovalbumin. The presence of NH_2-terminal acetylglycine was located by Margoliash[149] by identifying acetic acid upon gas chromatography of the sulphuric acid hydrolysates of the NH_2-terminal peptide obtained by digestion with chymotrypsin. Kreil and Tuppy[150] isolated from peptic digests acetylglycylaspartate, and the peptide bond was cleaved selectively by heating at *p*H 2·0.

Margoliash[140] analysed the sequences from divergent species (Charts 6 and 7) to compare the extent of variation of these structures with the known phylogenetic relations of species. In an evolutionary sense, all these proteins are truly homologous structures. The largest differences are found between phylogenetically distant species like the vertebrate and yeast, while a few differences are observed between closely related species. Certain sequences in the polypeptide chains are invariant while others are prone to variation. The extent of variation of the primary structure can give some approximations of the time elapsed since the lines of evolution leading to any two species diverged. Similar studies have also been carried out by Doolittle and Bloomback[151] who compared the sequences of fibrinopeptides isolated from divergent species.

Lysozyme (*Muramidase*)

Egg white lysozyme has been studied mainly by two groups of investigators[152-155] in recent years culminating in the successful elucidation of the primary structure of the protein (mol. wt 14,307, 129 amino acid residues), including the correct assignment of the —S—S—bridges, which latter was also accomplished by Brown[155] employing the diagonal technique[67]. The proposed structure is given in Chart 8. Lysozymes, including egg white lysozyme, have lytic action on bacteria and split the cell wall by hydrolysing the β (1-4) linkages between N-acetylmuramic acid and N-acetylglucosamine. Various groups in lysozyme, such as indole, imidazole, methionine, free amino groups, etc., are known to be essential for the biological activity of lyso-

6
Lys-Val-Phe-Gly-Arg-CyS-Glu-Leu-Ala-Ala-Ala-Met-Lys-Arg-His-Gly-Leu-Asp-Asn-Tyr-Arg-Gly-Tyr-Ser-
30
Leu-Gly-Asn-Try-Val-CyS-Ala-Ala-Lys-Phe-Glu-Ser-Asn-Phe-Asn-Thr-Gln-Ala-Thr-Asn-Arg-Asn-Thr-Asp-
64
Gly-Ser-Thr-Asp-Tyr-Gly-Leu-Leu-Gln-Ile-Asn-Ser-Arg-Try-Try-CyS-Asn-Asp-Gly-Arg-Thr-Pro-Gly-Ser-
76 80 94
Arg-Asn-Leu-CyS-Asn-Ile-Pro-CyS-Ser-Ala-Leu-Leu-Ser-Ser-Asp-Ile-Thr-Ala-Ser-Val-Asn-CyS-Ala-Lys-
115
Lys-Ile-Val-Ser-Asp-Gly-Asp-Gly-Met-Asn-Ala-Try-Val-Ala-Try-Arg-Asn-Arg-CyS-Lys-Gly-Thr-Asp-Val-
127
Gln-Ala-Try-Ile-Arg-Gly-CyS-Arg-Leu

Chart 8 — Amino acid sequence of egg white lysozyme which contains 129 amino acid residues. The disulphide alignments are 6 → 127, 30 → 115, 64 → 80 and 76 → 94

zyme[153,154,157-159]. With the structure of the protein known it is to be hoped that studies on the nature of the active site will be placed on a firmer footing by the characterization and location of the important groupings in relation to the known structure. One example of such a fruitful study is the identification of the single Try residue, at position 62, in lysozyme, which on modification with N-bromosuccinimide causes the enzyme to lose almost all activity[160].

Tobacco Mosaic Virus Protein

In the past decade intensive research has been done on the various aspects of structure, assembly, stability and biological activity of the tobacco mosaic virus (TMV) particle. The progress in this field, arising from the two groups working in Berkeley and Tubingen, have been amply surveyed[161-165]. The complete covalent structure of TMV protein is known and well documented (Chart 9) and a great deal of information on chemical mutagenesis and its consequences on the amino acid sequence has emerged from these studies[166,167].

Ferredoxin

Ferredoxin, the non-heme iron-containing protein from *Clostridium pasteurianum*, is involved in electron transport and the protein has been isolated also from some plants and numerous anaerobic bacteria. The structure of the *Clostridium pasteurianum* ferredoxin, of molecular weight approximately 6000 and containing about 55 amino acid residues, has been the subject of several studies[168-170] (Also, Tanaka, M., Benson, A. M., Hower, H. F. & Yasunobu, K. T., personal communication). The protein contains eight cysteine residues, six or seven moles of labile sulphide S and seven atoms of Fe. The Fe and S are incorporated in a poly-ferric-sulphide chain in which there are two groups, comprising two and five, respectively, Fe atoms which are non-equivalent. At least two of the iron atoms are in the Fe^{3+} state. There are 6 inorganic sulphide bridges between the various iron atoms, and in the poly-ferric-sulphide structure in ferridoxin seven S atoms, from cystine residues in the peptide chain, alternate with the six

Acetyl-Ser-Tyr-Ser-Ile-Thr-Thr-Pro-Ser-Gln-Phe-Val-Phe-Leu-Ser-Ser-Ala-Try-Ala-Asp-Pro-Ile-Glu-Leu-Ile-Asn-Leu-CySH-Thr-Asn-Ala-Leu-Gly-Asn-Gln-Phe-Gln-Thr-Gln-Gln-Ala-Arg-Thr-Val-Gln-Val-Arg-Gln-Phe-Ser-Gln-Val-Try-Lys-Pro-Ser-Pro-Gln-Val-Thr-Val-Arg-Phe-Pro-Asp-Ser-Asp-Phe-Lys-Val-Tyr-Arg-Tyr-Asn-Ala-Val-Leu-Asp-Pro-Leu-Val-Thr-Ala-Leu-Leu-Gly-Ala-Phe-Asp-Thr-Arg-Asn-Arg-Ile-Ile-Gln-Val-Gln-Asp-Gln-Ala-Asn-Pro-Thr-Thr-Ala-Gln-Thr-Leu-Asp-Ala-Thr-Arg-Arg-Val-Asp-Asp-Ala-Thr-Val-Ala-Ile-Arg-Ser-Ala-Asp-Ile-Asn-Leu-Ile-Val-Glu-Leu-Ile-Arg-Gly-Thr-Gly-Ser-Tyr-Asn-Arg-Ser-Ser-Phe-Glu-Ser-Ser-Ser-Gly-Leu-Val-Try-Thr-Ser-Gly-Pro-Ala-Thr

Chart 9 — Amino acid sequence of the 158 residue-long polypeptide chain in tobacco mosaic virus

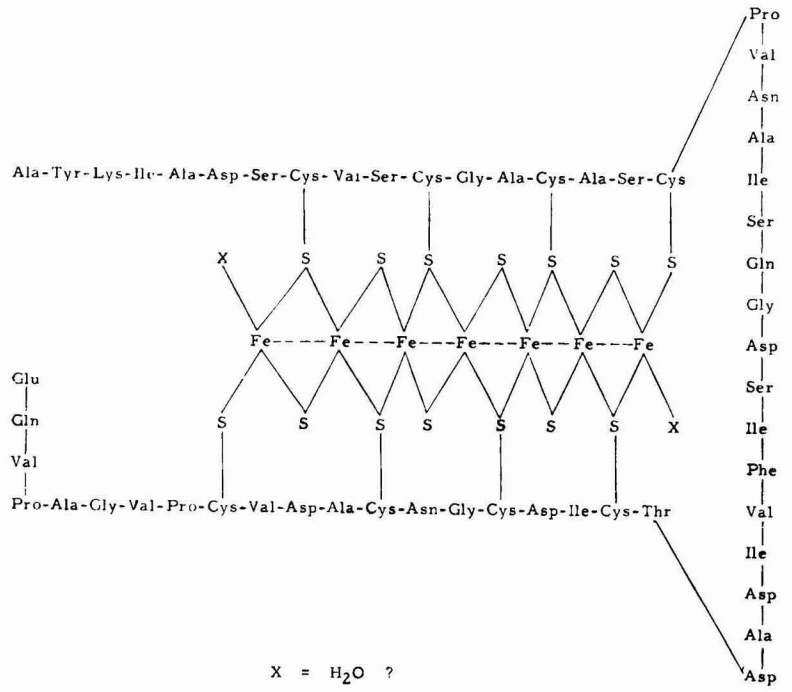


Chart 10 — Structure of ferredoxin

10
Ile-Pro-Glu-Tyr-Val-Asp-Trp-Arg-Gln-Lys-Gly-Ala-Val-Thr-Pro-
20 30
Val-Lys-Asn-Gln-Gly-Ser-Cys-Gly-Ser-Cys-Trp//Ala-Phe//$(Ile)_2$//
40
Arg-Asn-Thr-Pro-Tyr-Tyr-Glu-Gly-Val-Gln-Arg-Tyr-Cys-Arg-Ser-Arg-
50 60
Glu-Lys-Gly-Pro-Tyr-Ala-Ala-Lys-Thr-Asp-Gly-Val-Arg-Gln-Val-
70
Gln-Pro-Tyr-Asn-Gln-Gly-Ala-Leu-Leu-Tyr-Ser-Ile-Ala-Asn-Gln-
80 90
Pro-Ser-Val-Val-Leu-Gln-Ala-Ala-Gly-Lys-Asp-Phe-Gln-Leu-Tyr-
100
Arg-Gly-Gly-Ile-Phe-Val-Gly-Pro-Cys-Gly-Asn-Lys-Val-Asp-His-
110 120
Ala-Val-Ala-Ala-Val-Gly-Tyr-Asn-Pro-Gly-Tyr-Ile-Leu-Ile-Lys-
130
Asn-Ser-Trp-Gly-Thr-Gly-Trp-Gly-Glu-Asn-Gly-Tyr-Ile-Arg-Ile-
140 150
Lys-Thr-Gly-Asn-Leu-Asn-Gln-Tyr-Ser-Glu-Gln-Glu-Leu-Leu-Asp-
160
Cys-Asp-Arg-Arg-Ser-Tyr-Gly-Cys-Tyr-Pro-Gly-Asp-Gly-Trp//Ser-
170 180
Ala-Leu//Val-Ala-Gln-Tyr-Gly-Ile-His-Tyr-Arg-Gly-Thr-Gly-Asn-
190
Ser-Tyr-Gly-Val-Cys-Gly-Leu-Tyr-Thr-Ser-Ser-Phe-Tyr-Pro-Val-Lys-Asn

Chart 11 — Tentative amino acid sequence for papain [Residues in sequences within slant lines are unassigned peptides whose relative positions are not known]

10
Ala-Glu-Cys-Ser-Val-Asp-Ile-Gln-Gly-Asn-Asp-Gln-Met-Gln-Phe-Asn-
20 30
Thr-Asn-Ala-Ile-Thr-Val-Asp-Lys-Ser-Cys-Lys-Gln-Phe-Thr-Val-Asn-
40
Leu-Ser-His-Pro-Gly-Asn-Leu-Pro-Lys-Asn-Val-Met-Gly-His-Asn-Trp-
50 60
Val-Leu-Ser-Thr-Ala-Ala-Asp-Met-Gln-Gly-Val-Val-Thr-Asp-Gly-Met-
70 80
Ala-Ser-Gly-Leu-Asp-Lys-Asp-Tyr-Leu-Lys-Pro-Asp-Asp-Ser-Arg-Val-
90
Ile-Ala-His-Thr-Lys-Leu-Ile-Gly-Ser-Gly-Gln-Lys-Asp-Ser-Val-Thr-
100 110
Phe-Asp-Val-Ser-Lys-Leu-Lys-Glu-Gly-Glu-Gln-Tyr-Met-Phe-Phe-Cys-
120
Thr-Phe-Pro-Gly-His-Ser-Ala-Leu-Met-Lys-Gly-Thr-Leu-Thr-Leu-Lys-

Chart 12 — Amino acid sequence for azurin

sulphide S atoms and thereby provide points of attachment for the poly-ferric-sulphide chain to the protein molecule. The proposed structure for ferredoxin is indicated in Chart 10.

There is the possibility that this structure may have to be modified, since there are reportedly observations suggesting that Fe can be removed from the protein without loss of sulphide.

Another protein, rubredoxin, involved in electron transport has been isolated from *Cl. pasteurianum*[171]. The structure for the protein is not available but it is of size comparable to ferredoxin while not containing any alanine or serine.

Papain

A tentative structure of papain has been postulated recently[172] (Chart 11). The total number of amino acid residues is about 200 which is appreciably higher than the 178 reported previously[173].

It is of interest to compare the amino acid sequences around the active site of papain with that of ficin[174], which, like papain, is also a —SH enzyme. The two sequences are shown:

Papain *Pro*-Val-Lys-Asn-*Gln-Gly*-Ser-*Cys-Gly-Ser-Cys*-
Ficin *Pro*-Ile-Arg-Gln-*Gln-Gly*-Gln-*Cys-Gly Ser-Cys*-

The close identity of these sequences probably implies their direct involvement in a mechanism of action common to both enzymes.

Azurin

The copper protein, azurin (blue protein), from *Pseudomonas fluorescens* was investigated by Ambler[175,176]. The metal was removed by precipitation of the protein with trichloracetic acid. Peptides from conventional enzymic digests as well as those obtained by cleavage with cyanogen bromide were isolated and the sequences determined. The sequence of the apoprotein is given in Chart 12. The site of binding of copper in this protein is not known.

Miscellaneous Proteins

The structure of Bence-Jones proteins, corresponding in most instances to the light chains of the myeloma protein from the same patient, has received attention and a nearly complete sequence for the 212 (213) amino acid residues in Bence-Jones protein Cummings is available[177]. The structure work on Bence-Jones protein Ag has also progressed apace[178]. The structure of clupeine Z has been worked out[179].

The structure of porcine globin *a* is available[180]. Rattle snake heart cytochrome *c* contains 104 amino acid residues and the complete structure is known[181].

In isozymes such as lactic dehydrogenase, which is made up of four peptide chains, the sole differences seem to reside in the presence of varying numbers of two different types of peptide chains.

Small Molecular Weight Peptides

A vast array of polypeptides of relatively small size occur naturally, a few as hormones and the majority as bacterial polypeptide antibiotics. An idea of the fascinating structural variety in the latter group may be got from two published surveys[182,183]. The relatively small size of these peptides and the presence of unusual constituents such as D-amino acids and non-amino acid moieties naturally pose some questions relating to the mechanisms of biosynthesis. Analogies to protein biosynthetic mechanisms have been pointed to as relevant[184] and alternate schemes[185] have been proposed. Much work remains to be done in this area of mechanism(s) of antibiotic and other small polypeptide biosynthesis.

Gramicidin (Dubos) known to consist of at least three antibiotics[186] has now been demonstrated to contain a fourth constituent (gramicidin D[187]); and indeed yet another (also named ' gramicidin D ') containing amino acids not found in the others. The last has a very high partition coefficient, and a high activity in bioassays[188]. The structure of gramicidin A[189], a pentadecapeptide with a formyl group protecting the amino group of a terminal valine residue and an ethanolamine residue protecting the carboxyl group of a terminal tryptophan residue, is shown below:

1 5
OHC-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Try-

10 15
D-Leu-L-Try-D-Leu-L-Try-D-Leu-L-Try-$NHCH_2CH_2OH$

The structures of gramicidins B, C and D[187] have also been worked out[190]. Gramicidin D has a formyl-L-isoleucine residue replacing the formyl-L-valine at position one of gramicidin A. Gramicidin B contains a L-Phe residue, at position 11, replacing L-Try in gramicidin A, while in gramicidin C, L-Tyr replaces L-Try at position 11 in gramicidin A. Both gramicidins B and C are also found in lesser amounts in forms with L-Ile which replaces L-Val at position 1 (Sarges and Witkop[190]). Indeed the question of heterogeneity of gramicidin B had been raised earlier[187] on the basis of detection of non-stoichiometric amounts of isoleucine in amino acid analyses of gramicidin B. The synthesis of gramicidin A has been accomplished[191].

There is some confusion in relation to the nomenclature for the gramicidin components. One component isolated in 1963 (Ramachandran[187]) has been termed gramicidin D, and the same name given to another isolated in 1965 (Gross and Witkop[188]). This might have been avoided, and individual letters of the alphabet assigned to each component in the order of discovery as, for example, with the tyrocidines[192]. Such a system also would, unfortunately, not reflect well some of the structural relations that do exist in the gramicidins. Names such as valine-gramicidin A and isoleucine-gramicidin A have been used. To avoid such lengthy names we suggest that, while retaining the original[186] A, B and C designations for the three major peaks at 500 transfers, the corresponding naturally occurring minor components (analogues) with replacements of isoleucine for valine at position 1 be designated as gramicidins A_1, B_1 and C_1. Other arabic numeral subscripts may be used with the same letters of the alphabet to connote any related components containing other ' environmental ' amino acid substitutions which may be discovered in the future. The term D then can be assigned to the unique component, not related to gramicidins A, B and C, recognized recently[188].

Tyrocidine, previously known to contain components A and B, has now been fractionated to yield a third tyrocidine C[192]. Tyrocidine A contains one L-Phe and two D-Phe residues, of which one L-Phe is exchanged for L-Try in tyrocidine B while in tyrocidine C one more residue (D-Phe) is exchanged for a D-Try residue.

Other small peptides whose structures have been worked out in recent times include, amongst others, peptidolipine NA[193], ferrichrome[194] and ferrichrome A, albomycin[195], telomycin[196], etamycin[197], ostreogrycin B[198], cell wall glycopeptide(s) of bacteria[199], serratamolide[200], homoglutathione[201], mycobacillin[202], co-fibrin peptides A and B, bradykinin, and kallidin[203-205], maliformin A[206], and rufomycin (ilamycin)[207]. Partial progress in structure elucidation has been made on several antibiotics, viz. the complex thiostrepton[208] which has also been taken up for X-ray study. With some of these small but complex molecules, X-ray crystallography should considerably speed up structure elucidation. The extensive work on the actinomycins and other antibiotics has been well surveyed[209]. Interesting structure activity correlations in the actinomycin series have been made[210]. The synthesis and chemistry of several antibiotics and depsipeptides continues to receive attention[211,212].

Conclusion

Looking back, the progress during the past decade in the field of primary structure of proteins has been truly remarkable. The number of naturally occurring proteins and peptides whose structures are known is increasing by leaps and bounds. There has been no better time for studies on the role of gene action in relation to protein structure[213]. Aspects of secondary structure of proteins are better understood today, while an increasing number of investigators have taken on the more exacting problem of understanding the elements of tertiary structure of proteins.

The role of automation has been increasingly evident in amino acid analysis and in peptide fractionation and analysis. The sequential degradation of peptides remains an area where the advent of automation would effect dramatic economy in the time taken for elucidating a structure. The tedium of obtaining data with manual X-ray diffractometers, and literally the period of years taken for completing analysis, would seem to be avoidable in the near future. Automated diffractometers are becoming commercially available, and with computerization

the time taken for an X-ray analysis of structure may be cut by as much as 80-95 per cent.

A heartening sign of the times is the increasing interest the chemist has shown in the synthesis of the naturally occurring polypeptides and proteins. The record of progress is impressive[211,214]. Peptides synthesized range from glutathione, the simpler antibiotics[215], the peptide hormones of the pituitary[216,217] to insulin[218-220]. Significant strides are being taken towards automated peptide synthesis. All this brings us nearer to that day when complex proteins such as hormones and enzymes, some used in therapy, may all be conveniently made in the chemist's workshop*.

Summary

Recent progress in the elucidation of the primary structure of proteins, and innovations in techniques for studying protein structure are surveyed. In particular, our knowledge of the structures of ribonuclease (A, B, T), proteolytic enzymes of the pancreas, pancreatic trypsin inhibitor, myoglobin and haemoglobin, cytochrome *c*, lysozyme, tobacco mosaic virus protein, ferredoxin, papain, azurin, etc., and some of the bacterial polypeptides is summarized. The potentiality of current peptide synthesis procedures is brought out in the recent synthesis of insulin. The field of protein chemistry has developed to the point that one can hopefully anticipate, in the coming years, fascinating developments relating to the structure and synthesis of large protein molecules.

References

1. *Advances in protein chemistry*, Vols. 1-19 (Academic Press Inc., New York), 1944-64.
2. *Annual reviews of biochemistry*, Vols. 1-33 (Annual Reviews Inc., Palo Alto), 1932-64.
3. SANGER, F., *Science*, **129** (1959), 1340.
4. DU VIGNEAUD, V., RESSLER, C. & TRIPPETT, S., *J. biol. Chem.*, **205** (1953), 949.
5. DU VIGNEAUD, V., LAWLER, H. C. & POPENOE, E. A., *J. Amer. chem. Soc.*, **75** (1953), 4880.
6. DU VIGNEAUD, V., RESSLER, C., SWAN, J. M., ROBERTS, C. W. & KATSOYANNIS, P. G., *J. Amer. chem. Soc.*, **76** (1954), 3115.
7. DU VIGNEAUD, V., GISH, D. T. & KATSOYANNIS, P. G., *J. Amer. chem. Soc.*, **76** (1954), 4751.
8. DU VIGNEAUD, V., BARTLETT, M. F. & JOHL, A., *J. Amer. chem. Soc.*, **79** (1957), 5572.
9. CONSDEN, R., GORDON, A. H. & SYNGE, R. L. M., *Biochem. J.*, **41** (1947), 596.
10. ELLIOTT, D. F. & PEART, W. S., *Biochem. J.*, **65** (1957), 246.
11. LI, C. H., *Advanc. Protein Chem.*, **12** (1957), 269.
12. LI, C. H., DIXON, J. S. & CHUNG, D., *Biochim. biophys. Acta*, **46** (1961), 324.
13. BROMER, W. W., SINN, L. G. & BEHRENS, O. K., *J. Amer. chem. Soc.*, **79** (1957), 2807.
14. PERUTZ, M. F., *Nature, Lond.*, **185** (1960), 416.
15. PERUTZ, M. F., *Proteins and nucleic acids* (Elsevier Publishing Co. Inc., Amsterdam), 1962.
16. KENDREW, J. C., WATSON, H. C., STRANDBERG, B. E., DICKERSON, R. E., PHILLIPS, D. C. & SHORE, V. C., *Nature, Lond.*, **190** (1961), 666.
17. PERUTZ, M. F., in *Protein structure and function, Monograph* 13, *Brookhaven symposia in biology* (Brookhaven National Laboratory, Upton, New York), 1960, 165.
18. RAMACHANDRAN, L. K. & WITKOP, B., *Biochemistry*, **3** (1964), 1603.
19. PAULING & COREY, R. B., *Proc. nat. Acad. Sci., Wash.*, **37** (1951), 272.
20. RAMACHANDRAN, G. N. & KARTHA, G., *Nature, Lond.*, **174** (1954), 269.
21. RICH, A. & CRICK, F. H. C., *Nature, Lond.*, **176** (1955), 915.
22. RAMACHANDRAN, G. N., *Nature, Lond.*, **177** (1956), 710.
23. GOWAN, P. M., MCGAVIN, S. & NORTH, A. C. T., *Nature, Lond.*, **176** (1955), 1062.
24. BOEDTKER, H. & DOTY, P., *J. Amer. chem. Soc.*, **78** (1956), 4267.
25. KRANT, J., HIGH, D. F. & SIEKER, L. C., *Proc. nat. Acad. Sci., Wash.*, **51** (1964), 839.
26. STRANDBERG, B., TILANDER, B., FRIDBORG, K., LINDSKOG, S. & NYMAN, P. D., *J. mol. Biol.*, **5** (1962), 583.
27. LUDWIG, M. L., PAUL, I. C., PAWLEY, G. S. & LIPSCOMB, W. N., *Proc. nat. Acad. Sci., Wash*, **50** (1963), 282.
28. BLOUT, E. R., DE LOZE, C., BLOOM, S. M. & FASMAN, G. D., *J. Amer. chem. Soc.*, **82** (1960), 3787.
29. SIMMONS, N. R. & BLOUT, E. R., *Biophys. J.*, **1** (1960), 55.
30. ANFINSEN, C. B., in *Informational macromolecules*, edited by H. J. Vogel, V. Bryson & J. O. Lampen (Academic Press Inc., New York), 1963, 153.
31. ZUCKERKANDL, E. & PAULING, L., in *Horizons in biochemistry*, edited by M. Kasha & B. Pullman (Academic Press Inc., New York), 1962, 198.
32. MINER, R. W., editor, *Ann. N.Y. Acad. Sci.*, **47** (1946), 57.
33. CANFIELD, R. E. & ANFINSEN, C. B., in *The proteins*, Vol. I, edited by H. Neurath (Academic Press Inc., New York), 1963, 311.
34. MOORE, S., SPACKMAN, D. H. & STEIN, W. H., *Analyt. Chem.*, **30** (1958), 1185.
35. *The enzymes*, Vol. 4, edited by P. D. Boyer, A. Lardy & K. Myrback (Academic Press Inc., New York), 1960.
36. KATCHALSKI, E., SELA, M., SILMAN, H. I. & BERGER, A., in *The proteins*, Vol. II, edited by H. Neurath (Academic Press Inc., New York), 1964, 406.
37. WITKOP, B., *Advanc. Protein Chem.*, **16** (1961), 221.
38. WITKOP, B. & RAMACHANDRAN, L. K., *Metabolism*, **10** (1964), 1016.
39. PATCHORNIK, A., WILCHEK, M. & SARID, S., *J. Amer. chem. Soc.*, **86** (1964), 1457.
40. RUTTENBERG, M. A., KING, T. P. & GRAIG, L. C., *Biochemistry*, **3** (1964), 788.
41. TSUNG, C. M. & FRAENKAL-CONRAT, H., *Biochemistry*, **4** (1965), 793.
42. PARTRIDGE, S. M. & DAVIS, H. F., *Nature, Lond.*, **165** (1950), 62.
43. SCHULTZ, J., ALLISON, H. & GRICE, M., *Biochemistry*, **1** (1962), 694.
44. SANGER, F., *Biochem. J.*, **39** (1945), 507.
45. OKUYAMMA, T. & SATAKE, K., *J. Biochem. Tokyo*, **47** (1960), 454.
46. SATAKE, K., OKUYAMMA, T., OHASHI, M. & SHINODA, T., *J. Biochem. Tokyo*, **47** (1960), 654.
47. SATAKE, K., TANAKA, M. & SHINO, H., *J. Biochem. Tokyo*, **50** (1961), 6.
48. SIGNOR, A., BIONDI, L., TERBOJEVICH, M. & PAZETTA, P., *Gazz. chem. ital.*, **94** (1964), 619.
49. STARK, R. & SMYTH, D. G., *J. biol. Chem.*, **238** (1963), 214.

***Note added in proof**

There have been many interesting contributions published since this review was written. We cite only a few below. In haemoglobin no consistent correlation could be found between the positions of anti-helical amino acid residues in the chain and the non-helical regions, leading to the inference that there are other features of amino acid sequences not yet considered which are powerful in determining the secondary structure of the protein [Perutz, M. F., Kendrew, J. C. & Watson, H. C., *J. mol. Biol.*, **13** (1965), 669].

The structure of bovine parathyroid hormone (84 residues) has been almost completed [Potts (Jr), J. T., Auerback, G. D., Sherwood, L. M. & Sandoval, A., *Proc. nat. Acad. Sci., Wash.*, **54** (1965), 1743]. A large polypeptide (48 residues) having two intra-chain disulphide linkages has been isolated from bovine posterior pituitary, and its amino acid sequence is known [Preddie, E. C., *J. biol. Chem.*, **240** (1965), 4194].

Human insulin has been synthesized [Katsoyannis, P. G., Tometsko, A. & Zalut, C., *J. Amer. chem. Soc.*, **88** (1966), 166].

0. Gray, W. R. & Hartley, B. S., *Biochem. J.*, **89** (1963), 59 p.
51. Eriksson, L. & Sjoquist, J., *Biochim. biophys. Acta*, **45** (1960), 290.
52. Edman, P., *Acta chem. scand.*, **4** (1950), 283.
53. Isle, D. & Edman, P., *Aust. J. Chem.*, **16** (1963), 411.
54. Sjoquist, J., *Biochim. biophys. Acta*, **41** (1960), 20.
55. Hirs, C. H. W., Moore, S. & Stein, W. H., *J. biol. Chem.*, **235** (1960), 633.
56. Konigsberg, W. & Hill, R. J., *J. biol. Chem.*, **237** (1962), 2545.
57. Akabori, S., Ohno, K. & Narita, K., *Bull. chem. Soc., Japan*, **25** (1952), 214.
58. Redfield, R. R. & Anfinsen, C. B., *J. biol. Chem.*, **221** (1956), 385.
59. Anfinsen, C. B., Sela, M. & Tritch, H., *Arch. Biochem. biophys.*, **65** (1956), 156.
60. Goldberger, R. F. & Anfinsen, C. B., *Biochemistry*, **1** (1962), 401.
61. Marigan, T. C., Dreyer, W. J. & Berger, A., *Biochim. biophys. Acta*, **62** (1962), 122.
62. Ludwig, M. L. & Byrne, R., *J. Amer. chem. Soc.*, **84** (1962), 4160.
63. Itano, H. A. & Gotlieb, A. J., *Biochem. biophys. Res. Commun.*, **12** (1963), 405.
64. Lindley, H., *Nature, Lond.*, **178** (1956), 647.
65. Walsh, K. A., Kauffman, D. L., Sampathkumar, K. S. V. & Neurath, H., *Proc. nat. Acad. Sci., Wash.*, **51** (1964), 301.
66. Raftery, M. A. & Cole, R. D., *Biochem. biophys. Res. Commun.*, **10** (1963), 467.
67. Brown, J. R. & Hartley, B. S., *Biochem. J.*, **89** (1963), 59p.
68. Stahl, E., *Thin layer chromatography* (Academic Press Inc., New York), 1965, 409.
69. Meister, A., *Biochemistry of amino acids*, Vol. 1 (Academic Press Inc., New York), 2nd ed., 1965, 55.
70. Hagen, P. B. & Black, W., *Canad. J. Biochem.*, **43** (1965), 309.
71. Gehrke, C. W., Lamkin, W. M., Stalling, D. L. & Shahrokhi, F., *Biochem. biophys. Res. Commun.*, **19** (1965), 328.
72. Hirs, C. H. W., Moore, S. & Stein, W. H., *J. biol. Chem.*, **235** (1960), 633.
73. Spackman, D. H., Stein, W. H. & Moore, S., *J. biol. Chem.*, **235** (1960), 648.
74. Smyth, D. G., Stein, W. H. & Moore, S., *J. biol. Chem.*, **238** (1963), 227.
75. Gross, E. & Witkop, B., *J. Amer. chem. Soc.*, **83** (1961), 1511.
76. Scheraga, H. A. & Rupley, J. A., *Advanc. Enzymol.*, **24** (1962), 161.
77. Anfinsen, C. B., Aqvist, S. E. G., Cooke, J. P. & Jonsson, B., *J. biol. Chem.*, **234** (1959), 1118.
78. Takahashi, K., *J. Biochem. Tokyo*, **51** (1961), 95.
79. Takahashi, K., *J. Biochem. Tokyo*, **52** (1962), 92.
80. Takahashi, K., *J. biol. Chem.*, **240** (1965), PC 4117.
81. Plummer (Jr), T. H. & Hirs, C. H. W., *J. biol. Chem.*, **239** (1964), 2530.
82. Neurath, H., *Advanc. Protein Chem.*, **12** (1957), 319.
83. Desnuelle, P. & Rovery, M., *Advanc. Protein Chem.*, **16** (1961), 139.
84. Dixon, G. H., Kauffman, D. L. & Neurath, H., *J. biol. Chem.*, **233** (1958), 1373.
85. Walsh, K. A., Kauffman, D. L. & Neurath, H., *Biochim. biophys. Acta*, **65** (1962), 540.
86. Mikes, O., Holeysovsky, V., Tomasek, V., Keil, B. & Sorm, F., *Coll. Trav. chim. Tchecosl.*, **27** (1962), 1964.
87. Tomasek, V., Mikes, O., Holeysovsky, V., Keil, B. & Sorm, F., *Biochim. biophys. Acta*, **69** (1963), 186.
88. Mikes, O., Holeysvosky, V., Tomasek, V. & Sorm, F., *Coll. Trav. chim. Tchecosl.*, **26** (1961), 1048.
89. Hofmann, T., *Biochemistry*, **3** (1964), 356.
90. Walsh, K. A. & Neurath, H., *Proc. nat. Acad. Sci., Wash.*, **52** (1964), 884.
91. Walsh, K. A., Sampathkumar, K. S. V. & Kauffman, D. L., *Abstracts, Sixth international congress in biochemistry*, Vol. 4, New York, 1964, 100.
92. Hartley, B. S., *Nature, Lond.*, **201** (1964), 1284.
93. Keil, B., Prussik, Z. & Sorm, F., *Biochim. biophys. Acta*, **78** (1963), 559.
94. Siekevitz, P. & Palade, G., *J. biophys. biochem. Cytol.*, **7** (1960), 619, 631.
95. Keller, P. J., Cohen, E. & Neurath, H., *J. biol. Chem.*, **234** (1959), 311.
96. Bergmann, M. & Fruton, J. S., *Advanc. Enzymol.*, **1** (1941), 63.
97. Neurath, H. & Schwert, G., *Chem. Rev.*, **46** (1950), 70.
98. Hein, G. & Niemann, C., *Proc. nat. Acad. Sci., Wash.*, **47** (1961), 1341.
99. Hartley, B. S. & Kilby, B. S., *Biochem. J.*, **56** (1954), 288.
100. Gutfruend, H. & Sturtevant, B. M., *Proc. nat. Acad. Sci., Wash.*, **42** (1956), 719.
101. Bender, M. L. & Kaiser, E. T., *J. Amer. chem. Soc.*, **84** (1962), 1557.
102. Jansen, E. F. & Balls, A. K., *J. biol. Chem.*, **194** (1952), 721.
103. Schaffer, N. K., May, C. S. & Summerson, W. H., *J. biol. Chem.*, **202** (1953), 67.
104. Naughton, M. A., Sanger, F., Hartley, B. S. & Shaw, D. C., *Biochem. J.*, **77** (1960), 149.
105. Dixon, G. H., Neurath, H. & Pechere, J. F., *Annu. Rev. Biochem.*, **27** (1958), 489.
106. Koshland, D. E., Strumeyer, D. H. & Ray, W. J., in *Enzyme models and enzyme structure, Brookhaven symposia in biology*, **15** (1962), 101.
107. Schoellmann, G. & Shaw, E., *Biochemistry*, **2** (1963), 252.
108. Ong, E. B., Shaw, E. & Schoellmann, G., *J. Amer. chem. Soc.*, **86** (1964), 1271.
109. Smillie, L. B. & Hartley, B. S., *J. mol. Biol.*, **10** (1964), 183.
110. Sanger, F., *J. Polym. Sci.*, **49** (1961), 3.
111. Bender, M. L. & Kezdy, F. J., *J. Amer. chem. Soc.*, **86** (1964), 3704.
112. Crestfield, A. M., Stein, W. H. & Moore, S., *J. biol. Chem.*, **238** (1963), 2421.
113. Bruice, T. C. & Topping, R. M., *J. Amer. chem. Soc.*, **85** (1963), 1488.
114. Keller, P. J., Cohen, E. & Neurath, H., *J. biol. Chem.*, **230** (1958), 905.
115. Brown, J. R., Cox, D. J., Greenshields, R. N., Walsh, K. A., Yamasaki, M. & Neurath, H., *Proc. nat. Acad. Sci., Wash.*, **47** (1961), 1554.
116. Brown, J. R., Greenshields, R. N., Yamasaki, M. & Neurath, H., *Biochemistry*, **2** (1963), 867.
117. Brown, J. R., Yamasaki, M. & Neurath, H., *Biochemistry*, **2** (1963), 877.
118. Anson, M. L., *J. gen. Physiol.*, **20** (1937), 777.
119. Thompson, E. O. P., *Biochim. biophys. Acta*, **10** (1953), 633.
120. Bargetzi, J. P., Thompson, E. O. P., Sampathkumar, K. S. V., Walsh, K. A. & Neurath, H., *J. biol. Chem.*, **239** (1964), 3769.
121. Coombs, T. L., Omote, Y. & Vallee, B. L., *Biochemistry*, **3** (1964), 653.
122. Cox, D. J., Bovard, F. E., Bargetzi, J. P., Walsh, K. A. & Neurath, H., *Biochemistry*, **3** (1964), 44.
123. Sampathkumar, K. S. V., Walsh, K. A., Bargetzi, J. P. & Neurath, H., *Biochemistry*, **2** (1963), 1475.
124. Sampathkumar, K. S. V., Clegg, J. B. & Walsh, K. A., *Biochemistry*, **3** (1964), 1728.
125. Vallee, B. L., Williams, R. J. P. & Coleman, T. E., *Nature, Lond.*, **190** (1961), 633.
126. Vallee, B. L., Coombs, T. L. & Hoch, F. L., *J. biol. Chem.*, **235** (1960), PC 45.
127. Walsh, K. A., Sampathkumar, K. S. V., Bargetzi, J. P. & Neurath, H., *Proc. nat. Acad. Sci., Wash.*, **48** (1962), 1443.
128. Sampathkumar, K. S. V., Walsh, K. A., Bargetzi, J. P. & Neurath, H., *Aspects of protein structure* (Academic Press Inc., London), 1963, 319.
129. Sampathkumar, K. S. V., Walsh, K. A. & Neurath, H., *Biochemistry*, **3** (1964), 1726.
130. Kassell, B., Radicevic, M., Ansfield, M. J. & Laskowski (Sr), M., *Biochem. biophys. Res. Commun.*, **18** (1965), 255.
131. Finkenstadt, W. R., Ozawa, K. & Laskowski (Jr), M., *Fed. Proc.*, **24** (1965), 593.
132. Braunitzer, G., Gehring-Muller, R., Hilschmann, N., Hilse, K., Hoborn, G., Rudloff, V. & Wittmann-Liebold, B., *Hoppe-Seyl Z.*, **325** (1961), 283.
133. Konigsberg W., Guidotti, G. & Hill, R. J., *J. biol. Chem.*, **236** (1961), PC 55.

134. SCHROEDER, W. A., SHELTON, J. R., SHELTON, J. B., CORMICK, J. & JONES, R. T., *Biochemistry*, **2** (1963), 992.
135. HUNT, J. A. & INGRAM, V. M., *Nature, Lond.*, **184** (1959), 640.
136. SCHROEDER, W. A., *Annu. Rev. Biochem.*, **32** (1963), 301.
137. BRAUNITZER, G., HILSE, K., RUDLOFF, V. & HILSCHMANN, N., *Advanc. Protein Chem.*, **19** (1964), 1.
138. ROSSI FANELLI, A., ANTONINI, E. & CAPUTO, A., *Advanc. Protein Chem.*, **19** (1964), 74.
139. ITANO, H. A., *Advanc. Protein Chem.*, **12** (1957), 215.
140. MARGOLIASH, E., *Proc. nat. Acad. Sci., Wash.*, **50** (1963), 672.
141. AMBLER, R. P., *Biochem. J.*, **89** (1963), 341.
142. MATSUBARA, H. & SMITH, E. L., *J. biol. Chem.*, **238** (1963), 2732.
143. NARITA, K., TITANI, K., YAOI, Y. & MURAKAMI, H., *Biochem. biophys. Res. Commun.*, **77** (1963), 688.
144. STEWART, J. W. & MARGOLIASH, E., *Canad. J. Biochem.*, **43** (1965), 1187.
145. CHAN, S. K., NEEDLEMAN, S. B., STEWART, J. W., WALASEK, O. F. & MARGOLIASH, E., *Fed. Proc.*, **22** (1963), 658.
146. KREIL, G., *Hoppe-Seyl Z.*, **334** (1963), 154.
147. PALEUS, S. & TUPPY, H., *Acta chem. scand.*, **5** (1959), 389.
148. TUPPY, H., in *Symposium in protein structure*, edited by A. Neuberger (John Wiley & Sons, New York), 1958, 66.
149. MARGOLIASH, E., *J. biol. Chem.*, **237** (1962), 2161.
150. KREIL, G. & TUPPY, H., *Nature, Lond.*, **192** (1961), 1123.
151. DOOLITTLE, R. F. & BLOOMBACK, B., *Nature, Lond.*, **202** (1964), 147.
152. CANFIELD, R. E., *J. biol. Chem.*, **238** (1963), 2698.
153. JOLLES, P., *Angew. Chem. (Int. Ed.)*, **3** (1964), 28.
154. JOLLES, P., JAUREGUI-ADELL, J. & JOLLES, J., *C.R. Acad. Sci., Paris*, **258** (1964), 3926.
155. CANFIELD, R. E. & LIU, A. K., *J. biol. Chem.*, **240** (1965), 1997.
156. BROWN, J. R., *Biochem. J.*, **92** (1964), 13 p.
157. FRAENKEL-CONRAT, H., *Arch. Biochem. Biophys.*, **27** (1950), 109.
158. RAO, G. J. S. & RAMACHANDRAN, L. K., *Biochim. biophys. Acta*, **59** (1962), 507.
159. WEIL, L., BUCHART, A. R. & MAHER, J., *Arch. Biochem. Biophys.*, **40** (1952), 245.
160. HAYASHI, K., IMOTO, T., FUNATSU, G. & FUNATSU, M., *J. Biochem. Tokyo*, **58** (1965), 227.
161. FRAENKEL-CONRAT, H. & RAMACHANDRAN, L. K., *Advanc. Protein Chem.*, **14** (1959), 175.
162. TSUGITA, A., GISH, D. T., YOUNG, J., FRAENKEL-CONRAT, H., KNIGHT, C. A. & STANLEY, W. M., *Proc. nat. Acad. Sci., Wash.*, **46** (1960), 1463.
163. RAMACHANDRAN, L. K., *Bull. nat. Inst. Sci., India*, **24** (1963), 115.
164. ANDERER, F. A., *Advanc. Protein Chem.*, **18** (1963), 1.
165. CASPER, D. L. D., *Advanc. Protein Chem.*, **18** (1963), 37.
166. WITTMANN, H. G., in *Informational macromolecules*, edited by H. J. Vogel, V. Bryson & J. D. Lampen (Academic Press Inc., New York), 1963, 177.
167. TSUGITA, A. & FRAENKEL-CONRAT, H., *J. mol. Biol.*, **4** (1962), 73.
168. TANAKA, M., NAKASHIMA, T., BENSON, A., MOWER, A. F. & YOSUNABU, K. T., *Biochem. biophys. Res. Commun.*, **16** (1964), 442.
169. LOVENBERG, W., RAFTERY, M. A. & COLE, R. D., *Abstracts, 148th ACS meeting, Chicago, Aug.-Sept.* 1964 (American Chemical Society, Columbus, Ohio), 1964, 22C.
170. BLOOMSTROM, D. C., KNIGHT, E., PHILLIPS, W. D., WEIHER, J. F., *Proc. nat. Acad. Sci., Wash.*, **51** (1964), 1085.
171. LOVENBERG, W. & SOBEL, B. E., *Proc. nat. Acad. Sci., Wash.*, **54** (1965), 193.
172. LIGHT, A., FRATER, R., KIMMEL, J. R. & SMITH, E. L., *Proc. nat. Acad. Sci., Wash.*, **52** (1964), 1276.
173. SMITH, E. L., STOCKELL, A. & KIMMEL, J. R., *J. biol. Chem.*, **207** (1954), 551.
174. WONG, R. C. & LIENER, I. E., *Biochem. biophys. Res. Commun.*, **17** (1964), 470.
175. AMBLER, R. P., *Biochem. J.*, **89** (1963), 341.
176. AMBLER, R. P. & BROWN, L. H., *J. mol. Biol.*, **9** (1964), 825.
177. HILSCHMANN, N. C. & CRAIG, L. C., *Proc. nat. Acad. Sci., Wash.*, **53** (1965), 1403.
178. TITANI, K. & PUTNAM, F. W., *Science*, **147** (1965), 130.
179. ANDO, T., IWAI, K., ISHII, S., SAWADA, F. & AZEGAMI, M., *Proceedings of the fifth international congress of biochemistry, Moscow*, 1961, Symp. 2.
180. YAMAGUCHI, Y., HORIE, H., MATSUO, A., SASAKAWA, S. & SATAKE, K., *J. Biochem. Tokyo*, **58** (1965), 186.
181. BAHL, OM P. & SMITH, E. L., *J. biol. Chem.*, **241** (1965), 3585.
182. BRICAS, E. & FROMAGEOT, CL., *Advanc. Protein Chem.*, **8** (1953), 1.
183. MILLER, M. W., *The Pfizer handbook of microbial metabolites* (McGraw-Hill Book Co., New York), 1961.
184. HALL, J. B., SEDAT, J. W., ADIGA, P. R., UEMURA, I. & WINNICK, T., *J. mol. Biol.*, **12** (1965), 162.
185. MACH, B., REICH, E. & TATUM, E. L., *Proc. nat. Acad. Sci., Wash.*, **50** (1963), 175.
186. GREGORY, J. D. & CRAIG, L. C., *J. biol. Chem.*, **172** (1948), 839.
187. RAMACHANDRAN, L. K., *Biochemistry*, **2** (1963), 1138.
188. GROSS, E. & WITKOP, B., *Biochemistry*, **4** (1965), 2495.
189. SARGES, R. & WITKOP, B., *J. Amer. chem. Soc.*, **86** (1964), 1862.
190. SARGES, R. & WITKOP, B., *Biochemistry*, **4** (1965), 2491.
191. SARGES, R. & WITKOP, B., *J. Amer. chem. Soc.*, **87** (1965), 2020.
192. RUTTENBERG, M. A., KING, T. P. & CRAIG, L. C., *Biochemistry*, **4** (1965), 11.
193. LEDERER, E., *Proceedings of the plenary sessions, IUB*, Vol. 33, *International congress of biochemistry, New York*, 1964, 63.
194. EMERY, T. & NIELANDS, J. B., *J. Amer. chem. Soc.*, **83** (1961), 1626.
195. TURKOVA, J., MIKES, O. & SORM. F., *Experientia*, **19** (1963), 633.
196. SHEEHAN, J. C., DRUMMOND, P. E., GARDNER, J. N., MAEDA, K., MANIA, D., NAKAMURA, S., SEN, A. K. & STOCK, J. A., *J. Amer. chem. Soc.*, **85** (1963), 2867.
197. SHEEHAN, J. C., ZACHAU, H. G. & LAWSON, W. B., *J. Amer. chem. Soc.*, **80** (1958), 3349.
198. EASTWOOD, F. W., SNELL, B. K. & TODD, A., *J. chem. Soc.*, (1960), 2286.
199. TIPPER, D. J. & STROMINGER, J. L., *Proc. nat. Acad. Sci., Wash.*, **54** (1965), 1133.
200. WASSERMANN, H. H., KEGGI, J. J. & MCKEON, J. E., *J. Amer. chem. Soc.*, **83** (1961), 4107.
201. CARNEGIE, P. R., *Arch. Biochem. Biophys.*, **101** (1963), 364.
202. MAJUMDAR, S. K. & BOSE, S. K., *Biochem. J.*, **74** (1960), 596.
203. ERDÖS, E. G., editor, *Ann. N.Y. Acad. Sci.*, **104** (1964), 5, 35.
204. PIERCE, J. V. & WEBSTER, M. E., *Biochem. biophys. Res. Commun.*, **5** (1961), 353.
205. WERLE, E., TRAUTSCHOLD, I. & LEYSAITH, J., *Hoppe Seyl Z.*, **326** (1961), 174.
206. ANZAI, K. & CURTIS, R. W., *Phytochemistry*, **4** (1965), 263.
207. UEYANAGI, J., FUJINO, M., KAMIYA, T., IWASAKI, H., MIYAKE, A. & TATSUOKA, S., *Proceedings of the seventh symposia on the chemistry of natural products* (Kyushu University, Japan), 1963.
208. BODANSZKY, M., FRIED, J., SHEEHAN, J. T., WILLIAMS, N. J., ALCINO, J., COHEN, A. I., KEELER, B. T. & BIRKHIMER, C. A., *J. Amer. chem. Soc.*, **86** (1964), 2478.
209. BROCKMANN, H., *Fortschr. Chem. org. Naturst.*, **18** (1960), 1; **21** (1963), 121.
210. REICH, E., GOLDBERG, I. H. & RABINOWITZ, M., *Nature, Lond.*, **196** (1962), 743.
211. SHEMJAKIN, M. M., *Angew. Chem.*, **72** (1960), 342.
212. SCHWYZER, R., *Chimia*, **12** (1958), 53.
213. *Evolving genes and proteins*, edited by V. Bryson & H. J. Vogel (Academic Press Inc., New York), 1965.
214. SCHWYZER, R., *Annu. Rev. Biochem.*, **33** (1964), 259.
215. VOGLER, K., STUDER, R. O., LERGIER, W. & LANZ, P., *Helv. chim. acta*, **43** (1960), 1751.
216. RAMACHANDRAN, J., CHUNG, D. & LI, C. H., *Metabolism*, **13** (1964), 1043.
217. HOFFMAN, K., *Metabolism*, **13** (1964), 1275.
218. KATSOYANNIS, P. G., TOMETSKO, A. & FUKUDA, K., *J. Amer. chem. Soc.*, **85** (1963), 2863.
219. KATSOYANNIS, P. G., *Metabolism*, **13** (1964), 1059.
220. ZAHN, H., *Naturwissenschaften*, **52** (1965), 99.

Biosynthesis of Phospholipids

U. K. MISRA

Department of Radioisotopes & Biochemistry, V.P. Chest Institute, University of Delhi, Delhi 7

Functions of Phospholipids

THOUGH the knowledge of the metabolism of phospholipids is rapidly increasing, yet their function in a living cell is not clear. Bloch[1] recently commented that "In the future . . . one may expect a growing emphasis on the broader physiological problems which were temporarily overshadowed by the spectacular success of enzymology. It is already clear that the classical function assigned to lipids, to serve as long-term reserve and storage materials, is not the only one of importance. Presumably, this is a function confined to the triglycerides. The challenge facing the investigator is to find a role for the vast array of phospholipids, sphingolipids and glycolipids." Phospholipids are essential structural elements of the cell, and are typically in high concentration in membranous structures, such as endoplasmic reticulum and the mitochondria of plant and animal tissues, and in the protoplast membrane of certain bacteria. This wide distribution of phospholipids in nature sufficiently explains their structural role. Besides this, phospholipids have been assigned a role in the permeability of ions across the biomembranes[2]. For example, red cell membranes from several animal species, known to have varying permeability behaviour, differ greatly with regard to their lipid concentration. Removal of the phospholipids of mammalian liver mitochondria impairs oxidative phosphorylation. It has, therefore, been suggested that phospholipids may play a specific role in electron transport.

Recently, phospholipids have been implicated to play a role in biosynthesis of proteins by Hendler[3], Gaby *et al.*[4] and by Hunter and Godson[5]. Silberman and Gaby[6] and Godson *et al.*[7] and Hendler[3] observed that when protein synthesis in intact bacteria, bacterial protoplast or tissue preparation of hen oviduct was studied, a considerable amount of radioactivity was recovered from the lipid fraction as well as in the protein fraction. But Fukui and Axelrod[8] have shown that radioactive amino acids can be converted to lipid soluble forms in a reaction catalysed by a liver enzyme, which does not require the input of metabolic energy in the form of ATP and is unrelated to protein synthesis. To prove a definite role of phospholipids in protein biosynthesis much further work is needed. Experiments on the binding of potassium by lipids of the blood by Solomon *et al.*[9] seem to indicate that phospholipids specifically bind potassium. Hokin and Hokin[10-13] have shown that phosphatidic acid is involved in the transport of sodium ions across the cell membrane in the salt gland of certain marine birds and other tissues. Some support for the views of Hokin and Hokin comes from the observations of Karnovsky and Wallach[14]. These investigators noted that when leukocytes were allowed to injest starch particles, the pattern of labelling of the leucocytic phosphatides from ^{32}P was changed in a manner roughly reminiscent of the pattern obtained when secreting tissues are stimulated by acetyl choline. The hypothesis advanced by Hokin and Hokin on the role of phosphatidic acid in Na^+ transport has been questioned recently. Kirschner and Barker[15] presented evidence on the basis of compartment analysis of labelling pattern as to the possible involvement of triphospho inositide in Na^+ transport. The role of phospholipids in trapping iodine in thyroid gland has also been indicated. From the various reports available on the blood clotting mechanism it is quite clear that phospholipids play an important role in blood clotting. Recently, a number of workers[16-21] have reported that phospholipids play a role in the metabolism of spermatozoa. Phospholipids are also known to help in the absorption and transport process. The role of phospholipids has also been implicated in certain enzymatic activities. Phospholipids have been reported to be very effective in reactivating various cytochrome *c* oxidase preparations. Greenlees and Wainio[22] reported that purified preparation of cytochrome *c* oxidase required added phospholipids for activity and phosphatidyl serine was most effective. Ambe and Venkataraman[23] found phosphatidyl inositol to be the most effective phospholipid activator of a depolymerized cytochrome oxidase preparation. Lysolecithin has been reported to be a very good activator of an acetone-extracted cytochrome oxidase preparation[24]. A phospholipid preparation primarily of lysophosphatides (65 per cent ethanolamine containing 21 per cent serine and small amount of inositol) has been found to be very effective in activating the partially purified cytochrome *c* oxidase[25]. Sekuze *et al.*[26] reported that lecithin is specifically required for the activity of D(−)-β-hydroxy butyric-dehydrogenase isolated from beef heart mitochondria. Phosphatidyl inositol[27] has also been shown to be an activator for phospholipase C.

The specific structural role played by any given lipid in the complex structures observed in electron micrographs of the cell and of its particulate components is conjectural. Such conjectures have, for example, been based on the amphipathic nature of the lecithin molecule, which partly explains the layered structure present in the chloroplast[28], although the amount of lecithin present in the chloroplast is insufficient to fulfil this role. Phosphatidyl glycerol[29] and sulpholipids are the two new compounds which possess amphipathic properties[30]. Whether the mono- and digalactosyl glycerides play any part in the specific structural role of lipids is not known although they are present in considerable amounts.

Classification and General Properties of Phospholipids

Phospholipids have been classified under complex lipids. These compounds may be considered to be derivatives of L-α-glycerophosphate, substituted on the α′- and β-hydroxyl groups with long chain fatty acids. The parent glycerophosphatide, both from a structural viewpoint and in the biogenesis of these compounds, is the simple diacylglycerophosphate or phosphatidic acid. A large number of glycerophosphatides are now known in which the phosphatidic acids are further substituted by various residues such as choline, ethanolamine, serine, inositol, glycerol and others in phosphate diester linkages. These compounds are called phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, etc. The distribution of fatty acids in α′- and β-positions of glycerolphosphatide is highly specific. The saturated fatty acids are localized in α′-position[31,32]. Recently, Lands[33] has shown that the fatty acids in the α′- and β-position of such phospholipids may undergo turnover and metabolism independently of each other.

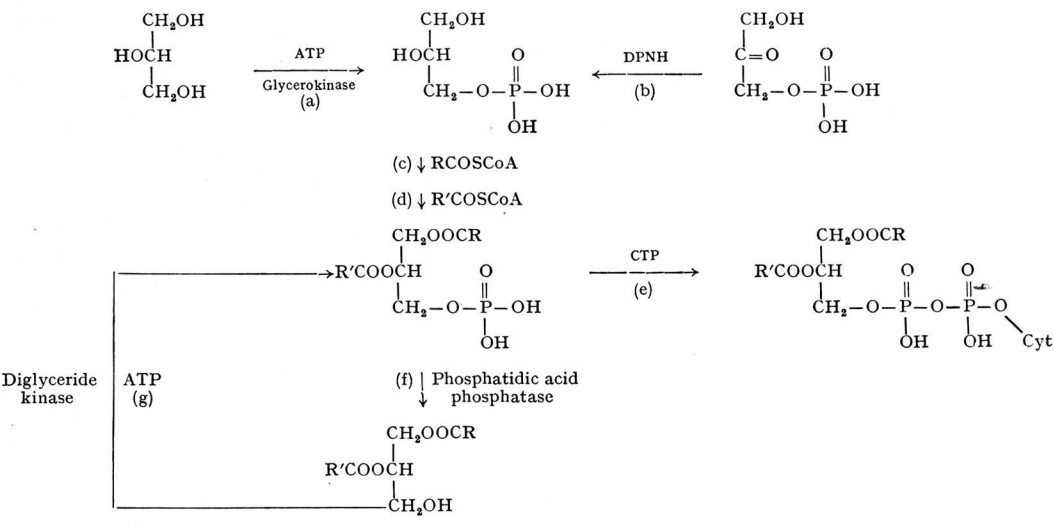
In plasmalogenic glycerophosphatides the fatty acid in α′-position is replaced by an α,β-unsaturated long chain alkyl ether[34,35]. Phosphatides containing saturated alkyl ethers in place of ester bonds have also been reported.

The use of labelled precursors has been one of the main factors in elucidating the biosynthetic pathways of phospholipids. The studies have revealed that phospholipids are synthesized by most tissues and that they are continually renewed.

Biosynthesis of Phosphatidic Acids

Phosphatidic acid is a diacyl-L-α-glycerophosphoric acid. Normally they are found in very small concentration in most naturally occurring tissues[36,37]. They are intermediates of great importance in the synthesis of other phospholipids, e.g. phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol, phosphatidyl glycerol, polyglycerophosphatide, etc. Phosphatidic acids are highly susceptible to decomposition under mild conditions but are quite stable for long periods when maintained over P_2O_5 *in vacuo* or stored as the sodium salt.

Kennedy[38] and Kornberg and Pricer[39] in their early studies demonstrated that L-α-glycerophosphate (but not free glycerol) was the precursor of the phosphatidic acids and other glycerophosphatides. The first step in the transformation of free glycerol to glycerophosphate is its phosphorylation to L-α-glycerophosphate. This reaction is catalysed by an enzyme glycerokinase. This enzyme was first reported by Kennedy[38] and later isolated in crystalline form by Wieland and Suyter[40]. Glycerokinase is quite limited in its distribution in mammalian tissues, and it has been found so far only in brain, liver, kidney and heart[41]. In tissues which do not possess glycerokinase, the major source of L-α-glycerophosphate must be from the reduction of dihydroxy acetone phosphate, which is catalysed by the enzyme, glycerophosphate dehydrogenase[42]. The next step in the biosynthesis of phosphatidic acid is the acylation of L-α-glycerophosphate by long chain fatty acids. To date, no convincing evidence for the direct acylation of free glycerol to long chain fatty acids thioesters of coenzyme A has been obtained. Kennedy[38] and Kornberg and Pricer[39] first demonstrated *in vitro* the acylation of L-α-glycerophosphate to phosphatidic acids. They observed that the acylation reaction requires long chain thioesters of CoA as donors of the fatty acyl residues. From the knowledge of the position specificity of the fatty acids



D-α,β-diglyceride

Chart 1 — Biosynthesis of phosphatidic acid

ositions, it was possible to postulate wo enzymes must be involved in acyla- n, that is one for putting saturated fatty acid at α′-position and the other for unsaturated fatty acid at β-position of L-α′-glycerophosphate. These reactions are shown in Chart 1. The two acylations are controlled by separate enzymes; the first (i.e. for putting saturated fatty acids at α′-position) is sensitive to SH-binding reagents, whereas the second one is not. Studies of Lands and Hart[43] on the formation of diacylglycerophosphoric acid in guinea-pig liver microsomes indicated an almost random system of acylation. If the phosphatidic acids are formed with a nearly random distribution of acids, as in guinea-pig liver, then the patterns observed in the triglycerides and different types of phosphoglycerides may arise from a redistribution of acids after the lipids are formed. Studies of Lands and Hart[43] on the formation of phosphatidic acid suggest that the diacylglycerophosphoric acid formed by other tissues could have a different distribution of fatty acids. Very little is known about the fatty acid composition of phosphatidic acid in tissues. Hübscher and Clark[44] have reported 60-77 per cent linoleate and 10-17 per cent oleate in mammalian liver phosphatidic acid. Alternatively, phosphatidic acid could be synthesized by the phosphorylation of D-α,β-diglyceride by ATP and an enzyme diglyceride kinase (Chart 1). Phosphatidic acids once formed may undergo at least two distinct metabolic reactions. First of these is a dephosphorylation by a specific enzyme, phosphatidic acid phosphatase[41] (Chart 1). This enzyme is found in liver, brain and other tissues and dephosphorylates phosphatidic acid to D-α,β-diglyceride. As pointed out earlier, D-α,β-diglyceride can be rephosphorylated with ATP by diglyceride kinase. The reaction of dephosphorylation of phosphatidic acid by the phosphatic acid phosphatase and rephosphorylation of its product, D-α,β-diglyceride by diglyceride kinase to form phosphatidic acid has been implied to be operating in the transport of cations across the cell membrane by Hokin and Hokin[45]. A system similar to that of animal tissues[39] for acylating L-α-glycerophosphate in plants has been found to be absent. This leaves open the question of how the *de novo* synthesis of glyceride bonds take place in plants. In another important reaction phosphatidic acids react with cytidine triphosphate to form cytidine diphosphate (CDP) diglycerides. The involvement of cytidine coenzymes in the biosynthesis of phospholipids was largely a serendipidity. Kornberg and Pricer[39] reported that phosphoryl choline could be converted to a lipid by rat liver enzymes requiring added ATP. Kennedy and Weiss[46] reported that phosphoryl choline was relatively ineffective as a precursor of lipid in cell free rat system in which ATP is not added as such, but was continuously generated from AMP by means of oxidative phosphorylation. Conversion of phosphoryl choline to lecithin took place rapidly when an impure preparation of ATP was added. Highly purified ATP was quite inactive whereas amorphous preparation stimulated the reaction. This observation has led to the very important discovery that cytidine coenzyme is involved in the phospholipid biosynthesis. It was soon realized that the function of cytidine coenzymes in the biosynthesis of phospholipids throughout nature is a general one and that there exists a class of these coenzymes of the general formula shown in Chart 2. Three types of cytosine containing coenzymes are now known to play an essential role in the biosynthesis of phospholipids. These are (i) cytidine diphosphate choline, (ii) cytidine diphosphate ethanolamine and (iii) cytidine diphosphate diglyceride. All coenzymes containing cytidine participate in the same type of enzymatic group transfer reaction leading to the formation of the phosphate diester bond as shown in Chart 3.

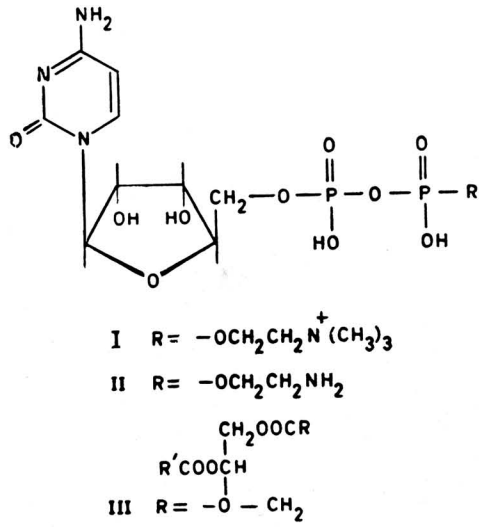


Chart 2 — Structure of cytosine containing coenzymes important in the biosynthesis of phospholipids

Several interesting reactions (discussed later) have been found in which one phospholipid may be converted to another. In all the reactions discovered to date, a net formation of phosphodiester bond in a phospholipid proceed by the mechanism

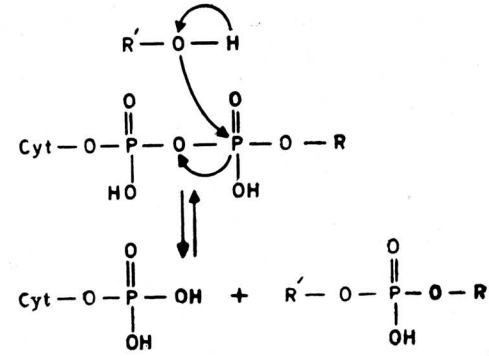


Chart 3 — General mechanism of formation of phosphodiester bonds in phospholipids

TABLE 1 — FUNCTION OF CYTIDINE COENZYMES IN THE BIOGENESIS OF PHOSPHODIESTER BONDS OF PHOSPHOLIPIDS[47]

Donor	Acceptor	Product
CDP-choline	D-α,β-Diglyceride	Lecithin
do	' Plasmalogenic diglyceride '	Choline plasmalogen
do	N-Acyl-sphingosine	Sphingomyelin
CDP-ethanolamine	D-α,β-Diglyceride	Phosphatidyl ethanolamine
do	' Plasmalogenic diglyceride '	Ethanolamine plasmalogen
CDP-diglyceride	Myo-inositol	Phosphatidyl inositol
do	L,α-Glycerophosphate	Phosphatidyl glycerophosphate

shown above. This type of enzymic group transfer reaction involving the cytidine nucleotides is very different from that exhibited by the uridine-diphosphate coenzyme series. In the cytidine coenzyme reactions, enzyme catalysed nucleophilic attack of the oxygen of hydroxyl group occurs with the displacement of cytidine monophosphate and formation of a monophosphorus diester linkage. This is in contrast with the series of group transfer reactions involving uridine diphosphate coenzymes (u-PP-R), in which UDP is the product of the reaction and the pyrophosphate bond remains intact, with the formation usually of a new glycosidic linkage. The reactions which have been observed to lead to the formation of the phosphodiester bonds of phospholipids are listed in Table 1. Phosphatidic acids as well as the cytidinediphosphate diglycerides and D-α,β-diglycerides derived from them are families of compounds c[illegible] acids in varying chain lengths and v[illegible] of unsaturation. The nature of any g[illegible] dual phosphatidic acid is dependent upo[illegible] specificities of the enzymes catalysing the steps *e* and *f*, and other reactions (Chart 1). For example, phosphatidic acid phosphatase may react more readily with phosphatidic acids containing a certain type of fatty acid residue, which will then tend to predominate in the D-α,β-diglyceride pool, while phosphatidic acids with other fatty acid residues may more actively form CDP-diglycerides. Further steps, which may allow specific control of the fatty acid pattern of lipids, are those in which the D-α,β-diglyceride pool is involved. Thus the enzyme which catalyses the formation of triglycerides from D-α,β-diglyceride has a pattern of specificity different from that of the enzymes catalysing synthesis of lecithin, which in turn may differ from that catalysing the synthesis of phosphatidyl ethanolamine. Similar considerations may apply to the enzymes involved in the further metabolism of CDP-diglycerides. The net result is the formation of lipids with a specific pattern of distribution of fatty acid residues, though all of them may have been derived originally from the same pool of phosphatidic acids[47,48].

Biosynthesis of Phosphatidyl Ethanolamine (PE)

Though PE is found widely distributed in nature, its isolation in highly purified form from natural sources has been a difficult task. Perhaps one of the chief difficulties encountered is the great sensitivity of these compounds to atmospheric oxidation and light. This may be attributed in some

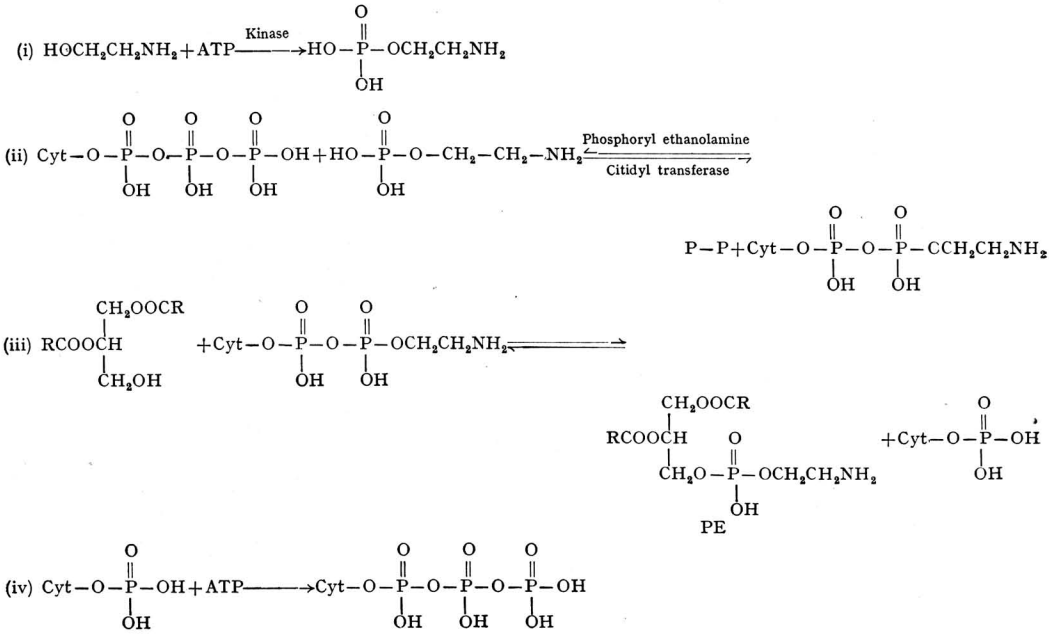


Chart 4 — Biosynthesis of phosphatidyl ethanolamine from ethanolamine

he presence of traces of pigments cally stimulate the oxidation of the fatty acids, that predominate in this olipid than in any other more commonly ntered phospholipids. A sequence of reactions, leading to the synthesis of PE in cell free extracts, is shown in Chart 4. Free ethanolamine is first phosphorylated with ATP by a kinase to form phosphoryl ethanolamine[49,50] which then reacts with CTP. This reaction is catalysed by an enzyme phosphoryl ethanolamine cytidyl transferase to form CDP-ethanolamine. This enzyme requires Mg^{2+} or Mn^{2+} ions and is highly specific for cytosine containing nucleotide triphosphate. In the next step, CDP-ethanolamine reaction with D-α,β-diglyceride to produce phosphatidyl ethanolamine with the liberation of CMP[49].

Recently, it has been suggested that phospholipids may act as obligatory intermediates in metabolic pathways, for example, conversion of phosphatidyl serine (PS) to phosphatidyl ethanolamine by decarboxylation reaction. This was first shown by Wilson *et al.*[51] and Bremer *et al.*[52,53]. These workers reported that serine must be converted to a phospholipid prior to decarboxylation. In such reactions PE is formed by decarboxylation of PS. Borkenhagen *et al.*[54] described two enzymes present in liver mitochondria and other tissues which catalyse a 'phospholipid cycle' in the decarboxylation of serine (Chart 5). In this cycle the free amino acid serine exchanges with ethanolamine moiety of PE to form PS. This phospholipid then undergoes decarboxylation to PE, with the release of CO_2. The PE then reacts with another mole of free serine yielding ethanolamine. The overall reaction is identical with the decarboxylation of free serine, but it differs in that the ethanolamine is not directly derived from the serine, but from the PE pool. The phosphatidyl serine decarboxylase is highly specific for the phospholipid form of serine and does not act on serine or phosphoserine. The cell may have a distinct advantage in such conversion reactions to form water insoluble forms or fixed to some subcellular structure prior to certain enzymatic transformation. PE can also be synthesized in the cell by an exchange reaction. In these reactions a single phospholipid component

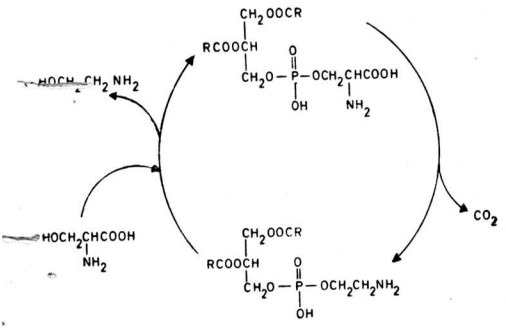


Chart 5 — A 'phospholipid cycle' leading to the decarboxylation of serine

is introduced. Reactions of this type are activated by Ca^{2+} ions and lead to the incorporation of free ethanolamine as well as that of free serine and choline[55]. Similarly, a Ca^{2+}-activated incorporation of free dimethyl ethanolamine into corresponding phospholipid has also been observed[56]. The physiological significance of these Ca^{2+}-activated reactions in the living cell, where the concentration of Ca^{2+} ion is probably very low, remains uncertain. Perhaps such reactions play a role in the transport across the cell membrane or from one type of cell structure to another. It may be that by such reactions a partial rejuvenation of the phospholipid, is achieved, the rest of the molecule being metabolized more slowly. Recently, Merkl and Lands[57] have shown that in rat liver microsomes, PE can be synthesized by the acylation of lyso PE. The enzyme requires ATP and Mg^{2+}. The saturated fatty acids (stearic acid) go predominantly to α'-position whereas unsaturated fatty acids (linoleate) go to the β-position.

Biosynthesis of Phosphatidyl Choline

This phosphoglyceride is the most typical and most abundant of the naturally occurring phosphoglycerides. Its widespread presence in plants, animals and microorganisms has led to considerable speculation as to its metabolic role. The reaction steps involved in the biosynthesis of lecithin are shown in Chart 6. Choline is phosphorylated by an enzyme, choline kinase, which requires ATP to form phosphoryl choline[58] which then reacts with cytidine-5-triphosphate to form CDP-choline. This reaction is catalysed by the enzyme phosphoryl-choline-cytidyl transferase found in liver, brain and other tissues. This enzyme is heat stable and requires Mg^{2+} or Mn^{2+} for optimal activity[59]. It is highly specific for cytosine containing coenzymes, but deoxycytidine diphosphate choline is also active. CDP-choline then reacts with D-α,β-diglyceride to form phosphatidyl choline and CMP. This reaction is catalysed by the enzyme 'phosphoryl-choline-glyceride transferase'[60]. This enzyme is completely inhibited by low concentrations of Ca^{2+} ions and shows a high degree of specificity both for cytosine nucleotides and diglycerides. Recently, Strickland *et al.*[61] have shown that L-α,β-diglyceride could also form lecithin *in vitro* in brain slices, but D-α,β-diglyceride is the natural acceptor. This step appears to be readily reversible and cytolysis of lecithin has been reported. The energy released by breaking the phosphodiester linkage in lecithin is apparently comparable to that released by breaking the pyrophosphate linkage of CDP-choline. The CMP which is set free upon the synthesis of lecithin may be rephosphorylated at the expense of ATP[62] to form CTP once again, thus constituting a cycle in which the metabolic energy released by the hydrolysis of ATP is used to form the phosphodiester bond of lecithin. One mole of D-α,β-diglyceride and one mole of phosphoryl choline are converted to lecithin at each turn of this cycle.

Methylation of PE to Phosphatidyl Choline

Stekol *et al.*[63] suggested the transfer of the methyl group of methionine in the liver to an acceptor which

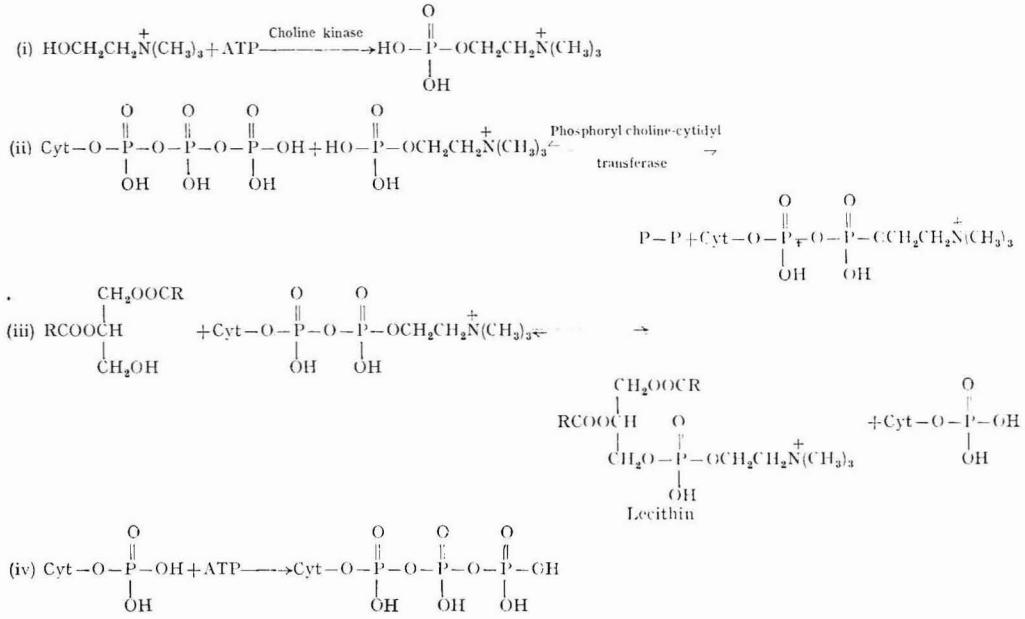


Chart 6 — Biosynthesis of lecithin from choline

is a component part of a phospholipid molecule. Studies on the precursors of methyl groups of choline[64-66] seem to indicate that all the methyl groups of choline arise by transmethylation from S-adenosyl methionine to PE, which in turn may arise by decarboxylation of PS[67,68]. The transmethylation of S-adenosyl methionine to PE is catalysed by a microsomal fraction of rat tissue and does not require the addition of synthetic, DL-dipalmitoyl cephalin. Incubation of microsomes with ethanolamine 1,2-^{14}C for labelling the bound lipid ethanolamine, and exhaustive dialysis and reincubation of the labelled microsomes with S-adenosyl methionine have shown that the bound lipid ethanolamine is indeed the methyl acceptor. Artom and Lofland[69] have now indicated a direct transfer of methyl group from S-adenosyl methionine to PE by rat liver homogenate. This reaction is irreversible[69]. Free dimethyl ethanolamine and S-adenosyl methionine do not affect an isotope dilution indicating direct conversion of phosphatidyl-dimethyl ethanolamine to phosphatidyl choline[69]. These studies show that methylation of ethanolamine to choline takes place via phosphatidyl ethanolamine, with phosphatidyl (N-methyl) ethanolamine, and phosphatidyl (N,N-dimethyl) ethanolamine as successive intermediates (Chart 7). Bremer and Greenberg[52] have confirmed these results in rat liver. The synthesis of lecithin also occurred when ^{14}C serine was injected to rats. Radioactivity was also found in amino-ethanol and choline labelled ^{14}C in both hydroxyl and methyl groups. This synthesis occurred by first decarboxylation of PS to PE. All these enzymes appear to be localized in the microsomal fraction of liver. It thus appears that phosphatidyl choline is the first form of choline to accumulate in tissues such as liver as a product of methylation of ethanolamine. The transformation of PE to lecithin constitutes an important alternate pathway for the biosynthesis of lecithin and for the utilization of the active methyl groups in animal tissues. The physiological importance of these reactions has not been established fully; however, the experiments of Groth *et al.*[67] suggest that this new pathway for lecithin synthesis is quantitatively of greater importance than the pathway elucidated by Kennedy and Weiss[68]. Through this sequence of reactions quaternary ammonium groups are introduced. Gibson *et al.*[70] speculated that the conversion of PE to lecithin alters the charge on a lipid membrane and thus has a pronounced effect on its structure and permeability. Since this system is located in the microsomes, it may be a determining factor in the lamellar structure of the endoplasmic reticulum. However, these reactions do not lead to a net synthesis of phospholipids by the living cell, but rather to the transformation of one kind of phospholipid into another. So far as is known at present, the net production of

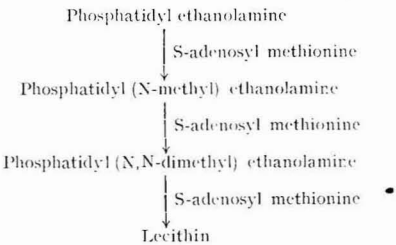


Chart 7 — Stepwise methylation of phosphatidyl ethanolamine

phosphodiester linkage in phospholipid is confined to reactions involving cytidine coenzymes as intermediates.

Acylation of Lysolecithin to Lecithin

Lecithin constantly breaks down to glyceryl phosphoryl choline via lysolecithin[71]. Lysolecithin could also be acylated to lecithin with fatty acid esters of CoA. This type of reaction has also been shown in human red blood cells, aortic walls and brain[72-76]. Phospholipase A hydrolysing diacylglycerophosphatides with the production of the corresponding lysoderivatives and free fatty acids has been shown in a number of tissues[72-76]. The lysolecithin so formed may then be further hydrolysed yielding glyceryl phosphoryl choline. Alternatively, the lysolecithin may react with a thioester of CoA to form lecithin once again. The reacylation of lysolecithin by a thioester of CoA is a reaction coupled to the release of free energy from ATP, which is needed for the activation of the fatty acid to form thioester. Lands[33] has discussed the possible importance of these two enzyme systems, phospholipase A and acylating enzymes, acting in a cylic manner, in relation to the independent turnover of fatty acids in the lecithin molecule. They point out that the combined effect could be a factor in determining the position of a symmetry of saturated and unsaturated fatty acids on the α'- and β-carbon atoms of the glycerol moiety.

Biosynthesis of Phosphatidyl Serine (PS)

Phosphatidyl serine is found naturally in most tissues and is always present in the form of Na, K, Ca or Mg salt. The exact mechanism of PS biosynthesis is still not clear. By analogy with the reaction described for the biosynthesis of PE, it might be presumed that serine would first be converted to phosphoserine, then to cytidine diphosphate serine and finally to phosphatidyl serine. No evidence, however, in support of such a reaction scheme has yet been obtained. Numerous workers have sought to establish the presence of serine kinase, or for direct phosphorylation of serine in animal tissue but all such efforts have been unsuccessful. Instead, it appears that serine arises from phosphoserine and which in turn is derived from phosphohydroxypyruvate by transamination. Hübscher *et al.*[77] have demonstrated the incorporation of uniformly labelled L-serine ^{14}C into phospholipids by liver enzymes. This reaction was stimulated by CMP and more so by Ca^{2+}, even in the absence of CMP or any energy source. This suggests the occurrence of an exchange reaction which does not require a source of metabolic energy. Recently, Miras *et al.*[56] have reported the presence of an enzyme system in the microsomes of human leucocytes which catalysed the incorporation of (3-^{14}C) serine into phosphatidyl serine. This system differed from the two other reported systems, viz. exchange reaction and decarboxylation reaction, in that its activity is absolutely dependent on the presence of added ATP and Mg^{2+} ions, in other words, a source of metabolic energy is needed rather than cytidine nucleotides. During recent years evidence has been forthcoming to suggest the occurrence of other amino acids in phospholipids. However, serine, present as phosphatidyl serine, is still the only well-authenticated example of such a combination. Recently, evidence has been presented for the existence of amino acids other than ethanolamine and serine such as ornithine and lysine in the phospholipids from several different sources[78-81]. Some functions have been suggested for this amino acid-phospholipid combination. For example, phospholipid may play a part in the supply of lysine for the synthesis of cell wall in *Staphylococcus aureus*[81]. If *p*H of the medium is lowered to 4·8 by adding glucose, accumulation of phosphatidyl glycerol lysine occurs. Another function may be balancing the positive charge of phosphatidyl glycerol in the lipid layer (s) of the membrane.

Biosynthesis of Plasmalogenic Phosphatides

The plasmalogens contain a fatty acid residue, an aldehydogenic (a vinyl ether) and glyceryl phosphoryl ethanolamine or choline. These compounds are found widely distributed in animal tissues, with particularly high concentrations in the myelin of brain and nerve, in heart and skeletal muscles, and in semen[82-95]. Kiyasu and Kennedy[96] have suggested that plasmalogen may be formed biologically by a sequence of reactions essentially similar to those described for lecithin and PE, as shown below:

1. CDP-choline + plasmalogenic diglyceride → Plasmalogenic phosphatidyl choline + CMP
2. CDP-ethanolamine + plasmalogenic diglyceride → Plasmalogenic phosphatidyl ethanolamine + CMP

They have obtained compounds having the structures shown in Chart 8 by the degradation of the plasmalogenic analogues of lecithin. These compounds are closely similar to D-α,β-diglycerides, and react with CDP-choline or CDP-ethanolamine in the presence of particulate enzyme from liver to form plasmalogenic phospholipids of lecithin and phosphatidyl ethanolamine types respectively. Presumably 'plasmalogenic phosphatidic acids' would be an essential intermediate in such a reaction sequence but there is no direct evidence for a compound of this structure. Recently, Baumann and Goldfine[97] have suggested that the diacyl phosphatides are converted directly into the corresponding acylalkenyl phospholipids. Although the glyceryl alkenyl ethers appear to be metabolically related to the acyl and alkyl analogues, the only recognized metabolic reaction of the alkenyl ether is that of hydrolytic cleavage. Warner and Lands[98] showed that alkenyl glyceryl phosphoryl-choline hydrolysis is catalysed by microsomal particles of rat liver, although the acylalkenyl glyceryl phosphoryl choline is not affected. The ethanolamine analogues react similarly[99,100], with liver microsomes, but Ansell and Spanner[101] have recently described on enzyme from rat brain which requires Mg^{2+} for maximal activity. Recently, Carter *et al.*[102] isolated a new ethanolamine containing phospholipid from egg yolk which they reported to be O-phosphoryl ethanolamine derivative of butyl alcohol. Karnovsky[103] and others have reported the existence of phospholipids analogous in structure to glycerol ethers in lipids in the tissues of marine and other animals. Their biological function is not yet known.

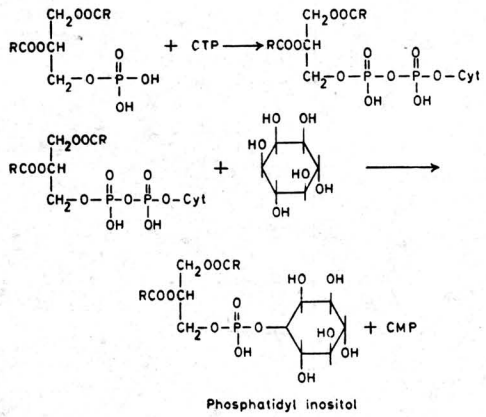


Chart 8 — Biosynthesis of phosphatidyl inositol

Biosynthesis of Phosphatidyl Inositol

The occurrence of carbohydrates as structural components of certain lipids of plants, animals and bacteria is now well established. Inositol phospholipids were first isolated from tubercle bacilli[104,105] and soybean[106-8]. Folch and Wooley[107] isolated inositol phospholipids from brain on the basis of the occurrence of either inositol mono- or di- or triphosphate in the hydrolysates of phosphoinositides. The terms monophosphoinositides, diphosphoinositides and triphosphoinositides have been used to describe these inositol containing phospholipids. Recently a phospholipid from brain has been reported to be a tetraphosphoinositide[108] but now this claim has been withdrawn. As these inositides are acidic substances, they usually occur as Na, Mg, K or Ca salts. The monophosphoinositides are present in rat, beef and pig liver and cardiac muscle where they constitute approximately 5-10 per cent of the total phospholipids. Phosphatidyl inositol has been shown recently to be an activator for phospholipase C_{27}. It has also been shown by Dawson[109,110] that phospholipase B of Penicillium hydrolyses lecithin only if phosphatidyl inositol or certain other lipid activators are present. These results suggest that perhaps the true substrate of phospholipases B and C may be complex between lecithin, and phosphatidyl inositol. By analogy with the scheme for lecithin biosynthesis one would surmise that in the pathway for phosphatidyl inositol biosynthesis, inositol monophosphate would be an intermediate. Attempts to show the presence of an inositol kinase in several animal tissues or in yeast have proved unsuccessful[111]. A partially purified yeast hexokinase has been reported to be able to phosphorylate inositol in the presence of ATP and Mg^{2+} but the rate of reaction is very low and therefore be of doubtful significance. The failure to demonstrate an active inositol kinase suggested a discrepancy in the postulate that phosphatidyl inositol synthesis follows a pathway similar to the mitochondrial phosphatidyl choline system. Agranoff *et al.*[112] studied the incorporation of tritium labelled inositol into lipids by guinea-pig kidney mitochondria. The incorporation into phosphatidyl inositol requires Mg^{2+} and CDP-choline or CMP. In some cases the incorporation of inositol is stimulated by phosphatidic acid but not by α,β-diglycerides. They suggested that phosphatidyl inositol is synthesized by transphosphorylation of CDP-choline and phosphatidic acid with the formation of CDP-diglyceride. Further a phosphorylytic reaction occurs between CDP-α,β-diglyceride and a hydroxyl group of inositol with subsequent formation of inositol phosphatide. The reactions are shown below:

(a) CDP-choline + L-phosphatidic acid ⟶ CDP-α,β-diglyceride + phosphoryl choline

(b) CDP-α,β-diglyceride + inositol ⟶ Inositol phosphatide + CMP

The stimulating effect of Mg^{2+} has been explained by assuming that it inhibits a phosphatidic acid phosphatase. Later work by Paulus and Kennedy[113,114] suggested that there are two pathways for the incorporation of inositol into the lipids. One is an exchange reaction which was put forward to explain the data of Agranoff *et al.*[112]. The second pathway leading to net synthesis involves CTP rather than CDP-choline, in the formation of CDP-diglyceride. This CDP-diglyceride is chemically unusual because it is not only a nucleotide coenzyme, but also it is a phospholipid and can be purified by silicic acid chromatography with organic solvents. In the schemes for the syntheses of inositol put forward[112-114] the liponucleotide-CDP-diglyceride is implicated. However, the reaction proposed for the formation of CDP-diglyceride differs. Agranoff *et al.*[112] suggested that CMP in CDP-choline is transferred from phosphoryl choline to phosphatidic acid, whereas Paulus and Kennedy[113,114] suggested that CTP and phosphatidic acid react to yield CDP-diglyceride and pyrophosphate. Paulus and Kennedy[113] found that free inositol could be incorporated into lipid by particulate enzyme from chicken liver by a reaction which is stimulated by CMP. At low Mn^{2+} ion concentration, a considerable stimulation by CMP is noted, while with saturating amounts of Mn^{2+}, CMP had little or no effect. In the latter case, an extensive incorporation of labelled inositol, almost linear with time, is observed in the complete absence of an added source of metabolic energy. For this reason the incorporation of labelled inositol into lipid as observed by Agranoff *et al.*[112] was ascribed to be an exchange reaction which did not lead to the net synthesis of phosphatidyl inositol.

Using L-α-glycerophosphate ^{32}P as a tracer it has been shown that phosphorus of phosphatidyl inositol is derived from L-α-glycerophosphate and that CTP is specifically required for this transformation while CDP-choline is quite inactive[113]. In contrast to this the phosphorus of lecithin and PE is derived from CDP-choline and CDP-ethanolamine respectively, since the phosphate of phosphatidic acid is removed by the reaction of phosphatidic acid phosphatase. Using CTP labelled with ^{32}P in the α-phosphorus atom, the enzymic formation of a radioactive compound having properties expected of CDP-diglyceride was observed. The addition of inositol prevented the accumulation of this compound. Synthetic CDP-dipalmitin reacted with

free inositol in the presence of chicken liver to form phosphatidyl inositol. The scheme proposed by Paulus and Kennedy[114] for the biosynthesis of phosphatidyl inositol is shown in Chart 8. These studies have recently been confirmed in all detail by Thompson *et al.*[115] in brain tissues. They showed that phosphatidyl inositol is readily formed from (^{3}H) inositol and (^{14}C) phosphatidic acid in brain dispersions. The fatty acids in phosphatidyl inositol may be distributed randomly between α'- and β-positions, but saturated fatty acids predominate at the α'-position, and unsaturated acids at the β-position of the phosphatidyl inositol from pigeon pancreas. Recently, Kennan and Hokin[116] have reported the acylation of lysophosphatidyl inositol by pigeon pancreas homogenates or guinea-pig brain microsomes. These enzymic reactions require ATP and CoA. These authors have reported that the enzyme acyl-CoA: acylglycerol-3-(1'-inositol-phosphate)-acyl transferase may control the specific positional distribution of fatty acids in the inositol lipids.

Biosynthesis of Polyglycerophosphatides

Phosphatidyl glycerol (PG) — In the past few years several types of nitrogen-free phospholipids have been shown to be present in many natural sources. Phosphatidyl glycerol was first shown to be present in *Scenedesmus* by Mauro and Benson[117,118]. This phospholipid is present in small concentration in most of the animal tissues but is more abundant in plants. Its biosynthetic pathways have been investigated by Kiyasu *et al.*[119]. It is synthesized enzymatically by a reaction between L-α-glycerophosphate and CDP-diglyceride. They found that on incubation of L-α-glycerophosphate and CDP-dipalmitin with liver particulate enzyme, a lipid was formed which was rapidly dephosphorylated by phosphatase to produce phosphatidyl glycerol. The two enzymes are readily distinguishable since the phosphatase is sensitive to inhibitors which attack sulph-hydryl groups; while the second enzyme catalysing reaction between L-α-glycerophosphate and CDP-diglyceride is unaffected by these reagents. The reaction sequence is shown in Chart 9.

Polyglycerophosphatides (cardiolipin) — This complex phospholipid was isolated by Pangborn[120] from beef heart and named cardiolipin. This compound is essential for the complement activity with the sera of syphilitic subjects. Besides heart, it is also present in other tissues. Recently, Marinetti *et al.*[121] have demonstrated the presence of cardiolipin in chlorella, *Rhodospirillum rubrum* chromatophores and higher plants. In chlorella, cardiolipin constitutes only a small portion of total lipids. However,

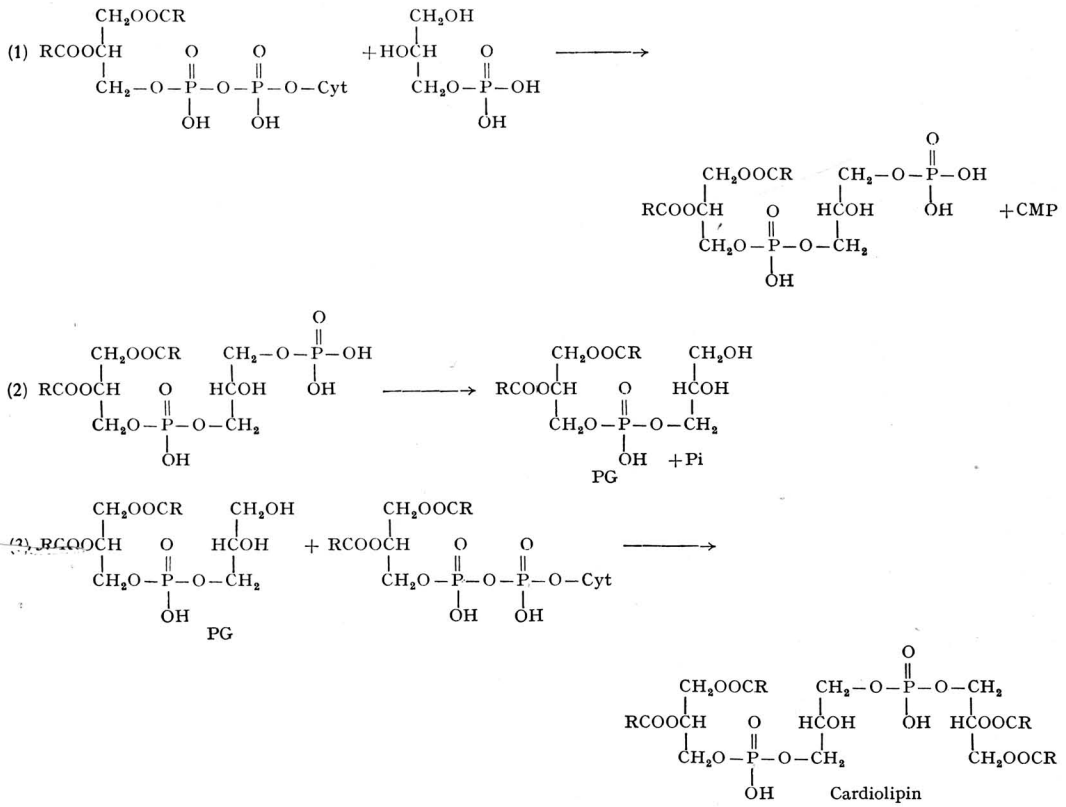


Chart 9 — Biosynthesis of polyglycerophosphatides

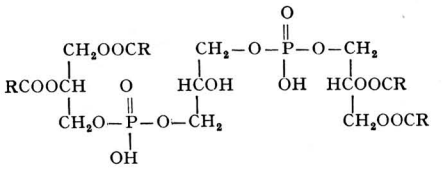


Chart 10 — Structure of cardiolipin

its concentration increases in phosphate deficient media. The chromatophores of *R. rubrum* may contain cardiolipin in concentrations as high as $1\text{-}5\times10^{-3}M$. The structure of cardiolipin is shown in Chart 10.

Biologically, cardiolipin is synthesized by a reaction between a mole of phosphatidyl glycerol and mole of CDP-diglyceride. The reaction steps are shown in Chart 9.

Biosynthesis of Sphingolipids

Sphingolipids, the name proposed for this group of lipids by Carter *et al.*[122], are defined as those lipids which contain the long chain amino alcohols sphingosine, dehydrosphingosine, phytosphingosine and dehydrophytosphingosine. Recently, several other long chain amino alcohols have been discovered. Complex sphingolipids containing sphingosine and dehydrosphingosine have been found so far only in animal tissues, while phytosphingosine and dehydrosphingosine appear to occur only in plant sources. Animal sphingolipids are present in particularly high concentrations in brain and nerve tissues and in lower concentrations in other tissues. Sphingomyelins are phospholipids in which sphingosine or a closely related base is bound by an amide linkage to a long chain fatty acid and by an ester linkage to phosphoryl choline.

Sphingomyelin

Isotope tracer studies *in vivo* by Zabin and Mead[123] and Sprinson and Coulon[124] revealed that carbon atoms 1 and 2 and the amino group of sphingosine are derived from serine, while carbon atoms 3-18 are derived from acetate, probably via 16 carbon intermediates. Ethanolamine was shown not to be an efficient precursor of sphingosine, eliminating the possibility that decarboxylation of serine occurs prior to its incorporation into the long chain base. An enzyme system which carries out the synthesis of free sphingosine from radioactive serine was found by Brady *et al.*[125,126] in the brain tissues of young rats. The system requires numerous cofactors for optimum activity, including CoA, ATP, pyridoxal phosphate, a source of NADPH and divalent cation. The primary product of this reaction appears to be dehydrosphingosine which on enzymatic dehydrogenation produced sphingosine. Later it was shown that palmitic aldehyde could replace CoA and NADPH.

Zabin and his colleagues[127] reported a partially purified enzyme from cell free extracts of brain, which catalyses the reduction of palmitoyl CoA to palmitic aldehyde and requires NADH in contrast to NADPH as reported in enzymatic transformation of ceramide (sphingosine amide) from serine. The cofactor requirements for this enzyme system are similar to those reported by Brady and Koval[125]. The exact stage at which the long chain fatty acid is formed still remains uncertain. An enzyme, phosphoryl choline ceramide transferase from liver, and other tissues catalysed the formation of sphingomyelin from CDP-choline and ceramide[128]. This enzyme requires Mn^{2+} ions for optimum activity and is highly specific for the sphingosine portion of the ceramide which acts as an acceptor for the phosphoryl choline moiety of CDP-choline. The reaction sequence is shown below:

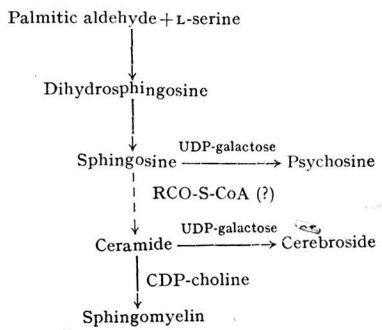
1. Palmitoyl CoA + TPNH $\longrightarrow$ Palmitic aldehyde + TPN + CoA

2. Palmitic aldehyde + L-serine $\xrightarrow[\text{Pyridoxal phosphate}]{Mn^{2+}}$ Dihydrosphingosine + CO_2

3. Dihydrosphingosine $\xrightarrow[\text{En}]{-2H}$ Sphingosine + 2H

Recent studies have shown that metabolic pathways for the synthesis of cerebrosides and sphingomyelin are common up to the stage of ceramide formation. Studies on (1-^{14}C) acetate incorporation have shown that the radioactivity of cerebrosides was higher than that of sphingomyelin. In the case of (1-^{14}C) palmitate, the radioactivity of cerebrosides and sphingomyelin was almost equal. But in the case of (1-^{14}C) lignoceric acid or (6-^{3}H) cerebronic acid the radioactivity of sphingomyelin is higher than that of cerebrosides. It may be assumed that metabolic system synthesizing sphingomyelins can utilize preformed fatty acids. In contrast, the systems synthesizing cerebroside utilize preformed fatty acids less rapidly, their fatty acid moiety being formed *in situ* from the low molecular weight precursors[129].

The significance and interrelationships of the enzymic transformations described above are not altogether clear at the present time. Thus free sphingosine acts as an acceptor for galactosyl units from UDP-galactose in the enzyme studied by Cleland and Kennedy[130], while a ceramide is apparently the acceptor in the reaction observed by Burton *et al.*[131]. Perhaps one reaction is part of a pattern leading to the formation of a simple cerebroside, while the other may be a part of sequence which produces a more complex ganglioside type of sphingolipid. Tentative pathways for the biosynthesis of sphingolipids on the basis of the present state of our knowledge on the subject are presented below:



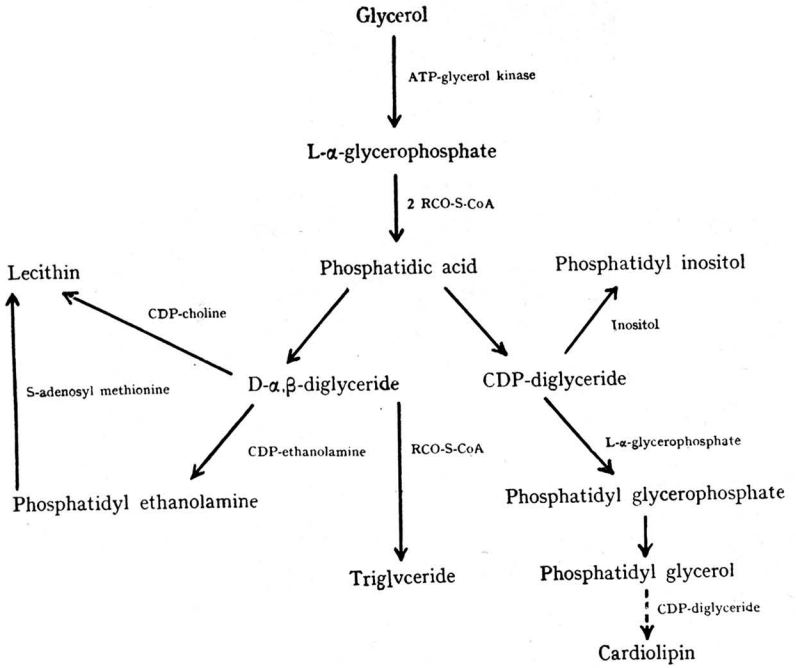


Chart 11 — Interrelationship in the metabolism of glyceride and glycerophosphatides

A summary of interrelationship between the biosynthetic pathways of phospholipids and glycerides is indicated in Chart 11.

Phospholipid Distribution

The vast amount of data accumulated show that the phospholipids occur in all biological membranes. As biological membranes contain phospholipids, it is suggested that the phospholipid content of a tissue would reflect of its membrane behaviour[132]. Some evidence to support this concept is provided by the brain tissue, which possesses the highest phospholipid content among the various tissue (82 mmoles/g. fresh weight), is remarkably rich in membrane structures, due largely to its myelin component[133]. The qualitative distribution of phospholipids is very similar in biological systems. Lecithin is the predominant phospholipid of mammalian tissues followed by phosphatidyl ethanolamine lipids. Next to these two lipids are phosphatidyl serine, phosphatidyl inositol and sphingomyelin, often present in much smaller amounts. In the mammalian plasma, lecithin is the predominant phospholipid, followed by sphingomyelin and phosphatidyl ethanolamine. The distribution of phospholipid in erythrocytes is very interesting. Erythrocytes from mammals exhibit remarkable differences. The ruminant red cell membranes contain less than 10 per cent lecithin as compared with 30 per cent in non-ruminant. The distribution also shows species differences[134-138]. Kochen *et al.*[139] have shown the existence of significant differences in phospholipid distribution between the myocardium, conducting bundles and valves in beef heart. Getz *et al.*[140,141] have analysed rat liver mitochondria, fluffy layer, microsomes and cell sap. Mitochondria exhibited equal amount (38 per cent of total) of lecithin and cephalin, whereas microsomes contained 60 per cent lecithin and 30 per cent cephalin. Fleisher *et al.*[142] reported the phospholipids of mitochondria and purified electron transport particles and fragments thereof. Recently, the presence of α-glyceryl ethers has been reported in nature and the subject has been reviewed recently by Bodman and Maisen[143]. The possible role of α-glyceryl ether in wound healing has been suggested. Also glyceryl ethers have been shown to possess erythropoietic, thrombopoietic and the granulopoietic activity[144]. Brohult has proposed that glyceryl ethers also function as bone marrow protective agents against irradiation[145] and as growth stimulators for some microorganism[146]. Glyceryl ethers have been isolated from fish oil[147], egg yolk[148], calf and human brain[149], human milk, liver, kidney, spleen, and bone marrow[150,151], rat brain, bovine red blood cells, bone marrow[152], ox heart[153] and human plasma[154]. Analogues of both phosphatidyl ethanolamine and phosphatidyl choline have been reported. Little is known about the biogenesis of glyceryl ethers. Ansell and Spanner[151] reported that in rat brain ^{32}P orthophosphate was incorporated into glyceryl ether lipids much faster than it was taken up by sphingomyelin. Thompson

and Hanahan[155] in their studies on the incorporation of glucose-6-^{14}C reported that α-glycerophosphate may be a direct precursor. Available evidence is insufficient to determine whether glyceryl ethers are functionally and biosynthetically related to the structurally similar plasmalogens. That such a relationship may exist is apparent from a comparison of the ether containing lipids of bovine[151] and human erythrocytes[156]. Blomstrand[157] studied the metabolism of glyceryl ethers fed orally to rats in the free and esterified forms. Both forms are readily absorbed by the intestine. When ^{14}C-chimyl alcohol was fed 50 per cent of the radioactivity was recovered as palmitic acid, proving that the ether linkage can be enzymatically cleaved. Enzymatic cleavage of the ether bond has also been demonstrated in the human[158].

Summary

Phospholipids are essential structural elements of the cell, and have been implicated to play an important role in various biochemical reactions of the cells. The biosynthetic pathways of phospholipids in animal tissues have been discussed. Phosphatidic acid, which occurs in small concentrations in various tissues, is the vital intermediate for the synthesis of other phospholipids, viz. phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol, polyglycerophosphatides. Free glycerol is phosphorylated to glycerophosphate with ATP by an enzyme glycerokinase. Glycerophosphate is then acylated at α′- and β-positions with fatty acids to produce phosphatidic acid. Phosphatidic acid once formed either may be dephosphorylated by an enzyme phosphatidic acid phosphatase to D-α,β-diglyceride or could react with CTP to produce CDP-diglycerides. D-α,β-Diglyceride could be phosphorylated by an enzyme diglyceride kinase to phosphatidic acid or acylated to form triglycerides. D-α,β-Diglycerides could also react with CDP-choline and CDP-ethanolamine to form phosphatidyl choline and phosphatidyl ethanolamine respectively. CDP-diglyceride could react with inositol, and L-α-glycerophosphate to produce phosphatidyl inositol and phosphatidyl glycerophosphate respectively. Phosphatidyl glycerophosphate can be dephosphorylated to produce phosphatidyl glycerol which could react with another mole of CDP-diglyceride to produce cardiolipin. Phosphatidyl ethanolamine and phosphatidyl choline are also formed by the acylation of lysophosphatidyl ethanolamine and lysophosphatidyl choline respectively. Phosphatidyl choline can also be formed by stepwise transmethylation of phosphatidyl ethanolamine. Phosphatidyl ethanolamine can also be formed by decarboxylation of phosphatidyl serine by the enzyme phosphatidyl serine decarboxylase. The exact mechanism of the synthesis of phosphatidyl serine in the body is unknown. It is formed in the body by an energy dependent exchange reaction. Calcium activated exchange reactions are also involved in the synthesis of phosphatidyl ethanolamine and phosphatidyl serine. The exact mechanisms of the synthesis of plasmalogenic phospholipids and sphingomyelin in the body are not yet fully understood.

References

1. Bloch, K., *Lipid metabolism* (John Wiley & Sons Inc., New York), 1960, vi.
2. deGier, J. & Van Deenen, L. L. M., *Biochim. biophys. Acta*, **49** (1961), 286.
3. Hendler, R. W., *Biochim. biophys. Acta*, **49** (1961) 297.
4. Gaby, W. L., Naughten, R. N. & Logan, C., *Arch. Biochem. Biophys.*, **82** (1959), 34.
5. Hunter, G. D. & Godson, G. N., *Nature, Lond.*, **189** (1961), 140.
6. Silberman, R. & Gaby, W. L., *J. Lip. Res.*, **2** (1961), 176.
7. Godson, G. N., Hunter, G. D. & Butler, J. A. V., *Biochem. J.*, **81** (1961), 59.
8. Fukui, T. & Axelrod, B., *J. biol. Chem.*, **236** (1961), 811.
9. Solomon, A., Bionetti, F. & Churran, P., *Nature, Lond.*, **178** (1956), 582.
10. Hokin, L. E. & Hokin, M. R., *J. gen. Physiol.*, **44** (1960), 61.
11. Hokin, L. E. & Hokin, M. R., *Biochim. biophys. Acta*, **67** (1962), 470.
12. Hokin, L. E. & Hokin, M. R., *Fed. Proc.*, **22** (1963), 8.
13. Lerner, S. H. & Hokin, L. E., *Amer. J. Physiol.*, **206** (1964), 136.
14. Karnovsky, M. L. & Wallach, D. F. H., *J. biol. Chem.*, **236** (1961), 1985.
15. Kirschner, L. B. & Barker, J., *J. gen. Physiol.*, **47** (1964), 1061.
16. Lardy, H. A. & Phillips, P. H., *Amer. J. Physiol.*, **133** (1941), 602.
17. Lardy, H. A. & Phillips, P. H., *Amer. J. Physiol.*, **134** (1941), 542.
18. Lardy, H. A., Hansen, R. G. & Phillips, P. H., *Arch. Biochem.*, **6** (1945), 41.
19. Hartree, E. F. & Mann, T., *Biochem. J.*, **69** (1958), 50P.
20. Lardy, H. A. & Phillips, P. H., *Arch. Biochem.*, **6** (1945), 53.
21. Hörschner, P., Kassenaar, A. A. H. & Querido, A., *Acta physiol. Pharmecol. Neerl*, **12** (1963).
22. Greenlees, J. & Wainio, W. W., *J. biol. Chem.*, **234** (1959), 658.
23. Ambe, K. S. & Venkataraman, A., *Biochem. biophys. Res. Commun.*, **1** (1959), 133.
24. Igo, R. P., Mackler, B., Duncan, H., Ridyard, J. N. A. & Hannahan, D. J., *Biochim. biophys. Acta*, **42** (1960), 55.
25. Cohen, M. & Wainio, W. W., *J. biol. Chem.*, **238** (1963), 879.
26. Sekuze, I., Jurtshuk, P. & Green, D. E., *J. biol. Chem.*, **238** (1963), 975.
27. Weiss, H., Spiegel, H. E. & Titus, E., *Nature, Lond.*, **183** (1959), 1393.
28. Rabinowitch, E. I., *Photosynthesis and related processes* (Interscience Publishers Inc., New York), 1945, 368.
29. Benson, A. A. & Mauro, B., *Biochim. biophys. Acta*, **27** (1958), 189.
30. Benson, A. A., Daniel, H. & Wiser, R., *Proc. nat. Acad. Sci., Wash.*, **45** (1959), 1582.
31. Hanahan, D. J., Brockorhoff, H. & Barron, E. J., *J. biol. Chem.*, **35** (1960), 1917.
32. Tattrie, N. H., *J. Lip. Res.*, **1** (1959), 60.
33. Lands, W. E. M., *J. biol. Chem.*, **235** (1960), 2232.
34. Rapport, M. M. & Alonzo, N., *J. biol. Chem.*, **217** (1955), 199.
35. Klenk, E. & Debuch, H., *Z. physiol. Chem.*, **299** (1955), 66.
36. Hokin, L. E. & Hokin, M. R., *J. biol. Chem.*, **233** (1958), 800.
37. Hübscher, G. & Clark, B., *Biochem. J.*, **72** (1959), 7P.
38. Kennedy, E. P., *J. biol. Chem.*, **201** (1953), 399.
39. Kornberg, A. & Pricer (Jr), W. E., *J. biol. Chem.*, **204** (1953), 345.
40. Wieland, O. & Suyter, M., *Biochem. Z.*, **329** (1957), 320.
41. Smith, S. W., Weiss, S. B. & Kennedy, E. P., *J. biol. Chem.*, **228** (1957), 915.
42. Bradbeer, C. & Stumpf, P. K., *J. Lip. Res.*, **1** (1960), 214.

43. Lands, W. E. M. & Hart, P. J., *J. Lip. Res.*, **5** (1964), 81.
44. Hübscher, G. & Clark, B., *Biochim. biophys. Acta*, **41** (1960), 45.
45. Hokin, M. R. & Hokin, L. E., *J. biol. Chem.*, **223** (1957), 915.
46. Kennedy, E. P. & Weiss, S. B., *J. biol. Chem.*, **222** (1956), 193.
47. Kennedy, E. P., in *Biosynthesis of lipids, Proceedings of the fifth international congress of biochemistry*, edited by G. Popjak, 1963, 113.
48. Kennedy, E. P., *Fed. Proc.*, **20** (1961), 934.
49. Wittenberg, J. & Kornberg, A., *J. biol. Chem.*, **202** (1953), 431.
50. Kennedy, E. P. & Weiss, S. B., *J. biol. Chem.*, **222** (1956), 193.
51. Wilson, J. D., Gibson, K. D. & Udenfriend, S., *J. biol. Chem.*, **235** (1960), 3539.
52. Bremer, J. & Greenberg, D. M., *Biochim. biophys. Acta*, **35** (1959), 287.
53. Bremer, J., Figard, P. H. & Greenberg, D. M., *Biochim. biophys. Acta*, **43** (1960), 477.
54. Borkenhagen, L. F., Kennedy, E. P. & Fielding, L., *J. biol. Chem.*, **236** (1961), PC 38.
55. Hübscher, G., Dils, R. & Power, W. F. R., in *Biochemistry of lipids*, Vol. 5, edited by G. Popjak (Pergamon Press, London), 1960, 89.
56. Miras, C. J., Mantoze, J. & Levis, G., *Biochim. biophys. Acta*, **84** (1964), 101.
57. Merkl, I. & Lands, W. E. M., *J. biol. Chem.*, **238** (1963), 905.
58. Borkenhagen, L. E. & Kennedy, E. P., *J. biol. Chem.*, **227** (1957), 951.
59. Kennedy, E. P., Borkenhagen, L. F. & Smith, S. W., *J. biol. Chem.*, **234** (1959), 1988.
60. Weiss, S. B., Smith, S. W. & Kennedy, E. P., *J. biol. Chem.*, **231** (1958), 53.
61. Strickland, K. P., Subramanyam, D., Pritchard, E. T., Thompson, W. & Rossiter, R. J., *Biochem. J.*, **87** (1963), 128.
62. Herbert, E. & Potter, V. R., *J. biol. Chem.*, **222** (1956), 53.
63. Stekol, J. A., Weiss, S., Hsu, P. T. & Smith, P., *Fed. Proc.*, **12** (1953), 274.
64. Bremer, J. & Greenberg, D. M., *Biochim. biophys. Acta*, **37** (1960), 173.
65. Wilson, J. D., Gibson, K. D. & Udenfriend, S., *J. biol. Chem.*, **235** (1960), 3213.
66. Gibson, K. D., Wilson, J. D. & Udenfriend, S., *J. biol. Chem.*, **236** (1961), 673.
67. Groth, D. O., Bain, J. A. & Pfeiffer, C. C., *J. Pharmacol.*, **124** (1958), 290.
68. Kennedy, E. P. & Weiss, S. B., *J. Amer. chem. Soc.*, **77** (1955), 250.
69. Artom, C. & Lofland, H. B., *Biochem. biophys. Res. Commun.*, **3** (1960), 244.
70. Gibson, K. D., Wilson, J. D. & Udenfriend, S., *J. biol. Chem.*, **236** (1961), 673.
71. Dawson, R. M. C., *Biochem. J.*, **59** (1955), 5.
72. Oliveria, M. M. & Vaughan, M., *Fed. Proc.*, **21** (1962), 296.
73. Mulder, E., DeGier, J. & Van Deenen, L. L. M., *Biochim. biophys. Acta*, **70** (1963), 94.
74. Stein, Y. & Stein, O., *J. Atheroscler. Res.*, **2** (1962), 406.
75. Robertson, A. F. & Lands, W. E. M., *Biochemistry*, **1** (1962), 804.
76. Webster, G. R. & Alpern, R. J., *Biochem. J.*, **90** (1964), 35.
77. Hübscher, G., Dils, R. R. & Pover, W., *Biochim. biophys. Acta*, **36** (1959), 518.
78. Macfarlane, M. G., *Nature, Lond.*, **196** (1962), 136.
79. Houtsmuller, U. M. T. & Van Deenen, L. L. M., *Biochim. biophys. Acta*, **70** (1963), 211.
80. Macfarlane, M. G., Reported at the NATO Advance Study Conference on Lipids, Cambridge, 15-21 September 1963.
81. Houtsmuller, U. M. T. & Van Deenen, L. L. M., *Biochim. biophys. Acta*, **84** (1964), 96.
82. Hainning, J. L., Fukui, T. & Axelrod, B., *J. biol. Chem.*, **235** (1960), 160.
83. Fukui, T. & Axelrod, B., *J. biol. Chem.*, **236** (1961), 811.
84. Hendler, R. W., *Biochim. biophys. Acta*, **25** (1957), 44.
85. Hendler, R. W., *Science*, **128** (1958), 143.
86. Hendler, R. W., *J. biol. Chem.*, **234** (1959), 1466.
87. Hendler, R. W., *Biochim. biophys. Acta*, **49** (1961), 297.
88. Hendler, R. W., *Proc. 5th int. Congr. Biochem., Moscow*, **9** (Abs. 28-55) (1961), 602.
89. Hendler, R. W., *Nature, Lond.*, **193** (1962), 821.
90. Hendler, R. W., *Biochim. biophys. Acta*, **60** (1962), 90.
91. Wolin, H. L. & Zajac, I., *Cancer Res.*, **20** (1960), 1508.
92. Wolin, H. L. & Zajac, I., *Fed. Proc.*, **19** (1960), 235.
93. Hunter, G. D., Brokks, P., Grathorn, A. R. & Butler, J. A. V., *Biochem. J.*, **73** (1959), 369.
94. Barnabei, O. & Ferrari, R., *Arch. Biochem. Biophys.*, **94** (1961), 79.
95. Sisakyan, N. M., Bezinger, E. N. & Marchukaitis, A. S., *C.R. Acad. Sci., U.R.S.S.*, **141** (1961), 748.
96. Kiyasu, J. & Kennedy, E. P., *J. biol. Chem.*, **235** (1960), 2590.
97. Baumann, N. & Goldfine, H., *Fed. Proc.*, **23** (1964), 221.
98. Warner, H. R. & Lands, W. E. M., *J. biol. Chem.*, **236** (1961), 2404.
99. Warner, H. R., *The structure metabolism of plasmalogens*, Doctoral thesis, University of Michigan, Ann Arbor, 1962.
100. Robertson, A. F. & Lands, W. E. M., *J. clin. Invest.*, **41** (1962), 2160.
101. Ansell, G. B. & Spanner, S., *Biochem. J.*, **90** (1964), 19P.
102. Carter, H. E., Smith, D. R. & Jones, D. N., *J. biol. Chem.*, **232** (1958), 681.
103. Karnovsky, M., *Biol. Bull*, **101** (1951), 23B.
104. Anderson, R. J., *J. Amer. chem. Soc.*, **52** (1930), 1607.
105. deSuto-Nagy, G. T. & Anderson, R. J., *J. biol. Chem.*, **208** (1939), 33.
106. Klenk, E. & Sakai, R., *Physiol. Chem.*, **258** (1939), 33.
107. Folch, J. & Wooley, D. W., *J. biol. Chem.*, **142** (1942), 963.
108. Calvo, E. S., Mule, S. J. & Hokin, L. E., *Biochim. biophys. Acta*, **70** (1963), 91.
109. Dawson, R. M. C., *Biochem. J.*, **68** (1958), 352.
110. Dawson, R. M. C., *Biochim. biophys. Acta*, **33** (1959), 68.
111. Hawthorne, J. N., *J. Lip. Res.*, **1** (1960), 255.
112. Agranoff, B., Bradley, R. M. & Brady, R. C., *J. biol. Chem.*, **233** (1958), 1077.
113. Paulus, H. & Kennedy, E. P., *J. biol. Chem.*, **235** (1960), 1303.
114. Paulus, H. & Kennedy, E. P., *J. Amer. chem. Soc.*, **81** (1959), 4436.
115. Thompson, W., Strickland, K. P. & Rossiter, R. J., *Biochem. J.*, **87** (1963), 136.
116. Kennan, R. W. & Hokin, L. E., *J. biol. Chem.*, **239** (1964), 2123.
117. Mauro, B. & Benson, A. A., *J. Amer. chem. Soc.*, **79** (1957), 4564.
118. Mauro, B. & Benson, A. A., *Fed. Proc.*, **17** (1957), 270.
119. Kiyasu, J., Paulus, H. & Kennedy, E. P., *Fed. Proc.*, **19** (1960), 233.
120. Pangborn, M. C., *J. biol. Chem.*, **168** (1947), 351.
121. Marinetti, G. V., Erbland, J. & Stotz, E., *J. biol. Chem.*, **233** (1958), 562.
122. Carter, H. E., Hains, W. J., Ladyard, W. E. & Norris, W. P., *J. biol. Chem.*, **169** (1947), 77.
123. Zabin, I. & Mead, J. F., *J. biol. Chem.*, **211** (1954), 8.
124. Sprinson, D. B. & Coulon, A., *J. biol. Chem.*, **207** (1954), 585.
125. Brady, R. O. & Koval, G. J., *J. biol. Chem.*, **233** (1958), 26.
126. Brady, R. O., Formica, J. V. & Koval, G. J., *J. biol. Chem.*, **233** (1958), 1072.
127. Hoshishima, K., Viganais, P. & Zabin, I., *Fed. Proc.*, **19** (1960), 234.

128. SRIBNEY, M. & KENNEDY, E. P., *J. biol. Chem.*, **233** (1958), 1315.
129. *Biosynthesis of lipids, 5th international Congr. Biochem.*, edited by G. Popjak, (Pergamon Press, London), 1963, in discussion, p. 201.
130. CLELAND, W. W. & KENNEDY, E. P., *J. biol. Chem.*, **235** (1960), 45.
131. BURTON, R. M., SOD, M. A. & BRADY, R. O., *J. biol. Chem.*, **233** (1958), 1053.
132. HANAHAN, D. J. & THOMPSON (Jr), G. A., *Annu. Rev. Biochem.*, **32** (1963), 215.
133. LUSE, S. A., *The biology of myelin*, 1959, 103.
134. DAWSON, R. M. C., HEMINGTON, N. & LINDSAY, D. B., *Biochem. J.*, **77** (1960), 226.
135. DEGIER, J. & VAN DEENEN, L. L. M., *Biochim. biophys. Acta*, **49** (1961), 286.
136. GRAY, G. M., *Biochem. J.*, **77** (1960), 82.
137. KATES, M. & JAMES, A. T., *Biochim. biophys. Acta*, **50** (1961), 478.
138. VEERKAMP, J. H., MULDER, I. & VAN DEENEN, L. L. M., *Biochim. biophys. Acta*, **57** (1962), 299.
139. KOCHEN, J., MARINETTI, G. V. & STOTZ, E., *J. Lip. Res.*, **1** (1960), 147.
140. GETZ, G. S., BARTLEY, W., STRIPE, F., NOTTON, B. M. & RENSHAW, A., *Biochim. biophys. Acta*, **81** (1961), 214.
141. GETZ, G. S., BARTLEY, W., STRIPE, F., NOTTON, B. M. & RENSHAW, A., *Biochem. J.*, **83** (1962), 181.
142. FLEISHER, S., KLOUSEN, H. & BRIERTEN, G., *J. biol. Chem.*, **236** (1961), 2936.
143. BODMAN, J. & MAISEN, J. H., *Clin. Chim.* being. (1958), 253.
144. LINMAN, J. W. & BETHELL, F. H., in *Proceedings of the Ciba foundation symposium on haemopoiesis*, 1960, 369.
145. BROHULT, A., *Nature, Lond.*, **193** (1962), 1304.
146. BROHULT, A., *Nature, Lond.*, **188** (1962), 591.
147. HALLGREN, B. & LARSSON, S., *J. Lip. Res.*, **3** (1962), 31.
148. CARTER, H. E., SMITH, D. B. & JONES, D. N., *J. biol. Chem.*, **232** (1958), 681.
149. KLENK, E. & HENDRICKS, U. W., *Biochim. biophys. Acta*, **50** (1961), 602.
150. HALLGREN, B. & LARSSON, S., *J. Lip. Res.*, **3** (1962), 39.
151. ANSELL, G. B. & SPANNER, S., *Biochem. J.*, **81** (1961), 360.
152. HANAHAN, D. J. & WATTS, R., *J. biol. Chem.*, **236** (1961), PC 59.
153. PIETRUSZKO, R. & GRAY, G. M., *Biochim. biophys. Acta*, **56** (1962), 232.
154. RENKONEN, O., *Biochim. biophys. Acta*, **59** (1962), 497.
155. THOMPSON (Jr), G. A. & HANAHAN, D. J., *Arch. Biochem. Biophys.*, **96** (1962), 671.
156. FRAQUHAR, J. W., *Biochim. biophys. Acta*, **60** (1962), 80.
157. BLOMSTRAND, R., *Proc. Soc. exp. Biol., N.Y.*, **102** (1959), 662.
158. BLOMSTRAND, R. & AHRENS (Jr), E. H., *Proc. Soc. exp. Biol., N.Y.*, **100** (1959), 802.

Fifth International Coal Preparation Congress

The Fifth International Coal Preparation Congress will be held in Pittsburgh, Pennsylvania, USA, during 3-7 October 1966. The congress is being jointly organized by the US Bureau of Mines and the American Mining Congress. There will be eight technical sessions devoted to (i) Preparation of fine coal by flotation procedure; (ii) Preparation of fine coal by means other than flotation; (iii) Automation or remote control of plant and equipment; (iv) Miscellaneous coal preparation research; (v) New developments; (vi) Control of product quality; (vii) Moisture reduction in coal; and (viii) Preparation trends.

Correspondence regarding the congress should be addressed to the Secretary, Fifth International Coal Preparation Congress, c/o Bureau of Mines, US Department of the Interior, Washington DC 20240, USA.

REVIEWS

OPTICAL MASERS: ADVANCES IN ELECTRONICS AND ELECTRON PHYSICS: Supplement 2, by George Birnbaum (Academic Press Inc., New York), 1965. Pp. xi+360. Price $ 9.50

This book presents a connected account of a large amount of information contained in the extensive literature concerned with the new and rapidly growing field of optical masers, or lasers as they are more popularly called. The growth of this field has resulted in a new look at optics and at the operation of many optical devices which are beginning to be understood clearly for the first time as a result of the striking properties of optical masers. In a field developing in such an explosive manner, a book in which the basic ideas are explained at a level which a physicist or an engineer with average background can comprehend deserves warm reception.

The book covers fairly well all important work done in this field up to the middle of 1964, the specific subjects dealt with being optical resonators, methods of pumping, theories of steady state and transient behaviours of optical masers, spectral characteristics of maser materials, principles of operation of various optical maser systems, characteristics of optical maser radiation and also the multiple quantum effects produced by it. A theory of oscillation in imperfect crystals, as claimed by the author, is presented in this book for the first time and a chapter includes discussion on the applications of optical masers. The emphasis is mainly on the fundamentals and, consequently, little is said about specific engineering design. Quantitative general relations are developed in such a way as not to interrupt the trend of thought significantly. Copious technical data are given for comparison with the analytical results. An extensive bibliography is also provided which should greatly help the investigator working on related problems not discussed in the book. In addition to the author and subject indices, five appendices are included at the end giving a summary of optical maser characteristics and dealing with the theory of spontaneous and induced transitions and also the mechanism of interactions between electromagnetic waves in a nonlinear dielectric. In the opinion of the reviewer, the author has done well by including these last two subjects in the form of appendices rather than in the main body of the book and by making each chapter as independent of others as is practicable. The printing and get-up are excellent.

The book should prove useful to anyone interested in optical masers. It should be particularly helpful to those who intend to prepare a syllabus for a course in optical masers which is at present offered by few institutions in India.

J. N. BHAR

APPLIED OPTICS AND OPTICAL ENGINEERING: Vol. 2 — THE DETECTION OF LIGHT AND INFRARED RADIATION edited by R. Kingslake (Academic Press Inc., New York), 1965. Pp. xiii+390. Price $ 15.00

The book under review is Volume 2 in a series of five volumes of fifty chapters edited by R. Kingslake, and deals with the detection of light and infrared radiation.

The last fifteen years have been a period of renaissance in the field of optics, which was previously just one branch of physics. A gradual transition from physics to engineering has started and is very much accelerated with the new discoveries on one side and new aids for design like computers and new methods of evaluation of optical systems like frequency response. Other branches of engineering like civil, mechanical, electrical and electronic have long back passed this transition stage and advanced so far as independent entities that their original link with physics is often not recognized by the present generation. Those who are interested in optics at present are more fortunate and can follow this very interesting change. We have already noticed the difference in the textbooks for geometrical and physical optics on one side and the later publications on technical optics by various authors. In the more recent times we have also noticed new vocabulary like 'Optical Physics' as against 'Physical Optics' as a result of the linking up of electronics to optics through various electro-optical detectors, and the development of various semi-conducting generators and detectors of infrared radiation. Technology of thin films is another exacting branch of physics in these developments contributing to these changes.

In view of these developments, the editor of the volume under review correctly claims that "a specific branch of engineering has arisen, devoted to the theory, design, manufacture, testing and use of optical instruments". There is a large demand for optical and electro-optical devices of "vital importance in industry and research, in medicine, in the armed forces, in space projects and in everyday life." The much needed data for this young branch of optical engineering "are scattered widely in many different places or are not available at all".

The five volumes are planned to answer this requirement. The stress has been mainly on the principles and operations of various devices described and equipment existing in the present time.

Chapter I deals with the eye and vision. The physical as well as physiological aspects of the eye are dealt with. Binocular vision, light and dark adaptation and the visual perception of the space are some of the other topics.

Chapter II deals with stereoscopic vision, with particular stress on stereoscopic cameras, viewers, etc. Methods of production of polychromatic anaglyphs and polarized anaglyphs have been described. This chapter ends with a description of the panoramic parallax stereogram for use in industrial photography.

Chapter III deals with photographic emulsions and films and summarizes their characteristics; data on a number of Kodak products are given.

Chapter IV is a short account of the principles of frequency response methods of image evaluation, with particular reference to photographic images, a short account of the calculations of image by point spread functions, the criteria of granularity, the Fonnier theory of detail reproduction, and measurement of modulation transfer functions. Finally, the application in practice of the above theory has been summarized.

In Chapter V, a concise account is given of illumination in the optical images which is an important consideration in the camera and projector systems; this chapter also gives very useful tips for the designer like " what distortion will give uniform illumination of the image " and " what are the tolerable distortion limits in detection systems where image quality is important ".

Chapter VI deals with various types of phototubes, photocells and television camera tubes. A short account of the vidicon tubes, uvicon, orthicon and the intensifier image orthicon is given. Characteristics of the image intensifier tubes and the infrared image converter tubes are also given.

In Chapter VII, the television optics have been dealt with in detail. Chapter VIII gives a short account of the wide variety of infrared detectors, while Chapter IX deals with infrared equipment.

The book is claimed to be a comprehensive treatise; it is probably more correct to call it an introductory volume to the topics discussed. As the editor says in the preface, the material has been divided ' arbitrarily ' into fifty chapters of five volumes, which inevitably results in a certain amount of imbalance between the scope of the subject and the space devoted. In a number of places too much is attempted in too small a space. One typical example is a sentence like this, " Lens-mirror combinations range from the simple Mangin mirror to Schmidt, Bouwers and Maksutov systems employing correcting lenses of glass, fused quartz, arsenic trisulphide, or other infrared materials suited to the spectral range ". The result is that the book is not adequately self-sufficient to serve the role of a text-book. Probably the scope of optical engineering has already gone too wide; if so, the need for more books giving a more detailed treatment is all the greater. However, the present volume forms an important landmark in the development of this young branch of engineering.

V. RAMAKRISHNA RAO

OPTICAL CIRCULAR DICHROISM — PRINCIPLES, MEASUREMENTS AND APPLICATIONS by J. Velluz, M. Legrand & M. Grosjean (Academic Press Inc., New York), 1965. Pp. xii+247. Price $ 10.00

After the demonstration of the power of optical rotatory dispersion measurements in the study of the stereochemistry of organic molecules it is natural that attention has been turned without much loss of time to the related phenomenon of optical circular dichroism (OCD). Although very few laboratories have the necessary equipment for OCD measurements, a substantial literature on their organic chemical applications has already come into being. Also commercial ' dichrographs ' are now becoming available. The appearance of the book under review is, therefore, most timely and it is sure to be widely welcomed, particularly as it is written by one of the most active groups of workers in this field.

With a brief historical introduction on rotatory power and circular dichroism in the first chapter the authors proceed in the second chapter to explain in detail the relationships between them. Chapter III which deals with measurements of OCD gives all pertinent details that will be of interest to those who aspire to build their own ' dichrographs '. An extensive survey of the application of OCD in the study of optically active carbonyl compounds is made in the next chapter. Methods of studying functions which do not absorb in the visible or accessible UV region are discussed next. Separate chapters are devoted to complex chromaphores in natural products and to the effects of physical factors on OCD. A brief account of molecular theories of optical activity forms the final chapter. The table of data obtained by the authors which is appended adds to the usefulness of the book.

This book is to be highly recommended on all counts. The translation is quite readable and the get-up of the book meets the standard of excellence to be expected from the publishers.

P. MADHAVAN NAIR

ITALIAN PHYSICAL SOCIETY COURSE 31, QUANTUM ELECTRONICS AND COHERENT LIGHT edited by C. H. Townes & P. A. Miles (Academic Press Inc., New York), 1965. Pp. viii+371. Price $ 16.00

The book is based on material presented at the International School of Physics on the subject organized in August 1963 by the Italian Physical Society with Prof. C. H. Townes as the Director. The proceedings have been edited by Prof. P. A. Miles. The book has about twenty papers and begins with two excellent surveys on optically pumped and solid state as well as semiconductor masers and lasers by A. L. Schawlow and B. Lax. The theory of optical resonators is treated next by G. Toraldo Di Francia. In their papers, W. E. Lamb and H. Haken, and H. Seuermann outline their theories of maser oscillators, while J. Gordon discusses in his paper the problem of noise in lasers. The paper by F. T. Arecchi is on thermal effects in a He-Ne optical maser. The dynamics of quantum oscillators is discussed in a paper by A. S. Grasiuk and A. N. Oraevskij. The subjects of Fourier transform spectroscopy and high resolution interferometric spectoscopy are discussed in two separate papers by P. Connes. W. Low and S. Yatsiv discuss the crystal field energy levels of iron group and rare earth ions and vibronic spectra of gadolinium compounds. The article by N. Bloembergen presents an overall picture of non-linear optical phenomena. O. Krokhin and O. Svelto treat the phenomena of saturation and photomixing in semiconductors. The paper by A. Javan outlines a theory of stimulated Raman effect based on density matrix approach. This is followed by a paper by B. P. Stoicheff on stimulated Raman scattering. The

theory of Raman and phonon masers is dealt with in the paper of R. Y. Chiao, E. Garmine and C. H. Townes. The operation of high intensity flash tubes is discussed in a paper by J. L. Emmett. J. Haisma describes in his paper the construction of a small He-Ne gas laser made of quartz. The last article by Z. Bay and H. S. Bayne deals with the problem of accurate measurement of the velocity of light with photobeats observed on a velocity modulated cathode ray tube.

Since the book is a collection of papers on a wide range of topics by different authors from different countries the task of editing must have been quite exhausting. Although the arrangement of the papers has been done remarkably well, yet in many articles grammar has been sacrificed and the printer is perhaps to be blamed only partly. There are quite a few printing errors (too many to list here) and even one page has been wrongly numbered (p. 339 instead of p. 239). Some of the reference numbers are also misplaced in text. However, these are only minor points. The editors and publishers are to be warmly congratulated on their efforts to bring the proceedings of the international school to a wider audience. To those interested in quantum electronics the present book will certainly serve as a useful source material.

P. T. NARASIMHAN

ADVANCES IN X-RAY ANALYSIS: Vol. 8, by W. M. Muller, G. R. Mallet & M. J. Fay (Plenum Press Inc., New York), 1965. Pp. xiii+472. Price $ 20.00

Volume eight of this continuing series is the proceedings of the Thirteenth Annual Conference on application of X-ray analysis, held during 12-14 August 1964 in Denver, Colorado, sponsored by the Metallurgy Division of the Denver Research Institute, University of Denver. It contains 42 of the papers presented at the meeting on the recent and most important developments in X-ray techniques for industrial and research applications. Most of the papers are related to metallurgical problems of importance. Topics included are crystal structure, analysis (structure of alloy carbides), stress determinations, microscopic and microprobe techniques, soft X-ray studies, X-ray diffraction and fluorescence analysis and satellite and non-satellite line studies. The book gives an account of the recent technological advances in the field mentioned above and presents some new apparatus for industrial applications. Mention may be made of a portable X-ray unit for measuring residual stress in alloy structures used particularly in aeronautics and of the vacuum spectrograph for ultrasoft X-ray fluorescence analysis.

In the field of soft X-ray analysis an interesting study on the effect of valence and coordination on K emissions in low atomic number elements will have far-reaching implications in the field of X-ray analysis by revealing much about electron configuration in molecular systems. The use of soap analysing films for dispersing soft X-rays in microprobe and macroprobe analysis of low atomic number elements down to boron will extend the scope of soft X-ray analysis.

All scientists concerned with X-ray analytical techniques will find this book very helpful. It can be used as an excellent reference book in courses involving X-ray analysis.

M. A. QUADER

DIGITAL INSTRUMENTS: MODERN ELECTRICAL STUDIES by K. J. Dean (Chapman & Hall Ltd, London), 1965. Pp. viii+181. Price 25s.

The book reviews the fundamentals of digital instruments and explains the principles of their working. In his apparent desire to make the book self-contained the author has also included — as the first chapter — an outline of the information theory and digital codes. The book explains switching logic and how transistors are used for the realization of these logical operations. The book explains various methods of digital voltage measurements, voltage-frequency conversion, etc., and discusses their relative merits and also outlines the principles and operation of some of the commercial digital instruments under current production.

The book is divided into nine chapters. A list of problems, a bibliography and solutions to the problems are also included at the end of the book.

The subjects covered in the various chapters are: (1) Information theory and digital codes; (2) Principles of transistor switching systems; (3) Input devices; (4) Output devices; (5) Measurement of frequency and time; (6) Digital voltmeters; (7) Ramp function digital voltmeters; (8) Voltage-frequency conversion; and (9) Other digital instruments. The appendix deals with the assessment of accuracy of digital voltmeters.

The book is well organized and well written. The explanations and discussions are clear and concise. Chapter I on information theory and digital codes is very well presented. The book should be very useful to the student, the practising engineer and the technical manager alike.

L. K. WADHWA

OLIGOSACCHARIDES by R. W. Bailey (Pergamon Press Ltd, Oxford), 1965. Pp. 178. Price 60s.

The chemistry of oligosaccharides has made rapid progress in recent years mainly due to development of efficient chromatographic techniques for the isolation of these carbohydrates in pure state from complex mixtures. The publication of this book, which forms the fourth volume of the International Series of Monographs on Pure and Applied Biology, Division — Biochemistry, has satisfied the need felt recently by workers in this field for a comprehensive reference book on this subject.

This monograph gives a coverage up to the end of 1962 of all the oligosaccharides which have been isolated in the pure state and whose structures have been conclusively established. Even data about compounds whose structures have not been completely established or which have been prepared only as derivatives have also been included provided they have been sufficiently distinguished from the known compounds.

The book is divided into eleven chapters. The first three chapters deal with topics of general interest such as nomenclature, source, preparation and general

properties of oligosaccharides and methods used for their structure determination. The rest of the chapters deal with different types of oligosaccharides classified by the author under Homogeneous oligosaccharides (Chapters 4-6), Heterogeneous reducing oligosaccharides (Chapters 7 and 8), Heterogeneous non-reducing oligosaccharides (Chapter 9), and Oligosaccharides containing amino sugars and uronic acids (Chapters 10 and 11). A classified index of oligosaccharides and a separate index of their trivial names have been included in addition to the general index for locating the required references easily.

Evolving a short but comprehensible system of nomenclature for oligosaccharides is a difficult job. In this connection one is reminded of the late Dr C. S. Hudson's advice, " If you can't name it, don't make it ". The author has followed Dr W. J. Whelan's system of nomenclature, which makes use of accepted and well-established trivial names of di- and trisaccharides and subscripts of numbers in the homo- and letters in the hetero-oligosaccharides. The configurational prefix, if *D*, and the ring form, if pyranose, are omitted in this system. Thus, *O*-β-*D*-fructofuranose (2→6)-O-β-D-fructofuranose (2→1)-α-D-glucopyranose is designated as 6^F-β-fructofuranosylsucrose. Space saving no doubt, but takes a little time to comprehend !

The printing and the get-up of the book are in keeping with the excellent traditions of the Pergamon Press.

J. L. BOSE

OSCILLOMETRY AND CONDUCTOMETRY by E. Pungor (Pergamon Press Ltd, Oxford), 1965. Pp. xvi+239. Price 70*s*.

This book is Volume 21 of an international series of monographs in analytical chemistry. Prof. Pungor is an eminent authority in this field and many commercial instruments of Hungarian origin are production models of equipment designed by him.

This monograph consists of five parts. The first part is devoted to an exposition of the fundamentals of electrical conductivity and dielectric constant of solutions and their determination along with relevant theory. Part II is the most valuable section of the book and gives details of the instrumental techniques including automatic titrators. Parts III and IV illustrate the applicability of oscillometry and conductometry in a wide variety of acid-base precipitation, complex formation and redox titrations. Part V gives application of these methods in kinetic studies, etc.

This book contains an extensive bibliography of current literature and is provided with an author index and a subject index. The newness of these instrumental techniques is shown by the dates of the references cited. Of the 374 references, none is earlier than 1950. As is to be expected only sophisticated electronic instruments are described. At least for conductometric titrations, the late Prof. N. Rae showed that the ' megger tester ' of the electrician is a suitable instrument to carry out a variety of titrations [Ref. Rae, N., A simple method of conductometric titration, *J. chem. Soc.*, (Part II), (1931), 3143 and other papers]. The reviewer has made a reference to this work so that institutions in India which may not have access to sophisticated instruments need not eliminate experiments in conductometry from their curricula.

A wide circle of university teachers, analysts and researchers will find this book useful. The price tag of 70*s*. makes one hope for a cheap paper-back edition of this book.

M. A. V. DEVANATHAN

SULFONATION AND RELATED REACTIONS by E. E. Gilbert (John Wiley & Sons Inc., New York), 1965. Pp. xi+529. Price $ 16.50

The Editor of the series has been fortunate in persuading Dr Gilbert to write this book, because Dr Gilbert was one of the pioneers in the development of stabilized SO_3 (' Sulfan ') and is the author of numerous patents, original papers and reviews on sulphonation. As one of the most important unit processes of organic synthesis, sulphonation has been treated extensively in books on synthetic dyes, but this is the first monograph on sulphonation. The title, however, is somewhat misleading, because the book deals with organic derivatives of sulphur trioxide rather than sulphonation. The wide scope of the ' related reactions ' covered by Dr Gilbert can be judged from the fact that he has discussed at considerable length the Bucherer reaction, the condensation of phenol sulphonic acids and aniline sulphonic acids to form polymers containing sulphonic groups, and the use of the condensation product of sulphanilic acid with cyanuric chloride as a sulphoarylating agent. One omission is sulphonation in the presence of boron fluoride, which has some advantages as a laboratory method. Mechanistic aspects receive less attention than in de la Mare and Ridd's Aromatic Substitution (nitration and halogenation). There is no reference, for instance, to the work of Brand and of Melander on the mechanism of sulphonation. However, the section in which kinetics, mechanism and orientation are discussed is clearly written and adequate as a background for studying technical sulphonation.

The eight chapters provide a very thorough coverage of the reagents, sulphonation with compounds of sulphur trioxide and sulphur dioxide, sulphonation by the oxidation of sulphur compounds, methods for indirect sulphonation such as sulphoalkylation, sulphation, sulphonation and desulphonation. There are nearly 3000 references to papers and patents, and the volume is the most valuable source of information on sulphonation and related reactions available at the present time.

K.V.

PROGRESS IN NUCLEIC ACID RESEARCH AND MOLECULAR BIOLOGY: Vol. 3, edited by J. N. Davidson & Waldo E. Cohn (Academic Press Inc., New York), 1965. Pp. xiv+363. Price $ 11.50

There are three outstanding features of this book: firstly, it contains several topics which have, to the reviewer's knowledge, never before been reviewed so comprehensively; secondly, the subjects chosen are well defined and represent areas on the frontiers of molecular biology and biochemistry; and thirdly, most, if not all, of the reviews are not merely a catalogue of information in the particular area but

contain a critical evaluation of the information available, which is presented in an integrated manner indicating areas in which knowledge is insufficient and where there is legitimate scope for speculation and experimentation.

The first review is by K. S. Kirby on isolation and fractionation of RNA and DNA. This is a most readable account of the various methods which have been used for the above purpose and should serve as an excellent source material when one is confronted with a specific problem in this field. The next chapter by D. M. Prescott on cellular sites of RNA synthesis documents evidence for the hypothesis that all RNA synthesis is DNA dependent and occurs in the nucleus. This chapter is well written and presented. The only criticism that could perhaps be made is that the possibility of *extra-nuclear*, DNA-dependent RNA synthesis, for example, in mitochondria and chloroplasts, has not been considered (although it has been mentioned that DNA is contained in chloroplasts). There is already some evidence for DNA-dependent synthesis of RNA in mitochondria. Therefore, the conclusion on page 54 that all RNA synthesis is restricted to the cell nucleus and that all cytoplasmic RNA must be derived from the nucleus cannot be accepted without reservation.

The review on ribonucleases T1 and T2, written by Prof. F. Egami and his colleagues, that follows is a delight to read. It summarizes the work done on the isolation of these two exceedingly important enzymes, their purification, their physico-chemical properties, the determination of their primary structure (which at the time of publication of the book was only partially worked out but now has been fully worked out by Prof. Egami), and the relationship between their structure and function. This is perhaps the first time that all these aspects of an enzyme have been studied in such great detail by a single group of workers and exemplifies the great tenacity, patience and attention to details which has characterized Japanese biochemical work in recent years.

The next review by J. J. Weiss is on chemical effects of radiation on nucleoproteins, and their precursors, the bases, nucleosides and nucleotides. The following review is by F. C. Neidhardt on the regulation of RNA synthesis in bacteria. The introduction to this chapter, where the problem is defined, is recommended for reading to all those who have access to this book. It very nicely sets the framework for the development of the important question of regulation of RNA synthesis. The subject has been subsequently dealt with in a most imaginative manner. Since out of all the fields reviewed this is one in which the least amount of definitive information is available, it allows the greatest speculation, and the author has fully recognized this 'merit' of the subject.

The comprehensive review on actinomycin and its effect on nucleic acid function would be welcomed by every worker in the field of molecular biology interested in using actinomycin D which, within a few years of its first use (only 2 out of 181 references cited are pre-1960), has proved to be one of the most useful tools in elucidating the molecular mechanisms underlying the biological role of DNA. The emphasis in this review is on the mechanism of action of actinomycin. The following chapter on *de novo* protein synthesis *in vitro* by B. Nisman and J. Pelmont is an orderly, well-presented and well-documented review of what is known of the formation from amino acids of completely new protein molecules in *in vitro* systems. The emphasis has been rightly laid on the question of the formation of functionally active protein molecules in such systems.

The coverage of the last review is very wide; it describes the discovery, isolation, fractionation, characterization and estimation of the free nucleotides from animal tissues and their qualitative and quantitative variations under various physiological and pathological conditions. This again should serve as a very good source material for those who are interested in free nucleotide pools. One may have liked to see some areas, like those describing methods used for the separation of individual components in the free nucleotide pools, covered in a greater detail than has been done, but this is compensated for by a carefully chosen set of references. The only minor error that came to the reviewer's notice is the citation of reference No. 269 in support of the statement that "genital organs have rather high contents of free nucleotides"; this reference, in fact, shows that spermatozoa (which are *not* an organ) are rich in free nucleotides.

In conclusion, the book is a very well-written and readable account of the state of our knowledge at the time of the writing of the review, i.e. about 1963, in several well-chosen, well-defined and important subjects of general interest to workers in the fields of nucleic acids and molecular biology. The material is, by and large, presented in a well-integrated manner with clear indication of where the major problems of the future in the particular field may lie.

The get-up of the book, particularly the type used, is excellent. The book is strongly recommended to be on the shelf of every worker in the fields of nucleic acids and molecular biology.

P. M. BHARGAVA

PRINCIPLES OF SENSORY EVALUATION OF FOOD* by M. A. Amerine, R. M. Pangborn & E. B. Roessler (Academic Press Inc., New York), 1965. Pp. x+602. Price $ 19.50

Taste and smell are sensations in response to a stimulation of the tongue and the nose by a material substance. As to how these two sensations arise is still little understood, though intensely studied. Knowledge gained so far in this study on the pathway to the final revelation yet far off has been substantial. Even to make a brief mention of the significant contributions up to 1965 has taken 600 pages of printed matter classified under: (1) Sensory evaluation problems of the food industry, (2) The sense of taste, (3) Olfaction, (4) Visual, auditory, tactile and other senses, (5) Factors influencing sensory measurements, (6) Laboratory studies: Types and principles, (7) Laboratory studies: Difference and

*First in the series of Monographs on Food Science and Technology edited by M. L. Anson, E. M. Mrak, C. O. Chichestor and G. F. Stewart.

directional difference tests, (8) Laboratory studies: Quantity-quality evaluation, (9) Consumer studies, (10) Statistical procedures, and (11) Physical and chemical tests related to sensory properties of foods. To document this vast and scattered literature, some of them obscure and not all easily accessible, is a commendable effort. The monograph as a literature survey is thorough, since even stray observations, if important, like the role of ear in taste, has not escaped the notice of the authors. The monograph is thus an important source book. Typical are Chapters 2 and 3 for completeness of the literature compiled and reviewed. In Chapter 2, for example, is elaborated, with illustrations where necessary, anatomy of the tongue and its functional part — the taste buds, the concept of the four fundamental tastes and their thresholds, factors which influence taste, especially the genetic factor so well proven with phenylthiocarbamide, our present understanding of taste stimulation in relation to the chemical configuration of the stimulant and theories regarding the mechanism of taste perception. Equally elaborate information on olfaction is provided in Chapter 3.

With the growing increase in the number and variety of processed and sophisticated foods, the need has arisen for their sensory evaluation, especially of their nuances. As long as taste and smell continue to be the factors determining acceptability of a food by the sentient, so will the primitive acuity of man to perceive subjectively these sensations continue to be the only means of assessing food quality. In recent times, a methodology, both experimental and statistical, has been built in into the sensory evaluation of food (Chapters 6-11) to provide a common basis for the expression of results by workers in this profession. Terms esoteric to this branch of science are explained in a glossary.

The architechtonics of production are the best traditions of an Academic Press publication.

M. SRINIVASAN

FOOD TECHNOLOGY THE WORLD OVER: Vol. 2— SOUTH AMERICA, AFRICA AND THE MIDDLE EAST ASIA edited by Martin S. Peterson & Donald K. Tressler (AVI Publishing Co. Inc., Westport, Connecticut), 1965. Pp. ix+414. Price $ 14.00 (US); $ 15.00 (Foreign)

The world population is increasing at a fast pace. Yet, it is possible to feed many times the existing population if concerted efforts are made to conserve and increase the available food resources by efficient use of modern technology and scientific achievements. The countries dealt with in this book are faced with problems of undernutrition as well as malnutrition. The first prerequisite, therefore, is to make a careful assessment of the resources and the technology, both traditional as well as modern, against the economic and sociological conditions. The editors have done useful service in pooling the information on different deficit regions, in the shape of articles contributed by leading workers in the field.

The subject matter has been distributed in four parts: (i) the establishing of a modern food industry; (ii) the food technology in South America; (iii) Africa and the Middle East; and (iv) Asia.

The first part gives brief, rapid, yet relevant look into the basic technical literature in food technology: it is supplemented with over 100 references.

The remaining parts deal with the food technology in different regions of the world. The articles are full of information on food resources, economy, levels of nutrition, technologies (traditional and modern), utilization of raw materials, existing state of the food industries, their problems, and the scope for development. Notes are also given on the training facilities, professional bodies, etc., in different regions. The matter is supplemented with commercial and production data.

The information presented provides sharp contrasts between ancient techniques and modern technology which exist in several of these deficit countries. Thus, while Latin America shows the coexistence of traditional and modern practices, tribal Africa still retains basically primitive food processing techniques. Egypt had a heritage of ancient food technology, but has begun to apply the most modern techniques. Israel and Taiwan have rapidly developing food industries, while Japan has already reached high standards of food technology.

The article on India shows that food technology in this country has developed rapidly after the second world war, with the result, one of the biggest centres of food technological research is situated here. The article is documented with references of Indian work done during the last fifteen years. The problem of foodgrain conservation, with special reference to the losses occurring from the stage of harvest to the consumers' table, and the technology available to prevent them, has not received adequate attention, though data are available. Similarly, milling technology, and modernization of equipment and machinery and other engineering aspects to put the food industry on a sound footing like in Japan should have received emphasis.

Food technology in the Philippines is still traditional; some suggestions have been made for its improvement. Brief account of the food technology in South Vietnam, Cambodia, Laos, Thailand and Federation of Malaya is also given.

The notes on the indigenous relishes and other food products given in some of the articles are of particular value to food technologists. A comprehensive subject index covers the entire matter. The book should prove valuable to students, planners, industries and all those concerned with augmenting the food supplies and ameliorating the deficiency states in the world. It should have a definite place on the shelf of every technical and science library.

H. A. B. PARPIA

D. N. WADIA COMMEMORATIVE VOLUME, Editor-in-chief A. G. Jhingron (Mining, Geological and Metallurgical Institute of India, Calcutta), 1965. Pp. xxiii+833. Price Rs 35

This is a volume of geological papers presented to Dr D. N. Wadia, doyen of Indian geologists, to felicitate him on his 81st birthday. It commences with several pages of congratulatory messages followed by an appreciation of the life and work of Dr Wadia by W. D. West. There are some 57 papers

covering various aspects of stratigraphy, palaeontology, structural geology, petrology, applied geology, etc., by authors from different countries.

Marvin Weller writes on palaeontology and evolution, while Stirton describes the cranial morphology of the Casteroides. Chakravarty and Deraniyagala deal with Indian Elephantoides and extinct hominids respectively. Invertebrate studies include papers on the Cretaceous ammonites and nautiloids by S. S. Sarkar and M. V. A. Sastry, on Myogypsinidae by Wright Barker and on a unique Baragwanathia specimen by Edmund Gill. Several interesting papers on stratigraphy and structure have been contributed by A. Desio, Percy Evans, Arogyaswamy, S. V. P. Iyengar and Alwar, D. Niyogi, B. Rama Rao, L. Rama Rau, H. Crookshank, Brian Engel, W. R. Danner and others. Several papers deal with petrology: A. I. Johnson writes on the hydrologic properties of volcanic rocks; L. R. Wager on injected granite sheers in Sikkim; A. K. Saha on variations in composition of granites; J. Harpum on the late Precambrian tholeiites of East Africa; S. C. Chatterjee on some anorthosites in Kalahandi; T. M. Mahadevan on the porphyritic granites of Bihar.

There are several papers on other aspects of geology. R. W. Boyle deals with the geochemistry of Galena Hill in Yukon; B. Dey and P. R. Sen Gupta describe the copper belt of Bihar; H. S. Pareek, M. N. Deekshitulu, S. P. Sanyal and S. M. Casshyap deal with coal geology and petrography; K. Kanchara gives an account of natural gas in Japan; Vasilenko on salt domes in Tadjikistan; G. C. Chaterji on recent data on subsurface geology and hydrology in India; A. K. Roy on ground water in Eastern Rajasthan; and N. K. Panikkar on the International Indian Ocean Expedition.

In a collection of papers of this nature, dealing with a variety of topics, it is scarcely possible to expect uniformity in presentation, scope or standards. Many of the papers are interesting and authoritative and provide a varied fare to satisfy a large circle of readers. Many of the papers by Indian authors contain material on recent advances in the knowledge of the geology of the Indian subcontinent, which is to be welcomed. The price of the publication is quite modest and the printing and get-up are good. The editors are to be complimented for the trouble they have taken in bringing out this publication.

M.S.K.

REVIEW OF JAPANESE LITERATURE ON BERIBERI AND THIAMINE edited by Norio Shimazono & Eisuke Katsura (Vitamin B Research Committee of Japan), 1965. Pp. 308.

This book, as the title suggests, is a comprehensive review of the work done on beriberi and thiamine in Japan. As the editors rightly point out, work done in Japan does not get abroad the recognition it merits; and this book should obviate such a defect.

The first three chapters are devoted to the clinical aspects of beriberi. Some of the noteworthy features here are the discussions regarding the contributory role of intestinal bacteria in the development of beriberi and the role of sodium in oedema formation in beriberi. Inouye and Katsura's classification of Type I and Type II beriberi is novel; their suggestion that the former is due to a dietary inadequacy of thiamine and the latter due to a disturbance of proper utilization of the vitamin is interesting.

The rest of the book deals with various aspects of thiamine and will prove of interest not only to the clinician and nutritionist but to the organic chemist, biochemist and bacteriologist alike. Many may not be aware of the discovery of allithiamine and the chapter written by Fujiwara should not be missed. If it is true that allicin, the principle in garlic, by formation of allithiamine facilitates better absorption and retention of thiamine in the body, it achieves great nutritional significance; and there will be much to commend the extensive use of garlic!

An extensive review such as this demands great effort and the editors of this book as well as the Vitamin B Research Committee of Japan need to be congratulated. This book, as the editors hope, is definitely illustrative of " how a study of one disease could contribute to the progress of science at large as well as to human welfare ".

KAMALI S. JAYA RAO & C. GOPALAN

PUBLICATIONS RECEIVED

LABORATORY PHYSICS: Part A — Berkeley Physics Laboratory (McGraw-Hill Book Co. Inc., New York), 1964. Pp. x+115

LABORATORY PHYSICS: Part B — Berkeley Physics Laboratory (McGraw-Hill Book Co. Inc., New York), 1965. Pp. ix+117.

ELECTRICITY AND MAGNETISM: Berkeley Physics Course, Vol. 2, by Edward M. Purcell (McGraw-Hill Book Co. Inc., New York), 1965. Pp. xviii +459

RADIOACTIVITY AND ITS MEASUREMENT by W. B. Mann & S. B. Garfinkel (D. Van Nostrand Co. Inc., New York), 1966. Pp. 168. Price $1.75

INFRARED RADIATION by Ivan Simon (D. Van Nostrand Co. Inc., New York), 1966. Pp. 119. Price $ 1.50

PLASMAS — LABORATORY AND COSMIC by Forrest I. Boley (D. Van Nostrand Co. Inc., New York), 1966. Pp. 164. Price $ 1.75

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

APPLIED MAGNETISM — A STUDY OF QUANTITIES by E. Olsen (Philips Technical Library, Eindhoven), 1965. Pp. x+144. Price Rs 33.66

MECHANICS: Berkeley Physics Course, Vol. 1, by Charles Kittel, Walter D. Knight & Malvin A. Ruderman (McGraw-Hill Book Co. Inc., New York), 1965. Pp. xviii+480

NOTES & NEWS

A new nuclear model

A nuclear model in which the protons and neutrons are arranged in clusters or spherons has been suggested recently by Dr Linus Pauling. These groups, each occupying a sphere of space, are close-packed in concentric layers about a single group or two groups. The spherons proposed are mainly helions (alpha particles) and tritons (one proton and two neutrons). The proposed model is compatible with the earlier liquid-drop and the shell structure of nuclear behaviour. The model built upon spherons offers a simpler explanation of the asymmetric fission of heavy elements such as uranium and predicts the magic numbers (elements whose atoms contain certain even numbers of either protons or neutrons) which intrigued nuclear modellers for three decades.

In the new Pauling model, spherons are part of either a core layer or a surrounding mantle. Heavier nuclei have both an inner and an outer core, the whole surrounded by the mantle. It has been suggested that three layers are sufficient for all but very heavy nuclei. In a very heavy nucleus, a single neutron appears to occupy the centre of the structure. Spheron assignment to the various layers is dictated by the geometric considerations of close-packing. These geometric considerations suggest that asymmetric fission should occur when the nucleus contains about 226 or more neutrons and protons which has been observed experimentally [*Chem. Engng News*, **43** (42) (1965), 23].

New method for increasing the resolving power of a spectrograph

A new method for increasing the resolving power of a spectrograph, based on the interference phenomenon, has been proposed at the Swedish Solar Observatory, Anacapri. The principle of the method and the set-up used in measurements are described below.

In a grating spectrograph having narrow entrance and exit slits a double image unit (composed of a Wollaston prism W of small deviation, a quartz retardation plate and two polarizers P_1 and P_2 in 45° position) is placed before the ray comes out of the exit slit S_2 after which the light is recorded by a phototube. If the two rays produced by the double image prism pass the slit S_2 simultaneously, interference can be observed by the phototube. The Wollaston prism acts as a Babinet wedge as well, and when it is displaced sideways intensity variations are recorded by the phototube. Each wavelength in the spectrum will appear as two adjacent images, and because of the diffraction image of the spectrograph (and limited resolving power of the grating and finite size of the slit) these images will be good enough to make it possible for some adjacent wavelengths to contribute to the light passing the slit S_2. Analysis of the intensity equations shows that the function $I_{\min.}(\lambda-\lambda_0)$ has a shape which could be altered by changing the separation between the double images. A comparison of the waveshapes of this function with and without the double image device clearly shows that this proposed interference method considerably increases the resolution. Experiments conducted using artificial spectral lines made up of two slits polarized in the perpendicular directions demonstrated the realization of the increased resolution. Experiments conducted at Anacapri by replacing the phototube with a photographic film showed interference patterns traced by the Fraunhofer lines which gave rise to increased resolving power of the spectrograph.

An interesting observation made from the inspection of the Fraunhofer lines was that in the maximum positions of intensity a single spectrum line appears as a doublet, whereas in minimum positions the same line appeared as a singlet. However, only extensive experiments will prove if a real gain is obtained with the suggested method. Even though there is bound to be a reduction of light intensity with the increased resolution, it may be possible to improve the intensity by the use of 'heliometer lenses' [*Nature, Lond.*, **207** (1965), 1284].

Thin films without substrate

In electron microscopy, thin films or membranes to serve as supporting substrates are prepared from some plastic material such as collodion, formbar, or polystyrene or evaporated light metals (beryllium or aluminium) or from oxides (SiO or SiO_2). The plastic membranes are easier and less time consuming to make. Such membranes can be used as initial supports for thin metallic films and can be subsequently dissolved in the proper solvent leaving behind the required metallic film. Sodium chloride and potassium chloride crystals can also be used for the same purpose.

Membranes of collodion can be quickly and conveniently prepared from USP collodion (12 per cent nitrocellulose dissolved in ether alcohol) diluted with four parts by volume of normal amyl acetate. Formbar (a polyvinyl acetate resin) films of required thickness and strength can be made using 0·125-0·25 per cent formbar in ethylene dichloride.

The inflammability of nitrocellulose led to the development of cellulose acetate which is not inflammable though it will burn slowly. The researches carried out by Dreyfus led to the use of cellulose acetate 'dope' (commonly defined as rapid drying lacquer of cellulose type) as fabric covering of aircraft wings and fuselages. Membranes prepared out of such dopes have been found to be very useful initial supports for preparing 'films without substrate' required for use as targets in nuclear reactions or for the study of their electrical, thermal or optical properties. The method for preparing films without substrate as developed in this laboratory is as follows.

A proper solution of the 'dope' is prepared in dope thinner, the exact strength of the solution being judged by experiments. A drop of castor oil is also added

to lubricate. This is then poured on the surface of water in a wide-mouthed trough. A thin film spreads out, which is lifted on the proper mount (to be used in the actual experiment) having the aperture of the required size. After drying, the mounted plastic film is placed in a vacuum chamber where the required metal is deposited by vacuum evaporation. Care is taken that the plastic membrane is not placed too close to the filament. Several metals have been successfully deposited by this process; they stick uniformly without breaking the plastic membrane. The mount is then gently placed in a dish containing acetone, which readily dissolves the plastic membrane. On drying in air the thin metallic film without substrate is obtained. — R. G. SINGH, National Physical Laboratory, New Delhi

Ultrasonic grinding of natural graphite

The application of ultrasonic radiation to grinding of graphite in mortars and ball mills has been found to result in flat glancing faces and clear twin boundaries as revealed under an optical microscope, in contrast to the rough surfaces and close fringes of laminated steps in the basal plane characteristic of ordinary grinding process.

Graphite powder (50-65 mesh) made into a slurry in an aqueous solution of tannic acid is treated with 20 kc/s., 150 V.-amp. ultrasonic radiation in a cylindrical cell 26 mm. in diameter. The slurry enters the cell through a pipe and leaves through another, fitted in opposite sides and is circulated slowly with a gear pump. The ultrasonic power is supplied through the top of the cell which is the end plane of the oscillator horn. When successive stages of grinding were examined with X-ray powder camera using CoK_α radiation, it was found that the rhombohedral lines (100) and (110) increase progressively with increase in grinding time [*Nature, Lond.*, **207** (1965), 1189].

Electrochromatography

A simple electrolytic chromatographic technique developed at the Kyoto University can be used to determine trace elements such as copper, lead, cadmium and radioactive metals like the disintegration products of thorium, in very dilute solutions.

A small difference in electrode potential is applied along a glass column packed with electrode material like silver grains. The column is divided into several chambers by insulating materials such as glasswool. Controlled potentials can be applied to each chamber of the column. At the beginning of a sample run the inlet is kept at a more positive potential than the outlet; the more noble metal ions deposit on the inlet part and the less noble ones on the outlet part. This is the electrolytic deposition step. Then the column is eluted with a suitable solvent, for example, 0·1*N* hydrochloric acid plus 1 per cent hydrazine which depolarizes any anodic product formed. The potential gradient along the column is lowered. The less noble metals dissolve first, followed by the rest, and are swept away one by one into a detector where they are determined. An a.c. polarograph with a single dropping mercury electrode serves as a detector.

In one of the initial experiments, cadmium, lead and copper were separated from 100 µl. of a solution containing 10^{-2} moles/litre of each metal ion, the elution taking 132 min. in all.

The system involves a single operation and the composition of the electrolyte and potentials can be so adjusted as to meet any combinations of metal ions. Further work is directed towards quickening the elution process, exploring the possibility of estimating surface active organic reagents, introduction of detection systems, such as colour, radioactivity, etc., checking out new electrode materials like glass beads coated with silver or silver amalgam, and working out the parameters such as electrode potentials, scanning potential rate, electrode grain size and flow rates. The usefulness of this technique will increase manifold if a system wherein a continuous potential gradient can be applied along the column can be worked out [*Chem. Engng News*, **43** (32) (1965), 42].

A new reactor for high pressure oxidation of butane

A new fast-mixing and quenching reactor has been developed at Phillips Petroleum, USA, for the study of very rapid chemical reactions. This reactor makes possible the large-scale production of olefins by high pressure oxidation of butane in a region of reaction conditions not explored previously.

The reactor combines direct jet impingement for mixing with expansion through a de Laval nozzle for rapid quenching.

The reactor is fed with preheated butane and air. Water added upstream of the nozzle to avoid increase in the temperature of the reaction products in the subsonic zone following the nozzle gives a condensed aqueous phase in it. The aqueous and gaseous effluents from the first cyclone separator are sampled. The gas stream is then made to pass through a condenser to the second separator which removes the aqueous stream containing small amount of formaldehyde and formic acid to avoid corrosion of the steel flare line.

Preheat temperature, reactor residence time and butane-oxygen mole ratio are the three most important factors in the conversion of butane. Small reactors with 7 millisec. residence time cause reduction in the conversion of butane with increase in temperature, the butane-oxygen ratio being fixed. In the case of large reactors with residence time 20-40 millisec., increase in temperature above 820°F. causes increase in butane conversion. At about 820°F., there is an abrupt rise in conversion. The reaction here is exothermic and the compounds formed are mostly hydrocarbons having molecular weight lower than those of butane, carbon monoxide and water.

Various types of products are obtained under different conditions. For butane-oxygen ratio 2:5, butane yields are high at low conversions. Propylene yields are highest when the oxygen content in the feed is small. Propylene yield increases up to 50 per cent conversion of butane and then comes down slowly up to 70 per cent conversion. High yield of

ethylene is obtained with high converisons of butane and it reaches a maximum at about 80 per cent conversion. Although high conversion generally decreases the amount of CO, CO_2 and water-soluble oxygenated hydrocarbons in the product stream, the yields of methane and ethane are on the increase [*Chem. Engng News*, **43** (15) (1965), 66].

High sensitivity voltammetry: Spot electrolysis

The sensitivity of voltammetry can be increased either by modifying the electric current as in the case of differential polarography [Perone, S. P. & Mueller, T. R., *Analyt. Chem.*, **37** (1965), 2] and a.c. polarography [Brayer, B. & Bauer, H., *A.C. polarography and tensimetry* (Interscience Publishers Inc., New York), 1963] or by increasing the rate of movement of electroactive material to the electrode surface by introducing convective transfer as is the case with the rotated wire electrode [Laitinen, H. A. & Kolthoff, I. M., *J. phys. Chem.*, **45** (1941), 1079] and tubular electrode [Blaedel, W. J., Olson, C. L. & Sharma, L. R., *Analyt. Chem.*, **35** (1963), 2100].

An extension to the above methods has been given by Evans [*Analyt. Chem.*, **37** (1965), 1520] in which the electroactive material was already at the electrode surface. A small volume of the solution was applied to gold foil electrode whose surface was slightly etched and the solvent was allowed to evaporate, leaving a spot of the electroactive material adhering to the electrode. The electrode was mounted on a laboratory jack with the lower part submerged in the supporting electrolyte solution but the sample spot above the solution. After adjusting the potential the sample spot was brought into contact with the supporting electrolyte and the resulting current was measured as in the case of ordinary polarographic methods. This method has the advantage that no pre-electrolysis is necessary to deposit the electroactive material.

This method is tested with the reduction of Fe(III) in $IF.H_2SO_4$ supporting electrolyte and hydroquinone oxidation in $IF.H_2SO_4$ in 50 per cent ethanol. In the case of Cd(II) reduction, the gold electrode suffered the disadvantage of evolution of hydrogen with very little overpotential. So, a gold amalgam electrode having almost the same rough surface as the original gold electrode was used. The results are not dependent on the presence or absence of oxygen.

The exact physical behaviour of the process involved is quite complex, but reproducible results are always obtained. When the supporting electrolyte contacts the sample spot, the dissolution and electrolysis begin simultaneously.

The method has the advantage of high sensitivity. Less than 1 nanoequivalent of electroactive material ($< 0{\cdot}1$ μg.) is detectable; 0·5-2·0 μg. amounts are easily determined with good accuracy. No additional instrumentation is necessary and the method requires very little time (3 min.) and avoids the deaeration of the samples.—A. L. J. Rao, Punjabi University, Patiala

Determination of leaching rates of antifouling compositions

A simple biological method for assessing the leaching rates of a variety of toxic compounds, particularly the newer antifouling compositions based on organometallic compounds, has been reported [*J. appl. Chem., Lond.*, **15** (1965), 469]. The method consists in first determining, under controlled conditions, the critical toxicity (the minimum concentration of toxin required to inhibit growth completely in 50 ml. of an unstirred culture medium inoculated with a known concentration of an alga). The concentration of toxin in the leachate from the composition under test is then determined by diluting the leachate till it reaches the critical toxicity under identical conditions. The indicator organism used is the unicellular organism *Chlamydomonas*. The method works successfully at molar concentrations as low as $1{\cdot}32 \times 10^{-8}$, where all known chemical methods fail.

A new monoclinic pyroxene from meteorites

An emerald green mineral found as an accessory constituent in the iron meteorites from three places, Coahuila, Toluca and Hex River Mountains, has been identified as a new chromium member of the jadeite group. The mineral has been named ureyite after Harold Urey. The composition of this mineral has been found to be $NaCrSi_2O_6$. Unlike jadeite, a high pressure phase, ureyite can be synthesized from melts at 1 atm. presure. It has been synthesized by fusing SiO_2, Na_2SiO_3 and Cr_2O_3 at 1100°C.

Ureyite is monoclinic, optically negative, and strongly pleochroic. The optical properties of the three meteoric occurrences of ureyite are similar, but not identical, and the indices of refraction are somewhat lower than those of synthetic $Na_2CrSi_2O_6$. The reason for this is found in the presence of small and variable amounts of Ca, Mg and possibly Fe^{2+}, indicating a compositional variation analogous to that extending from aegirine, $NaFeSi_2O_6$, to aegirine-angite, $(Na_2, Ca)(Fe^{3+}, Mg, Fe^{2+}, Al)Si_2O_6$. Crushed grains exhibit a well-defined cleavage on (110) and a pronounced parting on (001). The cleavage angle was measured as $87°23' \pm 10'$ [*Science*, **149** (1965), 742].

Insect 'bitometer'

The methods generally employed to examine the mosquito bite are limited to visual observations or to the measurement of engorgement by changes in weight or radioactive tracer intake after mosquito bite. The method of visual observations is laborious, uncertain and in many cases impractical. In the method of engorgement, employed to determine the repellent and attractive properties of a compound, there is no possibility to determine whether a mosquito has penetrated and salivated in the skin of a host unless engorgement occurs. Since salivation is the vector by which mosquito-borne disease is disseminated, the measure of repellency of a compound by the degree of engorgement is seriously limited.

The difficulties encountered in the above methods are overcome in the new method described [*Nature, Lond.*, **208** (1965), 462] which employs an electronically

operated 'bitometer'. The various phases of mosquito bite from the initial penetration of the mouth parts to final withdrawal can be detected electronically by using the contact between mosquito and its host to form an integral part of an electrical circuit, where the insertion and withdrawal of the mosquito mouth parts from the host are used as the making and breaking by a switch in the circuit. A 50- or 100-mesh bronze screen is used to separate the mosquito from its host and the screen is connected by a soldered lead to a resistance (16 megohms) and then to a negative pole of a standard mercury cell (1·35 V.). The positive pole of the battery is connected to a recording instrument. The circuit passes through the recorder and is carried by another lead from the recorder to the tail of anaesthetized animal (host) where it is embedded via a hypodermic needle soldered to the lead.

When a mosquito bites the host, the mosquito holds on to the wire screen with its legs and passes its proboscis through the spaces of the mesh into the host and then the circuit is completed. The resulting flow of current is registered on the recorder. When the proboscis is withdrawn the circuit is broken.

By changing the mesh size of the screen or the host animal, the apparatus can be employed to determine the biting activities of many species of biting and blood sucking insects and the attractant, repellent and insecticidal properties of various compounds can be determined. The recorder can be used for portable operations with a battery converter and can be adapted for operation under field conditions.

Detection of soil microorganisms by catalase activity

Catalase, the enzyme catalysing the decomposition of hydrogen peroxide to water and oxygen, is uniformly found in plants, higher animals, aerobic bacteria and most of the facultative anaerobic bacteria. The detection and estimation of intracellular catalase under appropriate conditions, because of the prevalence of catalase in microorganisms and the insensitivity of activity to reaction conditions, should be a simple and convenient indication of the presence of microorganisms in soils. A reasonable correlation between catalase activity and bacterial content measured by plate count was observed in desert soils.

For the detection of catalase in soils it is required that: (1) the soil sample should be homogeneous; (2) a method be available for releasing enzyme from indigenous microorganisms into the surrounding medium; and (3) a method be available for assaying the catalase without the necessity of extracting or separating it from the other components of soil. Soil homogenates can be made by grinding with a mortar and pestle. Lysing of bacteria can be achieved by rupturing the cell walls, permitting the contents to disperse throughout the solution, partially by the addition of the enzyme muramidase (lysozyme), an enzyme which can hydrolyse muramic acid, a component of cell wall. The enzyme assay can be made by using differential manometers which were made by connecting together two Warburg manometers and using Krebs' manometric fluid.

The enzyme assay was made placing two equal weights of soil samples in the bottom of each tube. Buffer was added to the control and an equal volume of muramidase to the other. After allowing 20 min. for muramidase to react, the upper reagent vessels were turned, so that the hydrogen peroxide solution, previously measured into each, was poured into the tubes. If the muramidase released catalase, the excess oxygen produced from hydrogen peroxide increased the pressure on that side, causing the liquid in the U-tube to move.

The quantity of intracellular catalase found in an 'average bacterium' was calculated as follows, based on values found in the literature for pure cultures. If 10^{-9} to 10^{-10} moles of haem are found in 1 mg. of organisms and up to 10 per cent can be catalase, then 1 mg. of organisms contains 10^{-10} to 10^{-11} moles of catalase. One bacterium weighs approximately 10^{-12} g. This means one organism contains 10^{-19} to 10^{-20} moles of catalase. For this calculation the heavier organisms such as the algae and fungi which contain larger quantities of catalase are not accounted. The smallest quantity of enzyme detectable by this method was 10^{-16} moles of active enzyme producing 9 μl. of oxygen in 40 min. Taking into account the amount of catalase expected per microorganism the method can detect 10^{3} to 10^{4} microorganisms in a relatively short time.

With an improved technique for lysing the organisms (freezing and thawing, and grinding with glass, detergents and other lytic enzymes including the newly reported cell lysing enzyme, lysostaphin) and a more sensitive method of detecting oxygen (pressure transducers, quenching of fluorescence, or luciferin-luciferase reaction which depends on the oxygen for luminescence) it may even be possible to detect 1-10 lysed organisms.

This technique may also be applicable in detecting any enzyme which produces a gas, such as carbon dioxide or oxygen. Thus amino acid carboxylase might be detectable by this technique. It is also useful to the soil microbiologist for the relative determination of microorganisms in soil samples. This method of detecting organisms in soil may also be of use in detecting extra-terrestrial organisms, if instrumented for inclusion in a planetary probe [*Nature, Lond.*, **206** (1965), 1019].

Preparation of follicle stimulating and luteinizing hormones from horse pituitary glands

An improved and rapid method for the preparation of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from horse pituitary glands has been described by Dr B. B. Saxena and Philip H. Henneman of the Seton Hall College of Medicine, Jersey City, NJ [*Biochim. biophys. Acta*, **104** (1965), 496-502].

An improvement over the earlier method of the same authors has been effected by reducing some of the chemical steps. This

reduction has not affected significantly the yield or the activity of the FSH fraction and has permitted the recovery of LH. Horse pituitary gland (1 lb.) is ground and extracted with 33 per cent cold aq. ethanol 3 times and the *p*H of the extract adjusted to 6 with 0·1*N* HCl. The proteins in the supernatant having gonadotropic activity are precipitated at 1°C. by the addition of 2 vol. of acetone. The precipitate is dissolved in water, adjusted to *p*H 7, lyophilized and then filtered through a 4·2×30 cm. column of Sephadex G-25 in the cold.

After lyophilization, 1·2 g. of protein from the Sephadex G-25 gonadotropic fraction is fractionated into FSH and LH fractions by zone electrophoresis, using a 3·2×180 cm. acid alcohol-treated cellulose column, equilibrated with 0·037*M* phosphate buffer (*p*H 7·7) at 1°C. Secondary buffer chambers containing 0·02*M* phosphate buffer (*p*H 7·7) are used to minimize *p*H change and heat production during electrophoresis. The electrophoresis is performed for 72 hr at 1500 V. and 70 ma. The column is eluted with the same buffer, and 3 ml. fractions collected every 10 min. on an automatic collector. Protein peaks are detected by measuring the absorbancy at 278 mμ for each fraction. Protein fractions are assayed in female and male rats for FSH and LH activity. Out of the two, one fraction contains FSH plus LH activity and this fraction containing 15 g. of protein is further purified by vertical starch-gel electrophoresis using 0·05*M* phosphate buffer (*p*H 7·7) and a discontinuous buffer system. Resolution of protein components is better in the discontinuous buffer system. The electrophoresis is performed for 20 hr at 9 V./cm. at 10°C. The zones containing FSH and LH are determined by bioassays and these zones subjected to a second electrophoresis in an elution cell, specially designed to permit recovery of the hormones. FSH and LH fractions from several experiments are pooled, desalted by filtering through Sephadex G-25 and lyophilized. Contaminating soluble starch from the FSH and LH preparation is removed by ion-exchange chromatography on Dowex 2×8.

Progress Reports

School of Research and Training in Earthquake Engineering, Roorkee

The annual report of the school for the year 1964-65 records its activities in the fields of structural dynamics, soil dynamics, seismology and instrumentation.

An improved design of elevated water towers capable of resisting seismic forces has been finalized. A novel feature of the design is that it remains elastic during moderate earthquakes and absorbs energy in plastic condition when a very strong earthquake occurs. Studies of period and damping measurements on an elevated reinforced concrete water tower indicated very little change in period with different levels of water in the tank.

A model of a single storeyed RC frame with unreinforced brick walls has been tested. The study included determination of stiffness and the natural frequency of the model in two perpendicular directions. Vibration absorbers have been successfully used to reduce forces on the main structure due to steady state forced vibrations. A digital computer programme has been used for the analysis of earth dams subjected to vibrations.

Studies on the liquefaction of soils under vibrations have shown that the pre-pressures attain a maximum value prior to resonance, and at resonance these get partly dissipated. Liquefaction of the top layer occurs first and the bottom layers liquefy subsequently and the settlement of the deposit varies characteristically with acceleration of the table motion.

A comprehensive study on the behaviour of single piles and pile groups subjected to dynamic lateral loads has shown that the initial static strength of the pile under steady state dynamic loading is less than the dynamic strength. After the soil gets sufficiently compacted, the static strength is greater than the dynamic strength. But under transient loading, the static strength obtained before and after transient testing is less than the transient strength; damping of soil pile system is about 3 per cent.

The results of the study on the horizontal and vertical modulus of subgrade reaction in cohesionless soils indicate that the value of K, modulus of subgrade reaction in vertical direction, goes on decreasing as the diameter of plate increases. Also, K_h, modulus of subgrade reaction in horizontal direction, is not zero at ground surface and it decreases as the diameter of plate increases.

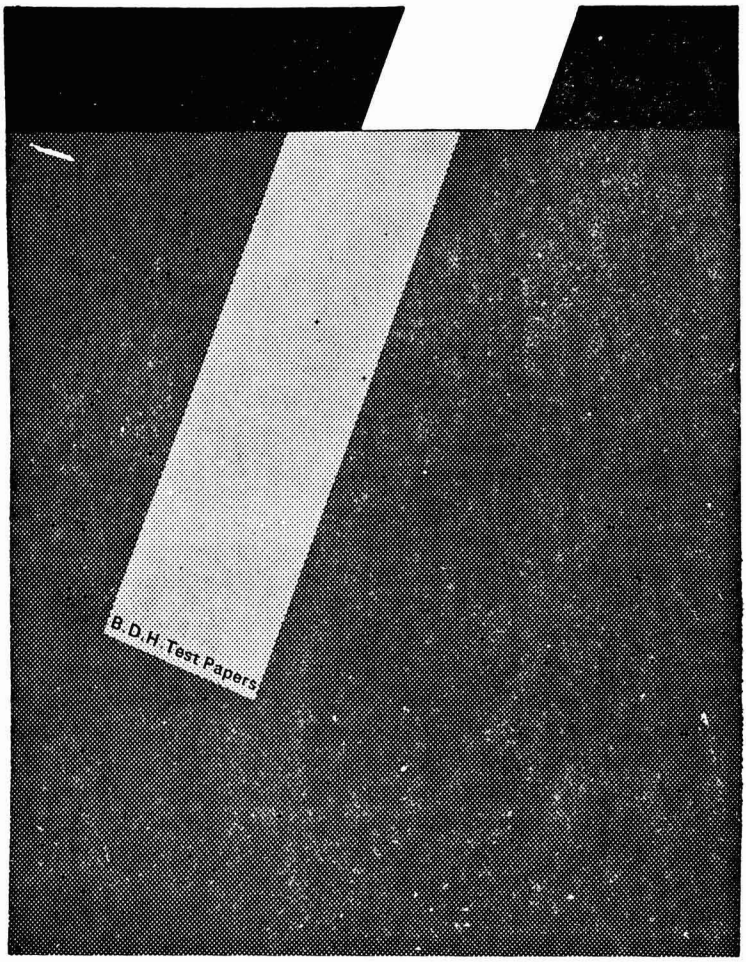
The behaviour of pile groups under lateral loads has been investigated. From tests on single piles in sound it has been observed that the resistance of negative battered piles to deflection increases by 0·22 β, where β is the angle of batter in degrees. The resistance of positive battered piles decreases by 0·4 β.

A study of resonant frequency of machine foundations has led to the conclusion that the first natural frequency of such a machine-foundation-soil system can be expressed by a simple expression in terms of the base area, height to base ratio, weight of the machine and the foundation and eccentricity factor. The amplitudes of vibration were found to be in good agreement with the theoretical values.

Among the new instruments designed and fabricated are: (1) a large shear box of 30×30 cm. size for studying shear characteristics of soils under dynamic loads; (2) a vacuum type triaxial equipment for transient loading; (3) a proton precession magnetometer for measuring magnetic anomalies for prediction of earthquakes in seismic zones; (4) a strainmeter for recording shearing strains in walls of buildings; and (5) displacement meters of linear variable differential transformer type of very low natural frequencies.

Announcement

▪ *The Third Asia and Oceania Congress of Endocrinology* will take place in Manila, Philippines, during 2-6 January 1967. Abstracts of papers to be presented should reach the Chairman, Organizing Committee, Third Asia and Oceania Congress of Endocrinology, Deparment of Physiology, College of Medicine, University of Philippines, Herren Street, Manila, Philippines, before 31 July 1966.

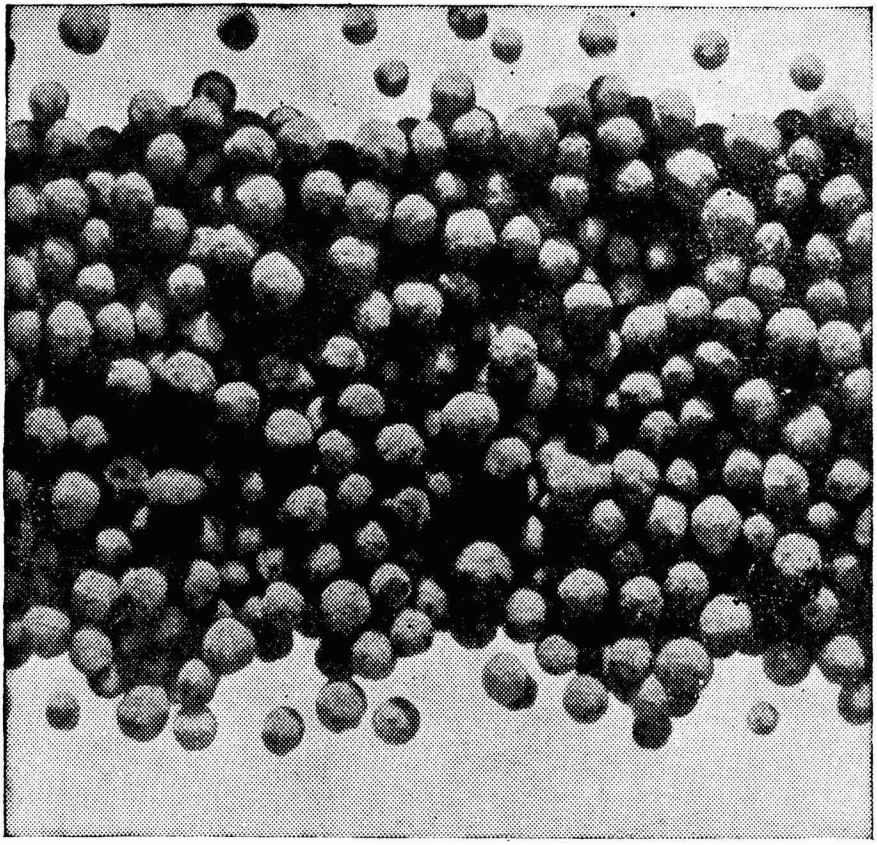


In laboratories and industrial plants, for analysis and process control, and in a host of other applications, these indicator papers are in continual use. They are the quickest and handiest means of making pH checks 'on the spot', the paper covering the appropriate range simply being dipped into the test solution; the colour which develops is compared with the standards shown on each book. It is often an advantage to use B.D.H. Wide Range pH indicator papers in the first instance, followed by the appropriate Narrow Range papers for an accurate pH determination. Where both the Wide Range and Narrow Range papers are in use the B.D.H. Multi-Range package is very convenient.

B.D.H. Narrow Range Indicator Papers □ B.D.H. Wide Range Indicator Papers
B.D.H. Multi-Range Indicator Papers □ B.D.H. Litmus Papers

BDH Laboratory Chemicals

British Drug Houses (India)
Private Ltd.,
Laboratory Chemicals Division,
8 Graham Road, Bombay 1



The first Pelletising Plants for Nickel Ore

The first plants for pelletising Nickel Ore will be built in New Caledonia for Messrs. Société Le Nickel S.A., second biggest Nickel producer of the world. POLYSIUS will supply all machinery and equipment for preparing and pelletising Garnierite from the Poro and Nepoui mines. Four units using the well-proven POLYSIUS Grate-Kiln process (Lepol process), will produce a total of 1.5 million tons of pelletised nickel concentrate. POLYSIUS engineers can solve your pelletising problems. Our pilot plant facilities permit testing on a semi-industrial scale of all ores regarding their adaptability to pelletising.

POLYSIUS

POLYSIUS GMBH

4723 Neubeckum/West Germany

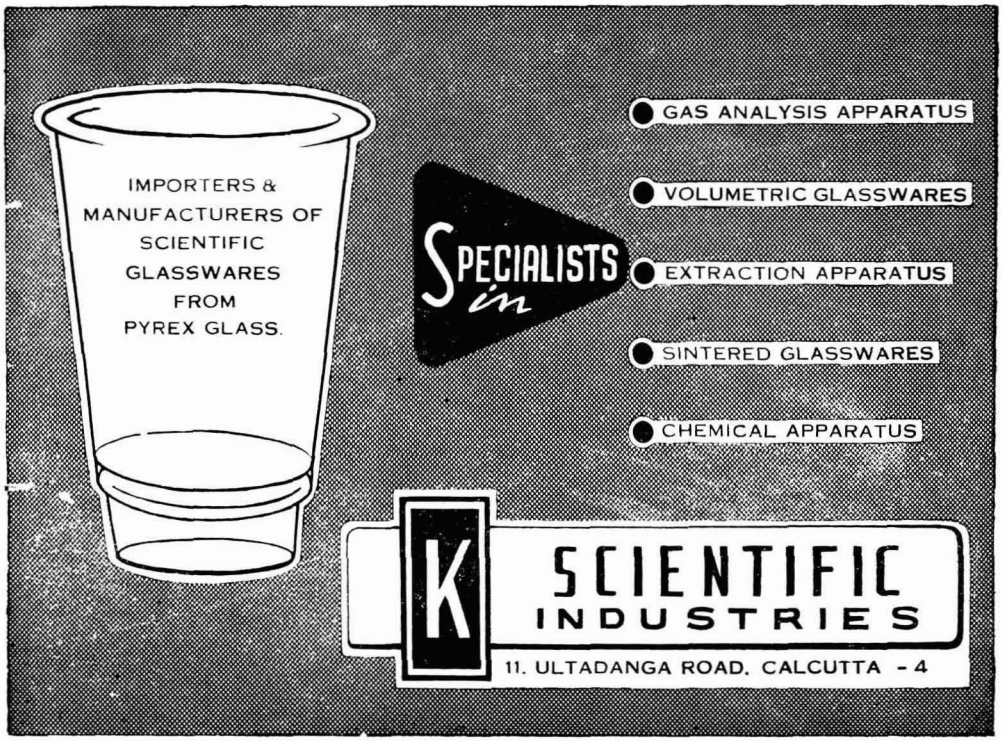
SALES REPRESENTATIVES:

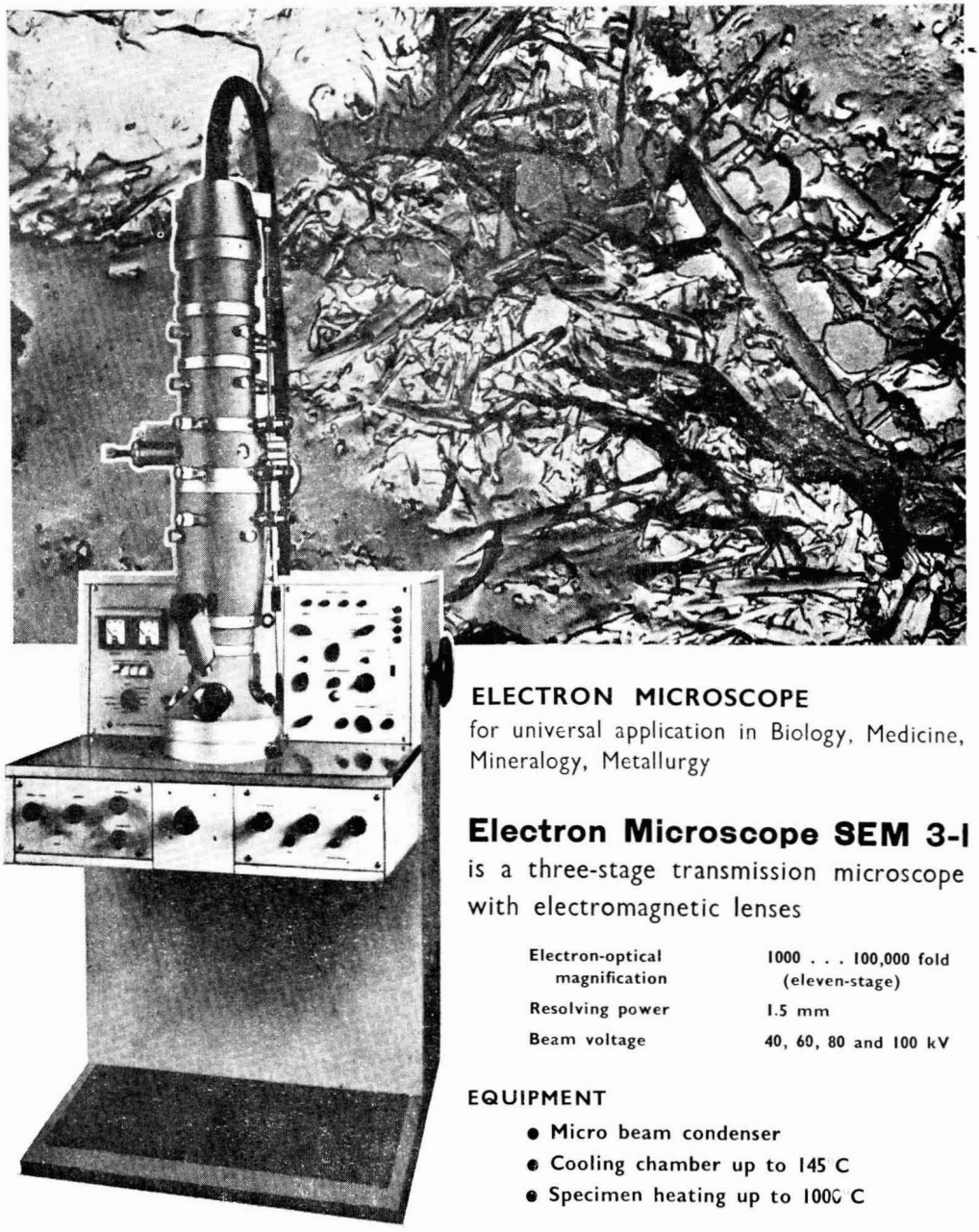
MOTWANE

PRIVATE LIMITED

127 Mahatma Gandhi Road, Post Box No. 1312 Bombay-1
Phone: 252337.Grams: 'CHIPHONE' all offices • Branches at: New Delhi, Calcutta, Lucknow, Kanpur, Madras and Bangalore.

ML-65





ELECTRON MICROSCOPE

for universal application in Biology, Medicine, Mineralogy, Metallurgy

Electron Microscope SEM 3-I

is a three-stage transmission microscope with electromagnetic lenses

Electron-optical magnification	1000 . . . 100,000 fold (eleven-stage)
Resolving power	1.5 mm
Beam voltage	40, 60, 80 and 100 kV

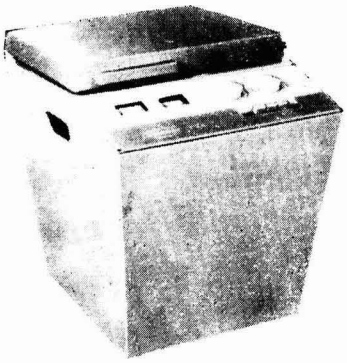
EQUIPMENT

- Micro beam condenser
- Cooling chamber up to 145 C
- Specimen heating up to 1000 C

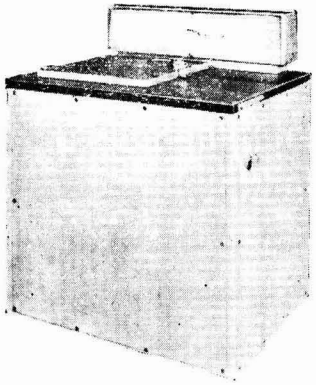
VEB WERK FÜR FERNSEHELEKTRONIK
116 BERLIN-OBERSCHÖNEWEIDE, OSTENDSTRASSE 1-5
GERMAN DEMOCRATIC REPUBLIC

AGENCY
MESSRS K. LAL BHAKRI
P.O. Box 487, New Delhi, India

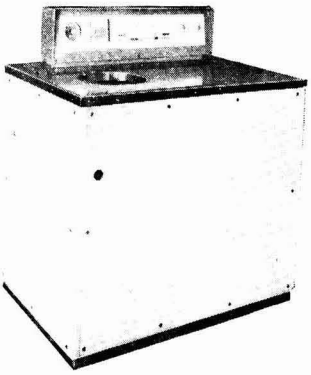
JANETZKI



TYPE T 24



TYPE S 60



TYPE VAC 60

JANETZKI CENTRIFUGES

a synonym for quality!

for medical, chemical and biological laboratories

Laboratory sugar centrifuges Type TZ 1

Rotating speed	2900 r.p.m.
Field of gravitation	520 g

Micro-haematocrite centrifuges Type TH 1

Rotating speed of	
Haematocrite rotor	max. 16,000 r.p.m.
Microrotor	max. 20,000 r.p.m.
Field of gravitation	
Haematocrite rotor	max. 18,500 g
Microrotor	max. 18,000 g

Laboratory table centrifuge Type T 4

Rotating speed	max. 3200 r.p.m.
Field of gravitation	max. 1200 g

Laboratory table centrifuge Type T 20

Rotating speed	max. 6400 r.p.m.
Field of gravitation	max. 4500 g

Laboratory table centrifuge Type T 22

Rotor freely swinging	max. 3400 r.p.m.
Angle rotor	max. 4800 r.p.m.
Field of gravitation	
Rotor freely swinging	max. 2000 g
Angle rotor	max. 3250 g

Laboratory table centrifuge Type T 23

Rotating speed	
(4 × 100 ml)	max. 6000 r.p.m.
(6 × 100 ml)	max. 6500 r.p.m.
Field of gravitation	
(4 × 100 ml)	max. 5600 g
(6 × 100 ml)	max. 5800 g

Laboratory table centrifuge Type T 24

Rotating speed	max. 16,000 r.p.m.
Field of gravitation	max. 21,500 g

Stand centrifuge Type S 60

Rotating speed	
Rotor freely swinging	max. 3500 r.p.m.
Angle rotor	max. 4500 r.p.m.
High-speed attachment	max. 16,000 r.p.m.
Field of gravitation	
Rotor freely swinging	max. 2600 g
Angle rotor	max. 4000 g
High-speed attachment	max. 21,500 g

Cooling centrifuge Types K 60 and K 60 S (similar to Type S 60)

Type K 60 with cooling aggregate	500 cal/h	
Type K 60 S with cooling aggregate	1250 cal/h	
Minimum temperatures	K 60	K 60 S
Rotor freely swinging		
4 × 1000 ml	± 0°C	−15°C
4 × 250 ml	−2°C	−22°C
Angle rotor		
8 × 250 ml and		
6 × 500 ml	−5°C	−25°C
High-speed attachment		
Rotor 6 × 20 and		
12 × 10 ml	± 0°C	−8°C
Rotating speed		
Rotor freely swinging	max. 3500 r.p.m.	
Angle rotor	max. 4500 r.p.m.	
High-speed attachment	max. 16,000 r.p.m.	
Field of gravitation		
Rotor freely swinging	max. 2600 g	
Angle rotor	max. 4000 g	
High-speed attachment	max. 21,500 g	

Preparative ultracentrifuge Type VAC 60

Rotating speed	max. 60,000 r.p.m.
Field of gravitation	max. 300,000 g
Vacuum in the rotor chamber	5×10^{-2} Torr
Minimum temperature in the rotor chamber	−20°C

Messrs Heinz Janetzki K.G.
Leipzig

AGENTS
MESSRS K. LAL BHAKRI
P.O.B. 487, New Delhi, India

Printed and published by Shri A. Krishnamurthi, Publications & Information Directorate, Council of Scientific & Industrial Research, New Delhi, at the Catholic Press, Ranchi, India

Regd No. PT-842