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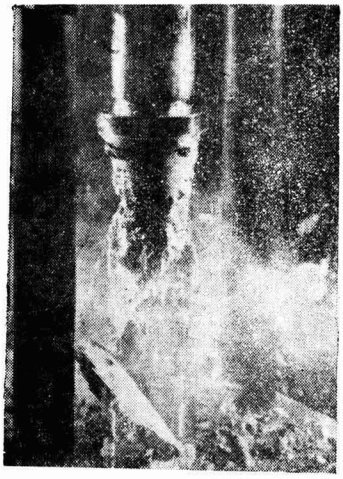
Journal of Scientific & Industrial Research

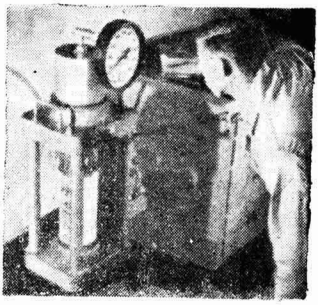
VOLUME 25 NUMBER 7 JULY 1966

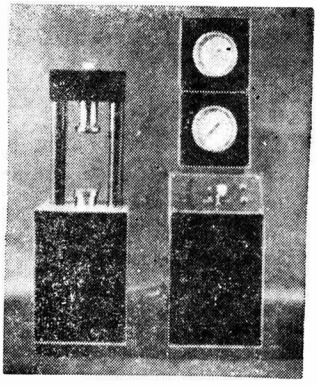
CONTENTS

For Index to Advertisers, see page A21

At what load did this concrete sample really fail?







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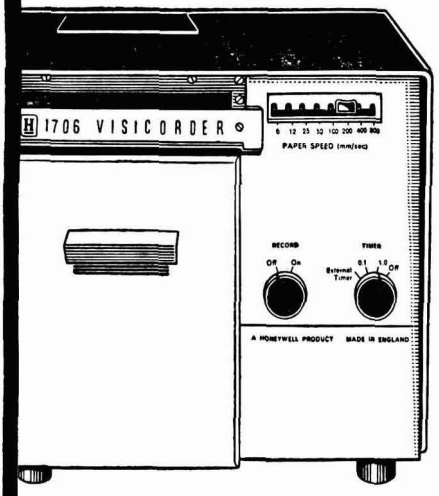

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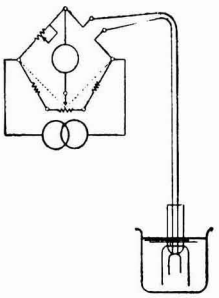

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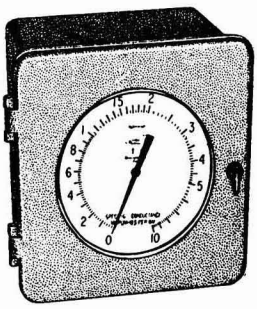

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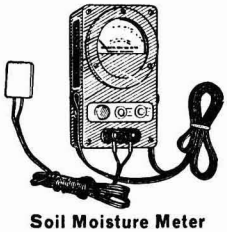

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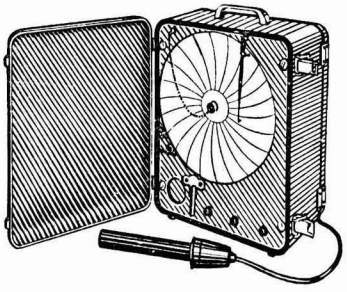

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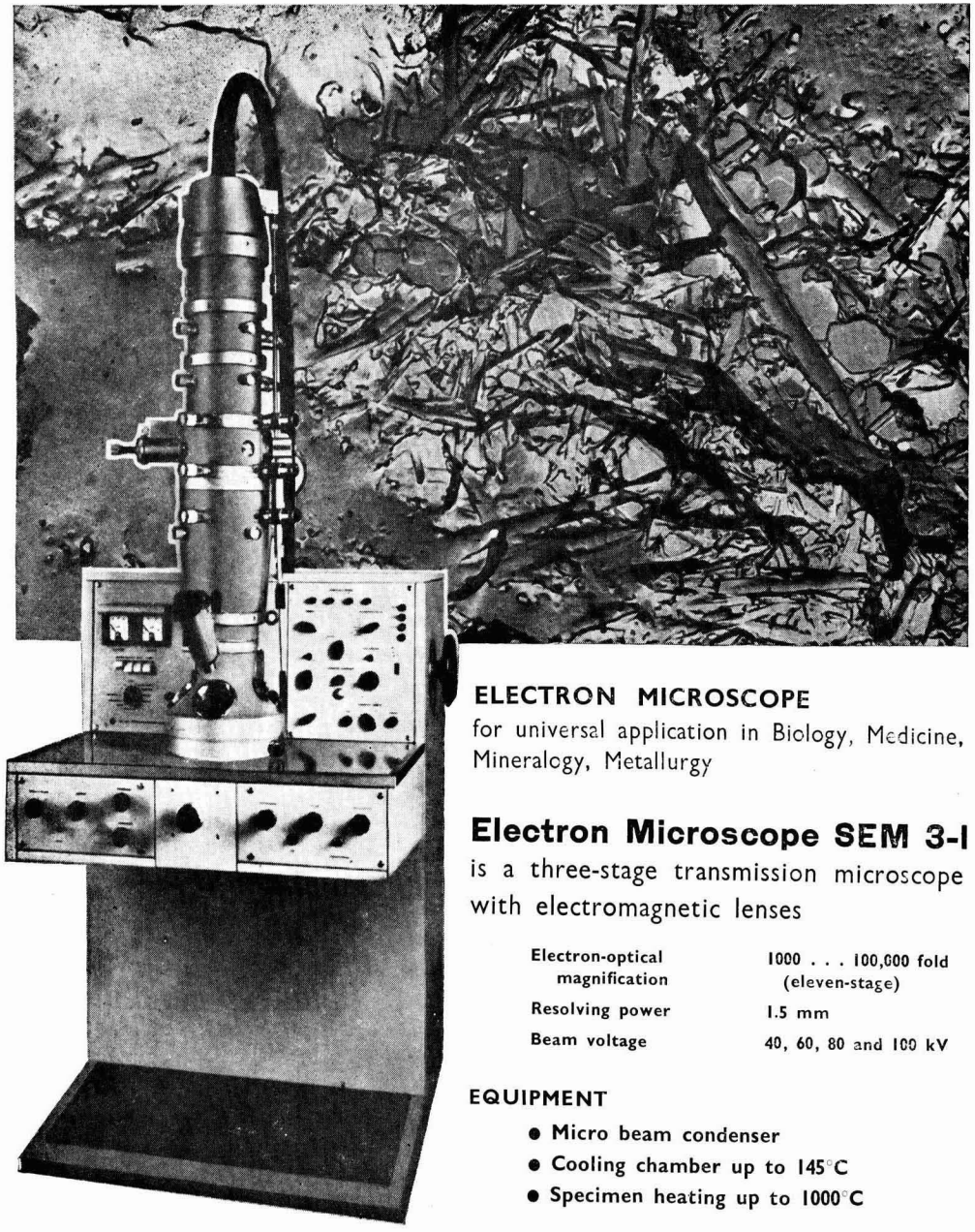

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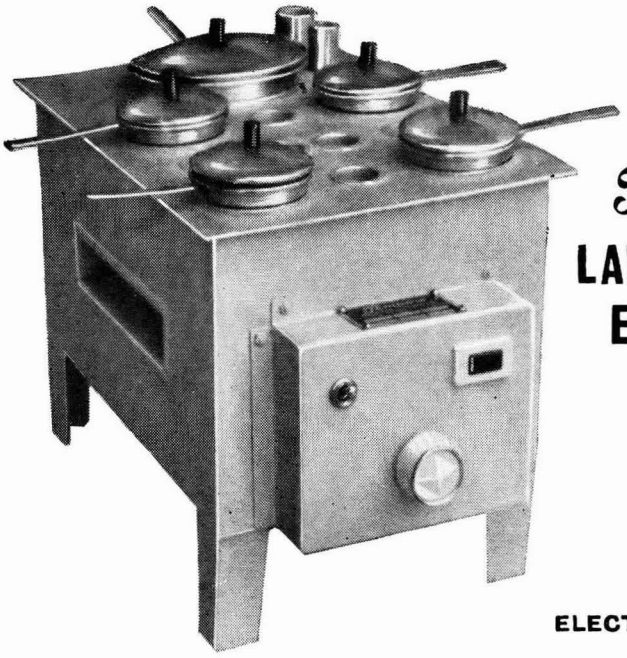

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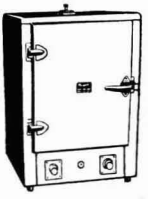

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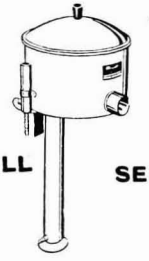

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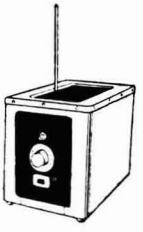

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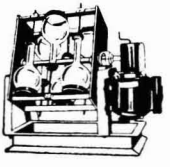

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Current Topics

Conservation of Foreign Exchange: Contribution of the National Laboratories

A REPORT* issued recently by the Research Survey and Planning Organization of the Council of Scientific & Industrial Research deals with an important problem concerning the country's current economic position, namely the contribution of the national laboratories in conserving foreign exchange. The determination of the extent of conservation of foreign exchange resulting from research effort in exact quantitative terms is a very complex problem, since, in most cases, it is the consequence of the combined impact of a variety of factors. The isolation of the impact of research effort from that of other factors and linking it up with proper quantum (monetary) of benefit (conservation of foreign exchange) is thus a formidable task. The present survey represents probably the first attempt in this direction. In fact, the purpose of the survey is more to 'build up a methodology which may serve as a working hypothesis for developing the subject further', rather than to arrive at numerically-backed definite conclusions.

The survey covers the five-year period 1958-59 to 1962-63 and the statistics provided are based on data obtained from 20 national laboratories. The two main aspects of saving of foreign exchange, viz. (i) saving through import substitution, visible as well as invisible (technical know-how and services of experts, etc.) and (ii) earning of foreign exchange by means of changing the quantum and structure of the commodities exported and/or raising the prices per unit of export, have been taken into consideration. In the classification model adopted the contributions from the national laboratories have been grouped under these two broad heads. Under direct sources of foreign exchange saving, contributions from activities like designing and fabrication of equipment, production of import substitutes, release of processes, teaching and training facilities, and exchange of literature are listed. The indirect sources include consultancy services and practical demonstrations through extension services, testing facilities and standardization of processes and products. Contributions from facilities considered to have been conducive to increasing the earning of foreign exchange by intensifying export effort have been grouped under: (i) sampling inspection of exportable goods; (ii) improvements in the methods of preservation and packaging of exportable commodities; and (iii) market research in foreign countries and commodity studies within the country.

The gross conservation of foreign exchange during the period under survey due to the research effort of the national laboratories covered as well as the other contributing industrial factors has been estimated at Rs 259·02 million. The net contribution of the research effort works out to Rs 76·91 million, i.e. about 30 per cent of the gross saving. The gross saving figures break into Rs 234·44 million as the saving through import substitution and Rs 14·58 million as the exchange earning. The gross contribution from direct sources of foreign exchange saving works out to Rs 183·83 million, the balance (Rs 75·19 million) being the contribution from indirect sources of saving. The corresponding figures for net saving are Rs 1·86 million and Rs 75·05 million respectively. The contribution arising from the utilization of research results has also been broken up according to the frequency of their occurrence in the production processes, viz. on a continuous basis or only once. The contribution of the continuous component runs as high as Rs 149·27 million and that due to the use of research results only once, Rs 109·75 million.

Laboratory-wise, the largest contribution to the gross conservation of foreign exchange is from the Central Food Technological Research Institute, Mysore (Rs 128·17 million), followed by the National Metallurgical Laboratory, Jamshedpur (Rs 47·45 million), the Central Fuel Research Institute, Jealgora (Rs 33·6 million), and the National Physical Laboratory, New Delhi (Rs 20·44 million). In terms of net conservation resulting from the research effort alone, the first position is occupied by the National Metallurgical Laboratory (Rs 45·0 million), followed by the Central Fuel Research Institute (Rs 30·13), the Central Food Technological Research Institute (Rs 1·28 million), the Central Glass & Ceramic Research Institute, Calcutta (Rs 0 129 million) and the Central Mining Research Institute, Dhanbad (Rs 0·10 million).

The results of the present survey provide a basis for conducting a more exhaustive and comprehensive survey covering not only the other national laboratories but also other centres of research in the country. Further surveys in this connection should cover those industries and others who have actually utilized the technical know-how.

A study on the conservation of foreign exchange by the national laboratories — Survey Report No. 4, Research Survey and Planning Organization, Council of Scientific and Industrial Research, New Delhi, 1966. Pp. 15.

Prof. P. Maheshwari, FRS

WE record with deep regret the sad demise of Prof. P. Maheshwari, Professor and Head of the Department of Botany, University of Delhi, on 18 May 1966 after a brief illness. Prof. Maheshwari, a pioneer in the field of plant morphology in India,

has made significant contributions to the study of embryo and its post-fertilization development. In his death India has lost an eminent botanist, held in high esteem by botanists all over the world.

Prof. P. Maheshwari, FRS

Born on 9 November 1904, Prof. Maheshwari had his education at the University of Allahabad, where he later worked as a research scholar under late Dr Winfield Dudgeon. He obtained the D.Sc. degree of the University of Allahabad in 1931. He began his teaching carrier as Lecturer in Botany at the Ewing Christian College, Allahabad (1928-30), and later at Agra College (1930-37) and the University of Allahabad (1937-39). In 1939 he was appointed Reader in Botany and Head of the newly started Biology Department at the University of Dacca, and was later promoted to the professorship. In March 1949 he was invited by Sir Maurice Gwyer, the then Vice-Chancellor of the University of Delhi, to join the University of Delhi as Professor and Head of the Department of Botany which appointment he held till his death.

Prof. Maheshwari's important contributions include studies on cryptogams, gymnosperms and angiosperms with special reference to taxonomy, anatomy, embryology and metabolism, on which nearly 300 papers have been published by him and his collaborators. He is the author of books entitled *Embryology of angiosperms*, *Gnetum* (jointly with his pupil Vimla Vasil) and *Dictionary of economic plants of India* (with his student Umrao Singh), and had in preparation a fourth publication on the *Morphology of gymnosperms*. He also edited *Proceedings of the summer school of botany: Darjeeling; Plant tissue and organ culture: A symposium;* and *Recent advances in the embryology of angiosperms*. He was Founder-Editor of *Phytomorphology*, official organ of the International Society of Plant Morphologists, of which he was the Founder-President.

Prof. Maheshwari was elected Fellow of the Royal Society, London, in March 1965 for his significant contribution in the field of botany, chiefly in the morphology and embryology of seed plants. He was elected Fellow of many national and international organizations. He presided over many international congresses as Vice-President and President. In 1959 he was the recipient of Birbal Sahni Memorial Medal of the Indian Botanical Society and in 1964 of the Sunderlal Hora Memorial Medal of the National Institute of Sciences of India. He received the honorary doctorate of the McGill University, Montreal.

Prof. Maheshwari travelled widely in Europe and USA. At the invitation of Unesco he visited Indonesia in 1952 and Egypt in 1954, and in 1958 he paid a short visit to the USSR as a member of a scientific delegation sponsored by the Government of India. In the summer of 1959 he was a visiting Professor at the University of Illinois. In 1961 he visited Germany and England, and in 1964 he gave lectures at many universities in USA. He had planned to visit some of the research centres in Japan, Hawaii, USA, England and Europe during May-June 1966, but fate prevented it.

Besides being an outstanding researcher he was an excellent teacher and was responsible for training a large number of students and research workers and establishing one of the best schools of botany in the country. During the last 15 years about 800 scientific papers have been published by the staff and students of his department; 65 students, including one from Buenos Aires, were admitted to Ph.D. degree. Prof. Maheshwari was a tireless worker. He expected from others the highest degree of efficiency and devotion. He aimed at nothing but the very best and never tolerated poor standards.

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

Summer School in Ferrous Metallurgy

B. K. SAXENA
National Metallurgical Laboratory, Jamshedpur

THE impact of modern technological developments in the metallurgy of iron and steel production has been felt markedly in the Indian iron and steel industry. The problems that confront India are complex, relating chiefly to difficulties of foreign exchange, raising of productivity potential, shortage of technically trained personnel and insular shortages of indigenous capital goods and maintenance spares. In spite of vast reserves of good grade iron ores, the vast expansion of the steel industry has revealed some basic metallurgical shortcomings in Indian raw materials for the steel industry. Unless these difficulties are overcome by the application of latest research results, the rationale of India's accelerated steel development would lose some of its force. To focus attention of ferrous metallurgists on the latest technological and research developments in the metallurgy of iron and steel and to provide a forum for discussions on their rational utilization, a two weeks' summer school on " Ferrous Metallurgy " was organized by the Ministry of Education, Government of India, at Shillong during 12-24 July 1965, with Dr B. R. Nijhawan, Director, National Metallurgical Laboratory, as its convener.

Seventeen scientists and metallurgists from different research laboratories, technological institutes and universities participated in the summer school, which was inaugurated by Dr Taylor, Ex-Vice-Chancellor of Gauhati University. While inaugurating the summer school, Dr Taylor expressed the hope that such a gathering of scientists and metallurgists will offer stimulation of thought and provide an ideal forum for exchange of technical ' know-how ' and ' know-why ' of diverse aspects of the complex subject of ' ferrous metallurgy '.

Technical Sessions

Twenty papers covering various aspects of research, production and planning in the field of iron and steel were presented and discussed in the technical sessions held from 13 to 24 July relating to: (1) preparation and use of the Indian raw materials; (2) techniques and choice of iron and steelmaking processes; and (3) growth of iron and steel industry in India and abroad.

The technical sessions of the summer school started with a paper on ' Status of iron and steel industry in India ' by Dr B. R. Nijhawan, followed by five technical papers on the preparation and use of the Indian raw materials. Dr Nijhawan referred to the heavy capital investment in the integrated iron and steel plants. He also discussed the different possible sites for the location of the fifth and sixth steel plants vis-à-vis the availability of raw materials in their vicinity, as well as the growth pattern of iron and steel industry in India. He stressed that while considering proposals for the establishment of coastal plants, such as at Goa, the import of high grade metallurgical coking coal or coke can be balanced against the export of iron ore pellets made out of beneficiated iron ore fines. It is a welcome feature of current Indian planning that the establishment of new steel plants is distinctly more ' raw materials oriented ' rather than ' equipment oriented '. He also illustrated the cost of production of 0·3 million tons/annum pig iron plant with a capital investment of Rs 2·5-3 crores. The total indigenous fabrication of such small plants in India today is a definite possibility and would encourage the growth of related engineering industries.

K. N. Gupta (NML) gave an appraisal of the raw materials for the blast furnace and about the investigations carried out at the NML on decrepitation and physical characteristics of some Indian iron ores, including porosity, abrasion and crushing strength at room and elevated temperatures.

S. K. Gupta (IIT, Bombay) gave an account on the interpretation of coke rates while varying charge bed heights during sintering fluxed charge. A layerwise heat balance was drawn according to the method suggested by A. A. Sigov. A similar heat balance was drawn in the case of fluxed charge using Durgapur iron ore. He also referred to the mineralogical structures of fluxed sinters from Rajhara iron ores.

J. Goswami (NML) in his paper on ' Indian coals and iron making ' gave the main classifications of Indian coals with their estimated reserves. The abundantly available non-metallurgical coals can be successfully used for the production of iron by suitable alternate methods. He also discussed the results of the use of non-metallurgical coals in different forms in the low shaft furnace of the NML.

Dr B. R. Nijhawan (NML) gave an account of the latest studies on the beneficiation of iron ores. He emphasized that mechanical mining of high grade iron ores liberates 30-40 per cent, $-\frac{1}{2}$ in. fraction containing as high as 20 per cent Al_2O_3 with a very adverse SiO_2/Al_2O_3 ratio. He also mentioned that the cost of beneficiation will amount to about Rs 12.00 per tonne inclusive of overheads. He indicated the difficulties of beneficiation of Indian iron ores containing high clay contents.

S. L. Malhotra (Department of Metallurgy, BHU) discussed the various direct reduction processes and reviewed the researches on the development of suitable alternate processes of iron ore reduction in view of inadequate coking coal resources, depleting resources of rich iron ores, non-availability of scrap, and the inherent high capital cost of the blast furnaces. During the discussion Dr Nijhawan discussed in detail the future prospects of the direct reduction processes in India and pointed out that these are not suitable for Indian conditions, largely because of the non-availability of natural gas.

B. K. Saxena (NML) gave an account of the recent trends in the blast furnace practice. He discussed the role of productivity and coke rate, the principal factors in the economy of the blast furnace. He also discussed the problems created by high alumina content and unfavourable Al_2O_3/SiO_2 ratio of the Indian iron ores, particularly in the ore fines, aggravated by heavy monsoon rains, and the high ash content of the metallurgical coals, etc.

Dr A. B. Chatterjea (NML) reviewed the technological aspects of low shaft furnace process. He mentioned the various chemical and physical characteristics of the burden, as well as the influence of grain size on heat transfer. He also discussed the furnace output, the rate of combustion of coke, the reduction of iron oxides by carbon monoxide and the temperature distribution in the low shaft furnace.

A. I. Djiakonov (Unesco Expert, IIT, Bombay) in his paper on electrical conductivity of molten open hearth slags and utilizing these data for controlling the process of steelmaking described a device for estimating the electrical conductivity of molten slag directly in the furnace and its correlation with desulphurization. J. Mohan (NML) gave an account of the different pneumatic steelmaking processes and their suitability to the Indian conditions. R. K. Dubey (NML) discussed the production of alloy steels, with special reference to the production of stainless steel and high speed tool steels. He emphasized that the development of oxygen lancing in the electric arc furnace for decarburizing the melt in the presence of high chromium has revolutionized the stainless steel melting practice. He also discussed the melting of high speed steels and the efficiency and economy achieved in different stages of manufacturing tool and die steels.

V. S. Bhandari (NML) reviewed the recent developments in cupola practice, with particular reference to the hot blast cupola. He also discussed the conversion of the cold blast cupola to the hot blast cupola along with its various advantages, with special reference to the hot blast cupola installed at the National Metallurgical Laboratory.

R. D. Gupta (NML) in his paper on 'Electro slag melting' discussed the studies made on the change in chemical composition, inclusion type and content and ingot structure of plain carbon, ball bearing, and high speed steels.

P. K. Sen (IIT, Kharagpur) presented a paper on the decarburization of open hearth melts and discussed some features of the kinetics of the decarburization process in the open hearth furnaces under different conditions. He also discussed the apparent activation energy of the process of oxidation of carbon in Fe-C melts. The effect of temperature on the rates of decarburization of Fe-C melts, subjected to two different oxidizing gaseous media, viz. still air and a mixture of 92 per cent argon and 8 per cent oxygen, have been studied in the temperature range 1450-1650°C. S. K. Goel (Department of Metallurgy, University of Roorkee) described the development of rotor process of steelmaking. A. N. Kapoor (NML) in his paper on 'Some observations on surface protection' gave a description of various surface cleaning methods prior to surface coating. He also described the advantages of aluminium coating and the possibility of replacing tinning by aluminium or chromium plating, with special reference to the raw materials available in India.

Dr S. S. Khanna (Heavy Engineering Corp., Ranchi) in his paper on 'Role of Heavy Engineering Corporation for the building of heavy capital equipments' described the programme and the salient features of the various projects under the Corporation.

A. I. Djiakonov in his paper on 'Iron and steel industry in USSR' dealt with the oxygen enrichment of the flame in open hearth, decarburizing by roof lancing with oxygen and the use of 100 per cent compressed air in steelmaking. He mentioned that for increasing the output of a basic open hearth furnace by 10 per cent, the use of compressed air will be more economical, but for higher output, oxygen lancing was desirable. He also discussed the expansion programme of the iron and steel industry in USSR.

Dr A. B. Chatterjea, in his talk on 'A glimpse of the iron and steel industry in Japan' gave a picture of the developments in the iron and steel industry of Japan, which imports both coal and ore and still ably competes in the world market. He gave an account of the Japanese blast furnace working and presented statistical data showing that Japan has the lowest coke rate.

Dr B. R. Nijhawan in his paper on 'Iron and steel industry in developing countries' gave an account of the growth and development of iron and steel industry in India and other developing countries. He cited examples of Pakistan, Burma, Ceylon and some African countries where no suitable raw materials are available for installing any iron and steel industry, but even then they are setting up integrated iron and steel plants, as it has become a prestige issue for them. Japan is developing her iron and steel industry with a modern base mainly to balance their trade with food imports. It is interesting to know that Japan is exporting 10 million tons of steel to Canada, UK and other countries from where they are purchasing the raw materials. He also discussed the iron and steel expansion programme of the United Kingdom.

At the concluding session, the participants expressed general satisfaction at the way the summer school was conducted. General appreciation was expressed of the role of the National Metallurgical Laboratory in putting the metallurgical research and developmental work in the country on a firm footing.

While presenting a résumé of the deliberations, Dr A. B. Chatterjea expressed appreciation for the valuable contributions on various topics.

Design of Cathodic Protection Installations

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IN an earlier publication[1], the criteria for cathodic protection were discussed in terms of the electrode reactions taking place on the metal surface under protection. It was pointed out how knowledge of the fundamental parameters such as Tafel slopes and exchange currents of the electrode reactions enables the calculation of the change in potential leading to a specific degree of cathodic protection. It was also mentioned that concentration polarization caused by the diffusion of the species taking part in the cathodic reaction may become a dominant factor in neutral solutions. In this communication, the complimentary aspect of this subject of cathodic protection, namely the influence of external parameters, such as the resistance of the electrolyte, the variation of the potential of the structure under cathodic protection, depending upon where the reference electrode is placed, and the spread of cathodic protection are discussed.

Electrolyte and Electrode Resistance

A cathodic protection installation (Fig. 1) is essentially a combination of an anode, the structure under protection which is made the cathode, and a source of supply of d.c., which will flow from the anode to the cathode through the electrolyte. When sacrificial anodes like zinc and magnesium are used, the current required for the installation is generated by the difference in potential between the anode and the cathode and no separate source of supply of current is used. On examining this system closely, it is seen that the driving voltage, V, will depend upon the current required as well as the total electrical resistance offered to the passage of current at each one of the component parts of the cathodic protection installation. The component parts are: (i) the anode, (ii) the anode-electrolyte interface, (iii) the electrolyte, (iv) the cathode-electrolyte interface, (v) the cathode, and (vi) the external connections between the cathode and the anode. The ohmic resistance of the anode may be expected to be negligible. Ordinarily, the anode-electrolyte interface would not contribute significantly to the total resistance. However, this becomes significant if anode passivation takes place or resistive deposits are formed on the anode. Since the resistance of the electrolyte, R, is given by $R=\rho.l/A$, where ρ is the specific resistance; l, the length; and A, the cross-sectional area of the electrolyte path, the geometry of the system has to be taken into account. The presence of a non-metallic coating may suggest that considerable resistance would be offered at the cathode-electrolyte interface. The resistance of the cathode itself may become significant in spite of the very high conductivity of the metal as compared to the electrolyte, if current has to flow over very long sections, as may be obtained in the case of cathodic protection of buried pipelines. The external connections are expected to contribute significantly to the total resistance of the system only when cables have to be taken over large distances from the source of supply of current to the structure under protection.

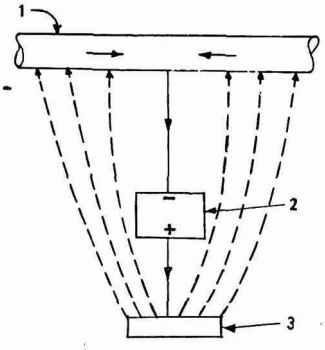

Fig. 1 — Cathodic protection installation [1, buried or submerged structure made the cathode; 2, d.c. source; and 3, anode surrounded by anode bed]

The electrolyte may be said to be homogeneous when protection is to be given in aqueous media. However, the electrolyte resistance may vary considerably from season to season in the case of estuarine and river water, while the resistance of sea water can be expected to vary considerably with the prevailing temperature. Gross heterogeneity of the electrolyte is exemplified in the case of protection underground. Large variations in resistivity may be observed between the different soil strata, and the resistivity of any particular part of the soil itself may vary considerably depending upon its moisture content. Theoretical calculations of the resistance that would be offered by the electrolyte, therefore, becomes particularly difficult in the case of underground structures. The resistivities of different media are as follows:

Medium	Resistivity ohm-cm.
Pure water	20,000,000
Rain water	20,000
Tap water	1000-5000
Clays	1000
Sea water	20-25
Chalk	3000
Coarse sand and gravel	300,000

Several general methods have been proposed for tackling the problem[2,3]. The resistivity of aqueous media can be readily measured using a Kohlrausch bridge; ready-made instruments are available for the purpose. Since this cannot be done in the case of soil resistivity, for this a soil

box[4] can be used. It is a cylindrical box made of lucite or perspex, in which four electrodes (usually of copper) are placed at equal distances from one another. A known current is passed through the outer electrodes and the potential difference between the inner electrodes is measured. The resistivity can then be calculated, applying Ohm's law, knowing the cross-sectional area and the distance of separation of the two central electrodes. This method has the serious drawback that it does not measure the mean resistivity of the soil through which current has to pass but only gives the resistance of an isolated sample. For measuring soil resistivity *in situ*, a number of methods have been proposed, of which the most widely used is the Wenner four-pin method[5]. In this case, four-point electrodes are introduced in the soil, such that these are equally spaced along a straight line and the resistance measured in the same way as in the case of the soil box. The resistivity, ρ, is given by

$$\rho=2\pi a.R \qquad ...(1)$$

where a is the distance of separation of the point electrodes; and R, the measured resistance. By adjusting the distance between the electrodes, the depth of the soil, for which measurement has to be made, can be varied. For example, if there are two strata of soil having resistivity ρ_1 (for the upper) and ρ_2 (for the lower), and if d is the depth of the upper stratum, then only ρ_1 will be measured so long as a is equal to or less than d. When a is very much greater than d, then only ρ_2 will be measured.

Resistance is given by the formula $R=\rho.l/A$. The geometry of the electrolyte, whose resistance is being measured, is conditioned by the size and shape of the anode and the cathode and their relative disposition. If this geometry is cylindrical, as for example, a vertical rod surrounded by a cylindrical cathode, it can be shown that the resistance R is given by

$$R=\rho/2\pi L.\ln(D/a) \qquad ...(2)$$

where D is the distance between the centre of the rod and the surface of the outer cylindrical cathode; a, the radius of the rod; and L, the length of the rod. The manner in which the resistance of such a rod varies with D/a is shown in Fig. 2. It is seen that while the resistance values continue to increase with D/a, the rate of increase is relatively small for values of D/a in excess of 300. On this basis, Eq. (2) can be simplified to

$$R=0{\cdot}0324\rho/L \qquad ...(3)$$

Eq. (3) has been tested in relation to lengths in excess of 64 times the diameter and the agreement with measured values is exceedingly good[2]. The resistance of the anode to a certain point in the ground has been considered because of its limited area. As compared to this, the resistance of the cathode to a similar point in the ground would be negligible because L will be much higher than for the anode.

On the other hand, if the anode has a spherical shape the resistance R is given by

$$R=\rho/4\pi.(1/a-1/D) \qquad ...(4)$$

Since D is very much greater than a, the expression reduces to

$$R=\rho/4\pi.a \qquad ...(5)$$

When the anode is close to the surface of the ground, only a hemisphere of the soil will be taking part in conducting the current. Hence

$$R=\rho/2\pi.a \qquad ...(6)$$

The equation developed for vertical anodes is also applicable to horizontal anodes, but the effect of proximity to ground surface has to be taken into account. When a rod is longitudinally placed on the ground, the soil through which current lines flow is a hemicylinder, and not a cylinder as in the earlier case and the resistance will not be like that of a vertical rod. This situation will be altered with the depth of burial of the anode. At depths greater than 10 times the diameter, the resistance will approximate to that given by Eq. (6), though a correction factor may still have to be used. But then, the uncertain and variable character of resistivity (ρ) in the case of a heterogeneous medium like soil is much greater than the small error which may be introduced by an approximate formula.

It is not generally practicable to use a single anode. Anode bed, be it horizontal or vertical, usually consists of several anodes connected in parallel. The theoretical formula for the resistance between two anodes and a pipeline at infinity has been propounded by Dwight[6] in terms of the capacity of the system. A practical test of the variation in resistance, as the number of anodes is increased, has been reported by Applegate[7]. The fact that the decrease in resistance with increase in the number of anodes is much slower than what would be expected from Ohm's law is illustrated in Fig. 3. It is also seen that the correction factor steeply increases with decrease in the spacing between the rods (Fig. 4) (ref. 8).

The electrolyte resistance is further modified by what is known as the 'backfill' at the anode

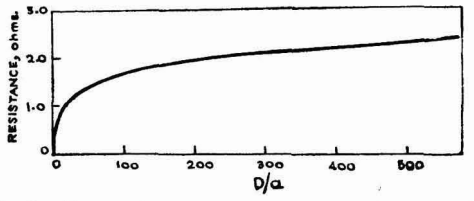

Fig. 2 — Increase in resistance between a vertical rod of radius a and ground with distance D from the rod

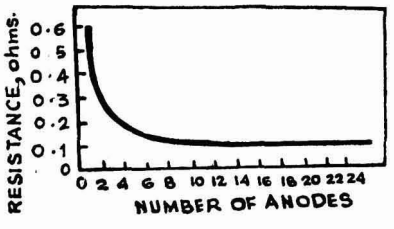

Fig. 3 — Relation between number of anodes and resistance [Soil resistivity, 200 ohm-cm.]

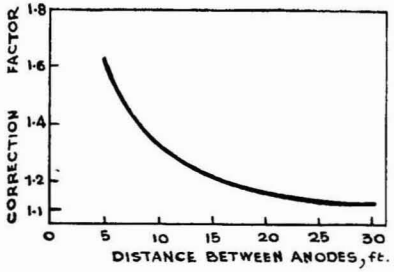

Fig. 4 — Relation between distance between anodes and the correction factor [No. of anodes, 5]

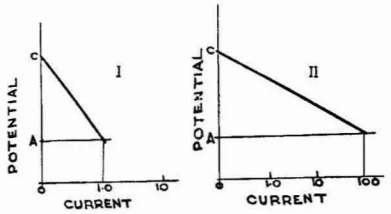

Fig. 5 — Relative values of protective current required for the coated (I) and uncoated (II) steel [C, cathode; and A, anode]

bed. The size of the anode bed is increased by placing the metal anode in a bed of coke-breeze or mixtures of gypsum and bentonite or common salt and bentonite. The ohmic resistance of such a bed may be considered to be negligible, as compared to the soil resistance and hence the whole bed can be looked upon as an enlarged anode and the resistance can be calculated taking into account the size and shape of the anode bed. However, care has to be taken to see that such an anode bed does not get dried up by electro-osmosis, when the resistance of the backfill will increase steeply.

The resistance of the cathode-electrolyte interface is governed by the type of coating applied to the structure under protection. It is, in fact, uneconomical to give cathodic protection to the structure in the bare condition because of the large consumption of power, and the high cost of maintenance of such a system. Under most circumstances, therefore, cathodic protection is combined with painting of the structure, the idea being that the area to be given cathodic protection will consist only of the holidays that are invariably present in almost all types of coatings. The current that would be required to bring steel coated with coal tar enamel to the protective potential as compared to a bare specimen of the same geometry is shown in Fig. 5.

It is evident that the distribution of current on a coated structure will be such that nearly all the current would be going to the bare areas of the coated structure. So the cathode-electrolyte resistance may be considered to be negligible. Further, as pointed out earlier[1], cathodic protection is obtained when

$$E'_a = E'_c - f(I_x/A_c) - I_x.R_c \qquad \ldots(7)$$

where E'_a is the open circuit potential of the anodic site; E'_c, the open circuit potential of the cathodic site; I_x, the applied current; A_c, the area of the cathodic site; R_c, the resistance of the cathode-electrolyte interface; and $f(I_x/A_c)$, the polarization effect, which is a function of the cathodic current density, (I_x/A_c). R_c is given by $\rho/2\pi L.\ln D/a$. On a pipeline, L is very large and D/a is very nearly one. When the holidays are few, I_x will also be small. Hence I_xR_c is negligible. So the coating resistance does not really figure in the calculation of the driving voltage. The economics of cathodic protection is governed by the driving voltage, because the power consumed is given by the product of the driving voltage and the current. By applying a coating, the current is reduced, and, though the driving voltage remains constant, the power consumption is reduced.

The resistance of the cathode becomes significant only in the case of long pipelines and cables. In such cases, there can be an appreciable voltage drop in the structure itself, so that the driving voltages available at the drainage point and further along the pipeline become different. This would naturally affect the current distribution over the structure. This will be considered again under the section 'Spread of Cathodic Protection'. Appreciable voltage drops at the several connections should be avoided as far as possible, since otherwise needless power loss would take place.

Potential of the Structure under Cathodic Protection

The set-up for the measurement of the potential of a structure under cathodic protection is shown in Fig. 6. The main problem is the location of the reference electrode for the measurement of the potential of the structure. The structure is brought to a more negative potential to give cathodic protection. The potential of the structure measured will vary with the position of the reference electrode. To understand how this variation is brought about, it is necessary to first consider the potential of a metal specimen dividing an electrolyte through which current is passing in one direction. Such a condition is illustrated in Fig. 7 (inset). The potential is plotted against the distance of the metal in either direction in Fig. 7.

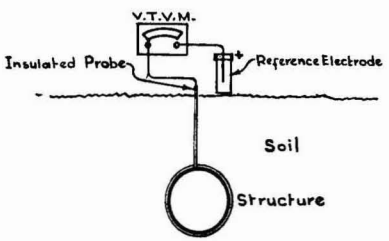

Fig. 6 — Set-up for the measurement of the potential of structure under cathodic protection [V.T.V.M., vacuum-tube voltmeter]

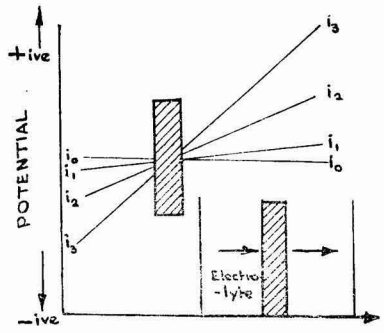

Fig. 7 — Variation of measured potential with relative position of the reference electrode

The manner in which the potential varies with distance will, however, not be as simple as shown in Fig. 7 in the usual case, because of the more complicated configuration of a structure under cathodic protection and of the medium surrounding the structure.

On a corroding structure, different potentials may be obtained in different regions, more negative potentials being obtained on anodic areas and more positive potentials being obtained on cathodic areas. If a reference electrode is brought inside the boundary of the corrosion cell and brought close to the anodic areas, it measures a certain potential. If it is moved to the cathodic areas, it measures a different potential. Therefore, the potential profile will show considerable variation, when the reference electrode is moved very close to the metal surface. However, if the reference electrode is placed farther away, then it will scan both the anodic and cathodic areas and hence will measure a potential, which is in between the two. When it is placed just outside the boundary of the corrosion cell, it will measure the compromise potential of the whole structure as a result of current flowing between the anodic and cathodic areas. This again emphasizes the importance of the correct location of the reference electrode. A simplified approach to this question may be made by a consideration of the potential variation of two cylindrical electrodes made of different metals (say copper and steel), which have been short-circuited, thus resembling a corrosion cell (Fig. 8).

The potential becomes more and more negative in the direction of A′, because cathodic current flows from the electrode C. Similarly, in the direction of A, the potential becomes more positive because anodic current flows from A. The potential variation in between the two electrodes is also shown in Fig. 8. It is seen that somewhere in between, along BB′ (outside the corrosion cell boundary) the compromise potential will be obtained. When cathodic protection is given, the potential of the structure is being changed in the negative direction. When the reference electrode is placed within the boundary of the corrosion cell, the potential will vary, depending upon whether it is close to the anode or the cathode. If the reference electrode is placed close to the anode, it will show the protective potential, before the cathode is polarized to this potential. If it is placed close to the cathode, when the protective potential is reached, the anode potential would have become more negative than the protective potential. It is, therefore, essential to make a reliable estimate of the half cell location before using the potential criterion. It should be placed such that it is just at the boundary of the corrosion cell, so that when the potential at this point becomes equal to the protection potential, no current flows in the corrosion cell and the potential is the same both at the anode and the cathode. In other words, the appropriate location will depend upon the geometry of the corrosion cell, resistive films including coatings and the resistivity of the electrolyte. If the size of the corrosion cell increases either due to an increase in the areas of the anode and the cathode or due to an increase in the distance separating the two electrodes, the reference electrode may be placed farther from the metal. If, however, the reference electrode is placed far away from the boundary of the corrosion cell, the *IR* drop would be included and a more negative potential would be measured before the cathode has been brought to the anode potential.

Three types of corrosion cells are indicated in the case of structures, e.g. buried pipeline: (i) corrosion due to differences between adjacent points, (ii) corrosion at the bottom of the pipeline due to the cell set up between the bottom and the top, and (iii) corrosion current flowing over long distances because of differences in soil conditions in different sections. These are shown in Fig. 9.

If the reference electrode is placed close to 1, for checking the attainment of protective potential, all forms of corrosion will be prevented, though the anodic area at the distant point and at the bottom may become overprotected. It if is placed over

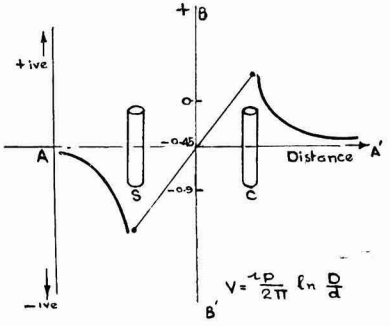

Fig. 8 — Potential variation of two cylindrical electrodes made of different metals [S, steel; and C, copper]

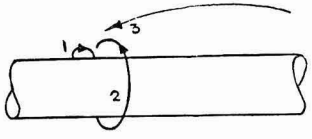

Fig. 9 — Corrosion cells developed in buried pipeline

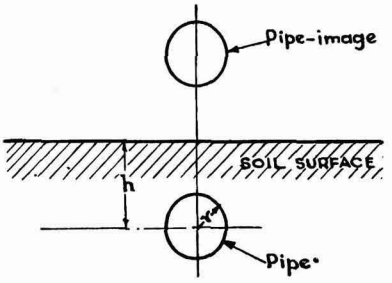

Fig. 10 — Swing in potential in the case of cathodic protection of large pipelines

this pipe section of the soil surface, the overprotection would be reduced, but the local cathode will not be made sufficiently negative. If the half cell is placed far away, the distant anode will not be overprotected, but the cathode potential will not be sufficiently negative; and localized corrosion will continue.

The location of the reference electrode becomes less critical, when the change in potential with distance is less because of less current flowing to the structure or the resistance of the electrolyte being less. Coatings reduce current density and hence the potential gradient in the electrolyte. Electrolytes having a specific resistance less than 100 ohm-cm. do not require accurate positioning.

In the case of large pipelines, the proximity of the pipeline to the surface of the soil has to be taken into account. This is done by imagining a situation, where above the surface also there is soil, just as below, in which case, it is expected that an electrical image of the pipeline will also be formed above the soil. This is represented in Fig. 10. The resistance of the soil can then be calculated in terms of the capacity of the buried structure as well as of its image, and then, half of the capacity value will correspond to that of the buried structure alone. In this way, it has been shown[9] that the potential difference between a point on the pipe and a point x on the surface of the earth is given by

$$V = \frac{i\rho}{2\pi}\left[\ln(h^2+x^2)+\ln\frac{M}{2rh}\right] \qquad ...(8)$$

where i is amp. per foot of the pipeline; ρ, the resistivity; x, the distance from the centre line on the surface of the earth of the cylinders to the point considered; h, the distance from the centre of the pipe to earth's surface; and r, the radius of the pipe and the coating-resistance has been provided for, in the mathematically convenient form of $\rho/2\pi \ln M$. On the vertical centre-line, the value of V will be given by

$$V = \frac{i\rho}{2\pi}\left[\ln(h+s)(h-s)+\ln\frac{M}{2rh}\right] \qquad ...(9)$$

where s is the depth at which the potentials are measured.

Spread of Cathodic Protection

For cathodically protecting long pipelines, they may be considered as made up of several short lengths of pipe and each section can be provided with suitable number of anodes. In general, a pipeline is protected by a single or a limited number of anode installations. The number of anode installations required depends on the spread of protection given by each installation. The spread of protection is affected by the potential drop along the pipeline itself, and also on the resistance of the coating. Even with good coatings, it has been found that the coating is damaged by current in excess of a few volts and so in practice, a shift in potential of two volts is taken as the maximum tolerance at the drainage point. This sets a limit to the spread of protection that can be aimed at. Taking into account the metal resistance r and the conductance of the coating g it has been shown[10] that the potential E_A, at the drainage point (where the negative lead of the power source is connected to the structure) is given by

$$E_A = E_m \cosh al \qquad ...(10)$$

where E_m is the minimum shift of potential required to achieve protection, $a = \sqrt{r.g}$ and l is half of the length of the pipeline getting protection. Since the change of voltage even at the drainage point cannot be allowed to go beyond two volts and the minimum shift in the potential of the structure required for protection can be calculated from the potential of the unprotected structure, the length of the pipeline for which protection can be given may be estimated from Eq. (10).

Summary

Factors influencing the economic design of a cathodic protection installation are discussed. It is shown that the geometry of the system and the size and shape of the anode have an important part to play in determining the electrical resistance of the cathodic protection circuit and, thereby, the power consumption. How the resistance of the metallic structure and the conductance of the coating determine the length of the pipeline which can be protected with a single anode bed is discussed. The variation of pipe to soil potential with distance of the reference electrode from the pipe and its significance are discussed.

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Adsorption & Other Related Phenomena in Paints

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THE phenomenon of adsorption at interfaces is of considerable importance not only in the paint industry but also in the ink, plastics, food and many other industries. The main aspects which concern the paint industry are (i) the effect of adsorption on the wetting and dispersion of pigments, (ii) the adhesion of paint films to the substrate, (iii) corrosion prevention of metallic substrates, (iv) the rheological properties of paint vehicles, and (v) the ageing and weathering properties of paint films.

Perhaps the most important factor in the adsorption of molecules on solid surfaces is the heterogeneity of these surfaces. Bikerman[1] has suggested that in surfaces consisting of more than one mineralogical component, as in the majority of alloys, the surface has a mosaic structure, since different compounds are exposed at various points on the surface. If the specimen is mineralogically uniform, its surface can still have various properties in different directions due to the presence of different faces, edges and corners. This heterogeneity accounts for the catalytic and adsorption properties, and the variation of thermionic and photoelectric work functions on the same crystal. Even one phase of the crystal does not have to be homogeneous as it normally contains hill-and-valley structure[2], kinks, jumps and cracks. Harkins[3] has pointed out that in all the work on adsorption and on energy relations for the surface of solids, no account has been taken of the possible effects of different crystal faces, edges, corners, etc., the observed effect being a statistical summation of all these.

The heterogeneous nature of pigment surfaces was studied by Dintenfass[4,5] by the adsorption of polar organic molecules from organic solvents on several industrial pigments and extenders such as TiO_2, talc, $BaSO_4$, $PbCrO_4$, etc. A selective polar adsorption pattern was observed which is different from physical capillary condensation type of adsorption and from chemisorption. Apparently, the surface of pigments, extenders, etc., consists of a number of distinct active areas, each of which selectively adsorbs a specific type of polar group. An independent and simultaneous adsorption of polar compounds containing different polar groups can take place, but the different polar compounds do not interfere with each others' adsorption. The active areas corresponding to various polar compounds, i.e. acids, alcohols, phenols, soaps, esters, primary and secondary amines, have been investigated.

The total surface area of a pigment appears to be the sum of the individual active areas and in some cases of non-active areas. Every pigment or solid surface can be characterized by the size, number and type of these individual areas. This gives a fingerprint of the pigment which apparently depends more on the origin and history of the pigment (or surface) than on the chemical structure. According to Dintenfass[6], in systems where compounds with identical polar groups and of different chain length are present, there is no preferential adsorption based on chain length; thus long chain compounds can be displaced by short chain length compounds with identical polar groups and vice versa. In each system, the ratio of amounts adsorbed on the surface is nearly the same as the ratio of the molecular concentration of the two species in the solution.

The adsorption of linseed oil fatty acids at an initial pH of 5·0-5·2 on magnetite during froth flotation was investigated by Kajanne[7]. It was found that more unsaturated fatty acids were preferentially adsorbed at low collector concentration. An increase in the amount of collector is accompanied by a decrease in the iodine value of the adsorbate. At low collector concentration, the amount adsorbed increases as the amount of collector is increased per unit weight of magnetite. A saturation point is reached and calculations of the specific surface show that the adsorbed layer is multimolar. A rod structure for the collector layer is proposed to accommodate the large number of fatty acid moles, while the hydrophobic nature of the layer is retained during its formation.

Ultrasonic waves were found to increase the adsorption of methylene blue[8]. The adsorption also increased with the power and frequency of the ultrasonic waves. The increase in adsorption was accounted for by the increase in the degree of fineness and change of electrochemical properties of the pigment. Studies on the adsorption of sodium oleate on pigments like TiO_2 and ZnO have revealed that in low concentrations of sodium oleate there is the usual increase in adsorption with concentration[9]. Near the critical concentration of micelle formation, especially at higher concentrations of sodium oleate, several points of abnormal behaviour in the adsorption of sodium oleate were observed. The comparison of the adsorption isotherms with those obtained by other workers showed that the adsorption of surface active agents on pigments depends only on the properties of the surface active agents in the solution and specific properties of the pigments have no effect.

Miller and Anderson[10] made apparent adsorption studies of oleic acid from coefficient of friction measurements. The coefficient of friction of solutions of oleic acid in paraffin oil (0-25 per cent by weight) were measured on a friction pendulum over the temperature range 60-140°C. The friction concentration isotherms were amenable to treatment by the kinetics of monolayer adsorption. As the temperature was increased from 60°C. the friction decreased to a minimum at 120°C. and then increased again. The decrease in friction with temperature was ascribed to the formation of a

close-packed soap monolayer that became complete at 120°C. The subsequent increase in friction was interpreted as being due to the destruction of the monolayer owing to the increased thermal agitation. Residue method for the study of adsorption of lauric acid by powdered TiO_2 was adopted by Petit *et al.*[11]. TiO_2 was used as a solid adsorbent for lauric acid in a mixture of saturated aliphatic hydrocarbons. At concentrations up to 5 per cent of lauric acid, the results agreed well with the classical method of adsorption determination, when lauric acid was replaced by alkyd resin modified by linseed oil fatty acids the observations were not as well defined as in the case of pure substances.

Surface Active Agents and Surface Chemistry of Pigments

Studies on the effect of surface active material on the oil adsorption of pigments have shown that the presence of oleic acid increases oil adsorption on zinc white and on Krivoi Roy red iron oxide pigments[12]. This effect has been attributed to the lessening in the energy of wetting of pigments and increase of the flocculation energy by the adsorption of oleic acid on the pigment particles, which in the presence of oil results in the formation of compact structures of pigment particles with the oil immobilizing the dispersed phase and lowering the fluidity of the system. The theory is supported by the results of measurement of the adsorption of oleic acid, the determination of sedimentation rates of dispersed phase in water and in mineral oil, measurement of adsorption when zinc oleate was added, measurement of the final pigment volume and calculation of the thickness of the oil film on the surface of zinc white. Butyric acid and saturated alcohols do not materially change the oil adsorption by the pigment.

Koelmans and Overbeek[13] divided surface active materials into two classes, non-ionic and ionic, according to the conductivity of their benzene solutions; fatty acids and alcohols being non-ionic and soaps, such as sodium dioctylsulphosuccinate, ionic. Ionic surface active compounds were found to help in the dispersion of pigments, whereas small concentrations of non-ionic materials failed in deflocculating the suspensions of one micron sized powders, including silica, titanium dioxide and calcium carbonate in xylene. A relationship was found between the adsorption of the solute, zeta-potential and deflocculation of the suspensions. They concluded that the observed electrokinetic potentials are theoretically sufficient to account for the deflocculation of suspensions of particles above one micron in diameter, but would be insufficient for smaller particles.

Fischer[14] investigated the pigment suspensions in solutions of high molecule binders used in paints, and found that stable suspensions are formed when solvents of low dielectric constant are used, the stability depending mostly on the electrical interfacial potential developed on pigment surfaces by the adsorption of molecules. He also observed a typical dependence of stability of the dispersion on the polymer concentration independent of the electrical interfacial potential.

Derjaguin and Karassev[15] have shown, by measuring the viscosity of liquids very near a solid boundary, the existence of layers up to 0·1 μ thick, which differ in viscosity from the remaining liquid and in some cases in structure also. Livine and Zisman[16] have described the properties of monolayers formed on solid surfaces from solutions. They correlated the contact angles formed between the frictional properties of monolayers and the chemical constitution of the adsorbates. Straight and branched chain acids and amines were amongst the compounds studied. The properties of monolayers of organic compounds with over 14 carbon atoms in a straight chain were independent of the nature of the adsorbent surface and the chain length, and depended essentially upon the chemical structure of the outermost radicals of the monolayers. Surface energy increased in the following order:

$$CH_3 > CH_2 > \text{ring} > \text{polar}$$

Viscometric studies on calcium carbonate suspensions in polybutene have produced evidence for the existence of immobilized layers up to 100 A. thick[17]. Viscometric evidence for the behaviour of irregular particles as spheres of large effective volume does not prove that the increased volume is caused by the adsorbed layer, since the effect might be due to mechanical environment. Zettlemoyer and Lower[17], however, demonstrated a decrease in the effective volume on fatty acid addition which is strong evidence that the effect was due to adsorption.

From their studies on the adsorption of surface active agents from non-aqueous solutions on carbon black, Abram and Parfitt[18] have pointed out that the adsorption of aerosol OT and octadecanol from benzene solution on carbon black follows Langmuir equation. The extent of adsorption of surface active agents increases with the amount of oxygen associated with carbon black. Aerosol OT has proved to be a very effective stabilizing agent, but with octadecanol very rapid flocculation took place. In similar work on the adsorption of surface active agents by carbon black in non-aqueous media, it was found that most carbon blacks show type I adsorption isotherms[19], but the adsorption isotherm on Neospectra carbon black exhibited a maximum after which the amount of adsorption decreased with the concentration.

Adsorption of Driers

Finne *et al.*[20] concluded from their experimental data that adsorption of drier by pigments is the combined effect of a natural precipitation of the drier from solution owing to the incompatibility of the drier salt with the solvent and the oxidizing effect of the pigment to form oxidized polymers which are insoluble and act as antioxidants. Some pigments such as ZnO, white lead and whiting have a reducing effect on the driers, while inert pigments have an oxidizing effect with a corresponding loss in drying during storage. A similar study on the adsorption of metallic driers was made at the Montreal Paint & Varnish Production Club[21].

The effect of physical properties of the pigment on the adsorption of drier has been stressed by Bryson[22]. According to him the shape of the pores and the nature of surface are more important than the porosity of the pigment. Generally, the pigments possessing a large specific surface inhibit drying. A series of containers each containing the same amount of the paint were taken, and increasing aliquots of drier were added to each and set aside for drying time tests at bimonthly intervals. A particular concentration of the drier, at which no increase in drying time is observed for 3 or 4 successive periods, corresponds to the correct amount to be added to the paint.

Wetting of Pigments

When a pigment comes in contact with a vehicle, the physical properties of such a system can be related to the extent and character of the interface between solid and liquid. The pigment-air interface is replaced by a new pigment-vehicle interface and the wetting of pigments follows. Wetting may be defined as the process by which a liquid comes in contact with a solid to form a solid-liquid interface. The nature of the process which takes place at the pigment-vehicle interface controls greatly the grinding and many other properties of the paints such as consistency, flow, levelling and gloss.

The wettability of a pigment by the vehicle has generally been considered as the most important characteristic associated with the stability of the paint suspensions and this was first studied by Harkins and Dahlstrom[23]. The total free energy of immersion was taken as the measure of wettability. It was found that if dry clean pigment is immersed in a liquid, heat is liberated, the quantity of which increases with increase of polarity of the liquid, or with increase of polarity of certain groups in the liquid molecules.

Pigments are often difficult to wet with oil, because of the envelope of air or gas around the particles that has been adsorbed by the pigment surfaces. Linseed oils of high acid value displace absorbed gases more readily than those of low acid value. If the pigments are first heated to remove moisture, they are wetted more easily. Carbon black pigments are very difficult to wet with water, but if they are first heated or so treated as to remove the adsorbed film of hydrocarbons and air, they are wetted more easily. The moisture adsorbed on the pigment may indeed be the main variable influencing the dispersion characteristic of commercial pigments. Pigment stocks are rarely given an oven drying treatment immediately before use. Consequently, the pigment has generally an adsorbed film of moisture. The quantity of water adsorbed is roughly equivalent to that necessary to form a monomolecular film on the solid surface.

Eide and Depew[24] reduced the reactivity of zinc oxide in various vehicles by adding phosphorus pentoxide or other dehydrating oxides to the vehicle before dispersing the pigment. The plastic viscosity of the finished dispersion is found to be reduced by this treatment and the paint is prevented from thickening. Presumably, the reaction between zinc oxide and fatty acids of the vehicle is considerably inhibited by dehydration of pigment before dispersion. The use of hygroscopic organic liquids with pigments in formulating lacquers has been postulated by Taylor and Chapman[25], as a means of preventing flocculation of pigments.

For wetting to occur, oil must be adsorbed on the surface of the pigment particles. More vehicle is required to wet a unit weight of fine particles than is required for coarse particles, because the amount of oil required for pigment saturation or wetting is directly proportional to the specific surface area of the pigment mass. The oil absorption factor being relative to the surface condition of the pigment is possibly to some extent independent of its chemical composition or specific gravity. As the surface of a pigment is being wetted with oil, the pigment mass remains relatively dry and crumbly, and will not smear when brought into contact with a piece of glass. At the point, however, where all pigment particles become wet, the mass immediately forms a soft paste, which will then smear when brought into contact with glass. The amount of vehicle required to wet a unit mass of the pigment is known as its ' oil absorption '.

The consistency of paints is affected to some extent by the oil absorption of the pigment. A high oil absorbing pigment gives greater consistency than a lower oil absorbing pigment, because it adsorbs more vehicle on the pigment surface, so that there is less free vehicle in the paint. Stieg[26] has suggested that the oil absorption of any white pigment can be a factor in determining the spacing relationship and hence the hiding power of the paint.

Freundlich[27] utilized the contact angle relationship to derive an expression for adhesion tension which is often taken as an indication of the degree of wetting

$$\gamma_{SA}-\gamma_{SL}=A_{SL}=\gamma_{LA}\cos\theta$$

where γ_{SA} is the surface tension of the solid; γ_{SL}, the interfacial tension of the solid and liquid; and γ_{LA}, the surface tension of the liquid. The term $\gamma_{SA}-\gamma_{SL}$ is defined as the adhesion tension; A_{SL} has the same dimension as surface tension. Since there are three phases present, solid, liquid and gas, it becomes apparent that three separate interfacial tensions play a part in determining the contact angle. The liquid-gas interfacial tension has always a positive value, and when the solid-gas interfacial tension is greater than the solid-liquid interfacial tension, the value of cos θ is positive which means that the contact angle is less than 90°. This is the result when a liquid wets the solid. On the other hand, when the solid-liquid interfacial tension is greater than the solid-gas interfacial tension the value of cos θ is negative and the contact angle lies between 90° and 180°. This is the result when the liquid does not wet the solid. According to Bancroft[28], a liquid is in actual contact with a solid only if it is adsorbed on the surface of the solid.

The normal types of solvent-based media, such as alkyd resins, are far from being pure liquids, and they contain a wide range of molecular species. Apart from the differences in the size of the

molecules, there is a range of polarity present in them. In an alkyd, the free groups are mainly carboxyl and hydroxyl, and the distribution of such groups is not uniform among the molecules of different degree of complexity, in fact, one would expect the smaller molecules to be more polar by virtue of having proportionately more end groups.

Calorimetric measurements of wettability of pigments in various organic liquids[29] confirm the belief that polar-nonpolar molecules in the solvent form a layer on the surface of the pigment with the polar group towards the pigment and the nonpolar group towards the liquid. The polarity of the liquid in which the pigment is suspended determines its degree of dispersion.

Dispersion of Pigments

First, the pigment must be wetted by the liquid used for dispersion. Secondly, mechanical work must be performed on the system to effect dispersion; in many cases the aggregates of the pigment are hard and require a large amount of energy to break them up. Thirdly, the resulting dispersion must be stabilized, i.e. the composition of the liquid phase must have been adjusted to provide conditions where there is no tendency for the pigment to flocculate to an undesirable extent. Dintenfass[4] considers that pigment dispersion occurs when a monolayer of sufficient thickness is adsorbed on the pigment to keep the particles at such a distance that particle-particle attraction is effectively negligible.

Every surface or interface has an energy associated with it. This can be expressed in ergs/cm.^{2}; in the case of liquid, this may be approximated to the surface tension in dynes/cm. With solids, however, the surface tension and the surface free energy are not necessarily equal as in creating a fresh surface of the solid, the rearrangement of atoms to an equilibrium configuration is hindered by the immobility of the surface region. Figures quoted for the surface energy of solids vary widely, depending on the method of measurement used, but a number of inorganic surfaces, such as Al_2O_3, $BaSO_4$, MgO, CaO, etc., appear to have surface energies of the order of 1000 ergs/cm.^{2}.

The surface energy of a powder will depend very largely on the extent of the surface, and, when the size of the individual crystals becomes very small, the edge energy factor, i.e. the energy associated with the junction of two planes of the crystal lattice, becomes important.

The cohesive strength of solids can be influenced by adsorbed layers of surface active agent. The part played by surface active molecules in milling is thus quite complex. Almost all paint media as such contain molecules which are themselves surface active, i.e. they contain polar groups which can interact with the solid surface in the same way as the specific surface active agents. Certain solvents like chloroform, butanol, nitromethane and xylene are effective in producing cracks, whereas aliphatic hydrocarbon such as heptane and petroleum ether are ineffective. It was then noticed that the liquids which caused cracks to develop were all liquids with a fairly high cohesive energy density (CED), while those without any effect had low CED values. The CED function is expressed as $(\Delta H - RT)/V$, i.e. the latent heat of vaporization of the liquid at the temperature concerned minus the work done in expanding the vapour against surrounding pressure expressed in terms of unit volume of liquid.

The amount of resin adsorbed from relatively dilute solutions increases with decreasing solvent power and with the oil length of the resin. Rothstein[30] made measurements from dilute resin solutions which allowed gravimetric study of adsorption, as well as separate study of bulk viscosity and adsorption on pigment dispersion. The value of the saturation adsorption was found to increase almost linearly with molecular weight of the resin. The same viscosity measurements that determined adsorbed resin thickness showed the effect of pigment surface coverage on the ease of dispersion of the suspension. There are three basic interactions which govern the physical characteristics of a suspension made by dispersing a pigment into a resin solution. First is the resin chain segment solvent interaction which determines the configuration of the resin chain in solution. The second interaction is the adsorption of the resin on the pigment surface, and the chain segment-pigment surface interaction which can determine the configuration of the chain on the pigment surface. These two interactions together determine the extent of the third interaction after initial dispersion and adsorption equilibrium has been reached, viz. the particle-particle interaction characterized by the presence or absence of flocculation.

Stabilization of Dispersion

To stabilize dispersions, it is necessary to prevent the solid particles from approaching one another too closely. There are various ways in which this can be brought about. In aqueous dispersions especially, electrical charges on the particles can cause a force of repulsion; this can also occur in nonpolar liquids to some extent. When a solid is present in a liquid, a potential difference exists across the boundary, the electrostatic potential. This potential exists whenever a solid surface is immersed in a liquid, if the liquid is ionizing, adsorption of ions occurs to form what is described as an electrical double layer. The principle is that with the charge on the surface of the solid particles there is associated a diffuse swarm of ions of opposite charge surrounding the particles. These ions entrap solvent molecules so that the effective sphere of action of the particles is greatly increased.

To investigate the state of pigments in oil-based paints when suspended in hydrocarbon medium, Khomikovskii and Revinder[31] prepared submadder varnish, pink varnish, yellow varnish and Prussian blue suspensions. Pigment dispersion in the suspension was measured with a vacuum sedimentater. The quantity of oleic acid adsorbed by the pigments and the change in the physical state of the pigments due to the adsorption of oleic acid were determined. All the pigments were well stabilized with oleic acid in hydrocarbon medium; the stabilization limit was reached when no more oleic acid could be adsorbed by the pigments. A simple relation was found to exist between the stabilization and wetting

of the pigment particles on the one hand and the quantity of stabilizer adsorbed on the other.

Viscosity of Pigment Dispersions

The viscosity of dispersion is affected by the quantity of pigment present and by the tendency of the particles to aggregate and develop structures which immobilize the vehicle. In addition, the pigment is thought to adsorb a layer of vehicle immediately around the particles and this in effect increases the pigment volume and reduces the amount of free vehicle. The viscosity effect contributed by the vehicle is influenced by the tendency of the molecule to coil and to form aggregates[32].

The relationship log $\eta_r = F/(1-F)$ between the sedimentation volume, F, and relative viscosity, η_r, holds for totally dispersed industrial pigment systems exhibiting Newtonian flow. F is a function of the shape of the pigment particles, vehicle viscosity and of the pigment phase (sum of the volumes of pigment and the adsorbed immobilized vehicle); and this relation is also true for thixotropic suspensions[33]. In concentrated pigment dispersions, the sedimentation volume F increases with increase in viscosity of both the dispersed pigment phase and the liquid phase. The F value of the flocculated pigment suspensions is not affected by temperature[34].

Thixotropy in Paints

Adsorption, flocculation and thixotropy in non-aqueous paint systems have been studied by Dintenfass[35]. The forces exhibited when a pigment is immersed in a vehicle result in the phenomena of adsorption, flocculation and thixotropy. When the adhesion forces between the pigment and the vehicle are less than the attraction between pigment particles flocculation results. When the adhesive force is greater, the pigment is dispersed. Thixotropy is a reversible state resulting when the flocculating pigments form a network, which immobilizes the vehicle. The degree of thixotropy depends upon the pigment concentration, the degree of flocculation and the viscosity of vehicle. Factors affecting the rate of dispersion and thixotropic build up have been discussed by Dintenfass[35].

A thixotropic paint is one which possesses a high consistency in the can, thus holding the pigments evenly dispersed. This high consistency, however, decreases under the action of mechanical treatment with the brush and after the application of paint, the consistency increases again. These anomalous changes in the consistency with variation in applied shearing stresses are termed together as the phenomenon of thixotropy. Since the consistency depends upon pigmentation of the vehicle, the optimum consistency on the pigment content of the 'thixotropic' corresponds nearly to the critical pigment volume concentration.

Levelling characteristics of paint are determined by proper formulations (pigmentation to the proper degree being an important parameter), so that the thixotropic rate of plasticity regain after application of the paint is fast enough to overcome the sagging tendency, but slow enough to allow the elimination of brush marks before the paint sets. The following relationship determines the levelling of the paint after application: $h = (d^2 f/8\Upsilon)$ where h and d are the depth and the breadth of the brush mark; f, the yield value; and Υ, the surface tension of the system. For better levelling, paints should have an f value 2-8 dynes/sq. cm.

Adhesion

The phenomenon of adhesion is due to forces of various types[36]. The forces that hold a paint film on a substrate include long-range body forces, such as gravitational, magnetic and electrostatic forces and intermolecular forces, loosely grouped under the title of van der Waals' forces and chemical bonds. The adhesion phenomenon must be differentiated according to the nature of contact, i.e. whether it occurs at points or over certain areas[37]. In the case of paint coatings, adhesion over an area depends on the structure of the metal surface and also on the presence of dirt and greasy material on the surface, which prevents the paint from coming into contact with and wetting the metal surface. The fact that forces between substrate and film are exerted through layers, only a few molecules thick, explains why it is essential to have a clean surface when applying paints to metals. If the oil material on the surface is fairly fluid and easily soluble in the solvent of the paint, all or most of this may be removed from the surface before the solvent evaporates, and adhesion may then be little affected[38].

Atoms on the surface of the solid have unsaturated valence electrons which are available to make bonds with reactive ingredients in the atmosphere, such as water vapour and oxygen. Robert[39] has listed the attractive forces that might exist between atoms as (1) chemical bonds including covalent bonds, (2) ionic bonds, (3) polymerization forces between pairs of dipoles or between an ion and dipole, and (4) dispersion forces between inert atoms. Polymerization and dispersing forces are frequently lumped together as van der Waals' forces, since these are the only forces which are effective between molecules of gases and liquids at room temperature. Bullet[40] suggested that the hydrogen bonds with energies of the order of 5-10 kcal./mole are responsible for the strong adsorption of water molecules on to the metal oxides but are unlikely to be the major element in adhesion.

According to Dintenfass[35], the adhesion of paint films depends on the tensile strength of the film and the total attractive forces between the coating and the substrate. Adhesion is affected by the number and type of the polar group available for adsorption. It may involve polar attraction, chemisorption involving electron exchange, and precipitation when the metal ions on the surface form soaps or chelated compounds with the vehicle. The cohesion component of adhesion depends upon the structure of the vehicle polymer.

According to Chatfield[38], as most surfaces are associated with negative charge, greater adhesion will be obtained by the use of an additive which in the presence of moisture tends to confer a positive charge by altering the zeta-potential. Such additives are cationic surfactants in their various forms,

which are sometimes so strongly adsorbed on certain surfaces that more than a simple tendency to neutralize the charge is required to account for it, and actual ion exchange at the interface is assumed. Another instance in which additives are desirable is in the application of paint to rusty and corroded metal surfaces. The long chain amine salts, particularly the acetates and oleates of primary amines, are used for this purpose, at about 0·5 per cent concentration on the paint weight. Provided the loosely adhering rust particles are removed by brushing before the application of the paint, a paint with such an additive can be applied direct to the rusty surface when the remaining rust particles will become incorporated into the paint in the same manner as the pigment itself.

Some metallic structures are protected by paint coatings and simultaneously by cathodic protection. Anderton[41] observed loss of adhesion of paint coatings on cathodically protected laboratory panels near standard coating breaks when the panels were immersed in sea water. He found that the loss of adhesion occurs initially near the coating breaks and increases at a uniform rate depending on the applied potential. The electrolyte solutions containing the alkali cations, potassium and sodium have been found to cause more loss of adhesion to coating on cathodically protected structures. Anderton suggested the mechanism that the diffusion of water into the film of wash primer at the coating break dissolves the soluble components of the coating. Under the influence of the electrical potential there will be an inflow of cations with large proportions of sodium ions, since sodium is the predominant cation in sea water. The presence of chromate ions and acid in aqueous solution near the coating break may produce soluble sodium or potassium compounds with the binder which may result in leaching out of the film at the break. The excess of hydroxyl ions at the cathode will develop an area of high alkalinity in the wash primer near the coating break which attacks the bond of the primer to the steel. Cations diffusing into the wash primer film at the break will carry water with them as hydrated ions and lead to loss of adhesion of the coating.

Summary

The various phenomena related to the adsorption of paint vehicles at solid-liquid interfaces which causes the dispersion of pigments from the standpoint of formulation of paints are reviewed. Factors such as the heterogeneity of surfaces, nature of paint vehicles, fineness of pigments, presence of surface active agents, driers, etc., which are closely associated with the phenomenon of adsorption and other factors which control the consistency, viscosity, flow, levelling and gloss of paints are also discussed. The wetting of pigments which mostly controls the grinding and stability of paints and factors such as the adhesion tension, the degree of wetting in terms of interfacial tension and the contact angle which determine the degree of dispersion and adhesion of the coating to the substrate have also been reviewed.

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Neural Control of Hormone Secretion*

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SOME neural mechanisms of hormonal control are easily definable in Sherringtonian terms. The pathways, for example, which serve the discharge of catecholamines from the adrenal medulla are divisible into the classical triad receptor, conductor and effector, in close analogy to the reflex arcs for exocrine glands. Stimuli of various kinds pursue 'private' pathways to reach internuncial neurones which, in turn, converge on another set of fibres providing the final common path. This ultimate stretch may differ from the intermediate common pathway only in that 'it exhibits communism in a still higher degree' (Sherrington[1]), but it may also contain neurones which elaborate specialized 'neurosecretory' products. Reflex arcs which contain such neurones (which, in vertebrates, are mainly situated in the hypothalamus) have in recent years proved to be the main apparatus which integrates neuro-endocrine relationships.

Afferent Pathways

Much is known about sensory stimuli which activate neuro-endocrine reflexes. In fact, it would seem that there is no type of external stimulus which, for example, may not give rise to the release of one or the other of the hypophysial hormones. Enteroceptive, blood-borne releasing stimuli are also known and they may be divided into two types: (i) hormonal feedback mechanisms by which messengers released from one of the 'peripheral' endocrine glands — say the gonads or the adrenal cortex — stimulate or depress some part of the central nervous system (CNS) functionally linked to the pituitary, and (ii) changes in blood constituents other than endocrines as, for example, a rise in plasma osmotic pressure which leads to the release of antidiuretic hormone from the neurohypophysis.

However, while the variety and efficacy of stimuli have been widely investigated, relatively little is known about that part of the reflex arc which connects the receptors with the fibres of the ultimate final path, that is to say, with the neurosecretory fibres which — by virtue of their secretory as well as their neural function — must be regarded as part of the efferent system.

The complexity of the problems involved is perhaps best illustrated by discussing two neuro-endocrine reflex systems which have been intensively explored. It is well known that suckling and vaginal stimulation lead to the release of oxytocin which contracts the mammary myoepithelium, thus causing the ejection of milk. Table 1 shows that numerous parts of the CNS may be activated by these stimuli. Table 2 shows the variety of structures of the CNS whose electrical stimulation causes release of oxytocin, and increase in uterine activity or the ejection of milk. Nauta[2] and Denamur[3] have recently outlined the neural pathways which seem to be involved in the milk-ejection and other neuro-endocrine reflexes. According to these workers, the afferent pathways to the hypothalamus pass essentially through the brain-stem reticular formation and the non-specific thalamic nuclei which act as a first order analysing-integrating system. Two other similar systems, in parallel

TABLE 1 — SITES OF ELECTRIC ACTIVITY IN CENTRAL NERVOUS SYSTEM EVOKED BY MAMMARY OR VAGINAL STIMULATION

Species	Structure	Ref.
Rabbit	Hypothalamus Thalamus Limbic system Septum Hippocampus Neocortex	57
Rat	Lateral hypothalamus Perifornical areas Thalamus Lateral preoptic areas Hypothalamus Medial forebrain bundle	12, 58, 59
Cat	Supraoptic nuclei Lateral preoptic areas Medial forebrain bundle Cingulate gyrus	60, 61

TABLE 2 — RELEASE OF OXYTOCIN RESULTING FROM ELECTRICAL STIMULATION OF VARIOUS PARTS OF THE CENTRAL NERVOUS SYSTEM

Species	Structure	Ref.
Goat	Medial lemniscus Mesencephalic reticular formations Anterior hypothalamus	62, 63
Rabbit	Mesencephalic cerebral grey matter Midbrain reticular formations Mamillary and supramamillary region Ventromedial hypothalamic nuclei Perifornical areas Limbic system including cingulate gyrus Amygdaloid nucleus	64-68
Cat	Mamillary and supramamillary region Ventromedial hypothalamic nuclei Medial amygdaloid nuclei Cingulate gyrus	61, 69, 70

*Dedicated to the memory of Heinrich Waelsch, late Professor of Biochemistry, Columbia University, New York, and Chairman of the Department of Pharmacology, New York State Psychiatric Institute.

and in series, seem also to be concerned. First, the limbic system-midbrain circuit of Nauta[2] to which the supraoptic and paraventricular nuclei are directly connected (there are also indirect connections by way of the lateral hypothalamus) and, secondly, the thalamo-cortical apparatus which contains the sensory pathways.

The other neuro-endocrine reflex I should like to discuss is that concerned with the liberation of vasopressin by osmotic stimuli. It differs from the milk-ejection reflex in not being initiated by sensory stimuli but by a blood-borne one. Since, in some species at least, it could be shown that an increase in plasma osmotic pressure enhances vasopressin release and a decrease inhibits liberation we are dealing with a feedback type of neuro-endocrine regulation, not very different from others in which the concentration of a hormone from a peripheral endocrine gland influences neurones which regulate the release of trophic hormones from the adenohypophysis. Verney[4] has demonstrated that in the water-loaded dog, small rises of osmotic pressure (of the order of 2 per cent) in the carotid blood inhibit water diuresis. He showed also that osmotic stimuli fail to act after removal of the posterior pituitary lobe. Further observations on the effects of increases in the osmotic pressure before and after ligation of the ipsilateral internal carotid led to the conclusion that the osmoregulators lie in the vascular bed normally supplied by that vessel. By evidence from blood distribution or degenerative cell loss, Jewell and Verney[5] restricted the site of osmoreception to a discrete region of the diencephalon. Their work has now been supplemented by experiments[6-8] in which the effect of hypertonic solutions on the electrical activity of neurones in the anterior hypothalamus were studied. It was found that the activity of the supraoptic neurones was always enhanced and that of the paraventricular cells inhibited. These results combined with the evidence for the origin of vasopressin-secreting neurones in the supraoptic nucleus, and the fact that hormone secretion remains normal after experimental isolation of the diencephalon[9], suggest strongly that the main site of the osmoreceptors is in — or very near to — the supraoptic nucleus. A contribution of other osmosensitive regions to the regulation of neurohypophysial activity can, however, not be excluded.

There is a difficulty. The effect of intracarotid hypertonic salt solutions on vasopressin release has been demonstrated in the dog and almost certainly also in the goat, the rabbit and in man, but so far it has not been possible to show this mechanism unequivocally in the rat. It is difficult to believe that osmotic stimuli in this species do not operate in their usual manner; the assumption that in the rat the main osmoreceptors are not within the reach of carotid blood seems preferable.

The Efferent Arc

Whether relatively short or highly complex, the efferent portion of central neuro-endocrine pathways seems to contain neurosecretory neurones as its essential constituent. The question may, therefore, be asked whether and in what manner this type of neurone differs from ordinary nerve fibres.

Electrophysiological Characteristics of Neurosecretory Fibres

Neurosecretory cells show Nissl substance, bipolarity, neurofibrils and dendrites like ordinary nerve cells, but their electric activity is relatively little explored. Where single-unit recordings are likely to have succeeded (leech neurohaemal areas[10], goldfish preoptic neurones[11], rat hypothalamic neurones[12], and others), action potentials of the usual kind have been observed. The action potentials of neurosecretory neurones of fishes and of the leech were of significantly longer duration than those recorded from nearby 'ordinary' neurones, but those from the mammalian hypothalamus cannot be so distinguished. This statement must at present be made with the reservation that neurosecretory neurones may not have been encountered in the latter recordings.

The postulate that neurosecretory fibres are functioning as both secretory and conductory unit is, however, difficult to avoid in the light of recent theories on the mechanism of release of their hormonal products. Depolarization of the nerve endings in the isolated posterior lobe by a high concentration of K^+ in the presence of Ca^{2+} has been shown to cause release of vasopressin and oxytocin[13,14]. Suspending the isolated hormone-carrying granules in solution at the same ion concentration did not lead to hormone release[14]. Acetylcholine releases hormones neither from isolated granules nor from pituitary lobes *in vitro* but it does so from the whole isolated hypothalamo-neurohypophysial complex[15]. The best fitting interpretation of these findings at present would seem to be that acetylcholine, liberated by the endings of the cholinergic fibres in synapse with the neurosecretory fibres, elicits an electric impulse in them which leads to changes in ion flux and in the permeability of the axonal boundary and — in turn — to hormone release.

Morphological Criteria for Neurosecretory Neurones

Diagnosis of neurosecretory function by light-microscopy, based upon the occurrence of apparent secretory inclusions in nerve fibres has proved to be unreliable. The classical staining method of Gomori-Bargmann and even the newer methods of staining with alcian blue or pseudocyanin are inadequate by themselves. For example, the neurones reaching the medium eminence which serve as the source of the 'releaser' substances regulating the output of adenohypophysial hormones have yet to be defined by light-microscopical methods[16,17].

Ultrastructural studies show that the perikarya and processes of neurone aggregations suspected of neurosecretory function contain electron-dense granules between 1000 and 3000 A. in diameter. Granules of these dimensions from the nerve endings in the posterior pituitary lobe have been isolated by differential centrifugation and the presence in them of vasopressin and oxytocin has been established[18-20]. However, similar evidence for other specialized neurosecretory systems has, so far, not been provided. It seems likely that such granules are characteristic of neurosecretory neurones, but in invertebrates at least similar organelles are also encountered in fibres which are not known to be secretory. Moreover,

catecholamines in peripheral nerves and acetylcholine in brain and nerves have also been shown to be stored in subcellular particles. The noradrenaline-containing granules isolated from beef splenic nerve, for example, have been described by von Euler[21] as being 300-2000 A. in diameter. Some of them contain electron-dense material which also appears in units of about 200 A. Similar small submicroscopic elements (diam. about 500 A.), called synaptic vesicles, have been seen in cholinergic nerve endings and have also been isolated[22].

Vesicles, morphologically quite indistinguishable from these 'synaptic vesicles', occur abundantly in the hypothalamo-neurohypophysial complex. They are characteristic for the median eminence of higher vertebrates[23] but occur also in the posterior lobe either in the same fibre swelling as the large, hormone-carrying granules or, less frequently, on their own[24]. The significance of these small vesicles is at present not established. Some workers[25,26] have suggested that they contain transmitter substances facilitating neuronal hormone release, others[27,28] think that they may represent breakdown products of the hormone-carrying granules.

Topographical Characteristics of Neurosecretory Systems

It used to be thought that neurosecretory systems could be characterized by their proximity to blood vessels and there can be no doubt that many — or probably most — show this intimate relationship. Such 'neurohaemal organs'[29] are the neurohypophysis, the urophysis of fishes, the corpora cardiaca of insects, the crustacean sinus gland and others. In the neurohypophysis the nerve endings may lie against the pericapillary basement membrane directly (as, for example, in the teleost fish *Lebistes*[30]) or abut on the perivascular (collagen) space.

However, evidence has recently been accumulating that neurosecretory products may reach their target cells not exclusively — or not primarily — through the blood. Neuronal processes of neurosecretory cells have been described in amphibians[31,32], mammals[33] and birds[34] which extend towards and into the lining of the third ventricle, suggesting that their products may be released into the cerebrospinal fluid. Löfgren[33] has postulated that material secreted in the nuclear complexes of the tuber cinereum is carried into the infundibular cavity and from there to the primary portal plexus and the anterior pituitary. Tinctorially (Gomori-negative, paraldehyde-fuchsin-positive) the neurosecretory material so considered does apparently not behave quite like that of neurohypophysial neurosecretion and may be suspected to represent or contain 'releaser' substances for the adenohypophysial 'trophic' hormones. However, the release of neurohypophysial hormones into the cerebrospinal fluid remains also a distinct possibility.

A neurosecretory pathway which does not involve transmission through blood has quite recently been well illustrated by Knowles[35]. Fibres which are neurosecretory by all accepted criteria — they are stainable with Gomori's technique and contain vesicles of about 1800 A. in diameter — have long been known[36-38] to originate in the preoptic nucleus of certain elasmobranch fishes and to enter the neurointermediate lobe. Knowles[35] has now shown that in the dogfish (*Scyliorhinus stellaris*) these fibres terminate on the secretory cells. Many such terminals were found to make intimate contact with the region of endoplasmic reticulum of the intrinsic secretory cells in a manner which suggests that a neurosecretory substance affects these cells directly.

There are several other arrangements, particularly in invertebrates, by which the products of neurosecretory cells reach their target tissue, but enough has been said to show that the older concept of defining neurosecretory neurones by their relationship to blood vessels can no longer be sustained.

Chemical Characteristics of Neurosecretory Products

The suggestion has been made[35] that there are two fundamentally different forms of neurosecretion, one concerned with the production of peptides and another with the secretion of non-peptide substances, and that the two types have ultrastructural correlates. Present information about the chemical composition of neurosecretory material, though scanty, is in favour of this concept. Only the products of one neurosecretory system can so far be said to have been intensively explored, namely those of the vertebrate hypothalamo-neurohypophysial complex. The biologically active products of these neurones seem to be a family of closely related octapeptides: The seven hormones whose chemical constitution has been ascertained differ from each other only by substitution of one or two amino acids in positions 3, 4 or 8. Their distribution ranges from the primitive jawless fishes, the cyclostomes, to the mammals.

Another hypothalamic neurosecretory system produces the so-called 'releasing factors' (RF), i.e. substances which mediate the liberation of the 'trophic' hormones from the adenohypophysis. Experimental evidence is extant for the existence of a corticotrophin-releasing factor (CRF), a thyrotrophin-releasing factor (TRF), a luteinizing hormone-releasing factor (LH-RF), a follicle stimulating hormone-releasing factor (FSH-RF)[39] and a growth hormone-releasing factor (GH-RF)[40,41]. It seems that TRF[42], LH-RF and FSH-RF[39] are small polypeptides with molecular weights slightly greater than those of the vasopressin or oxytocin. CRF presents a special problem since, according to Guillemin and Schally[43], there are two such substances: α-CRF which is chemically closely related to α-MSM (13 amino acids) and β-CRF, related to lysine vasopressin but with a somewhat larger molecule. Since the activity of LH-RF is not abolished by thioglycollate[44], and TRF and α-CRF, according to their published structure, could not be inactivated either, one would be tempted to assume that the releasing factors belong to a family of peptides different from the neurohypophysial hormones which have a disulphide bridge and are hence all inactivated by thioglycollate. β-CRF may be an exception: Guillemin and Schally[43] have reported its inactivation by thioglycollate, but this finding was not confirmed by Ramirez and McCann[44]. A further hypothalamic factor, the prolactin-inhibiting factor (PIF),

acting directly on the anterior pituitary where it inhibits prolactin secretion, has been described by Talwalker *et al.*[45]. Its chemical composition is unknown.

Relatively little is known about the chemical composition of the hormones produced by invertebrate neurosecretory complexes. Only two groups of higher invertebrates have been studied.

Insects — Groups of neurosecretory cells in the insect brain discharge their products into the corpora cardiaca and corpora allata. These neurosecretory cells differ from ordinary neurones by the presence of, usually, electron-dense granules. Moreover, the neurosecretory material stains with Gomori's technique and with aldehyde-fuchsin. The neurosecretory cells seem to be akin to the intrinsic glandular cells of the corpora cardiaca, organs which, in addition to producing hormonal substances of their own, serve also as centres for the storage and release of the neurosecretory material of cerebral origin[46]. A similar arrangement probably obtains for the corpora allata.

The hormone of the brain cells has been claimed by Kobayashi[47] to be a lipid, and even cholesterol. However, other Japanese workers[48] have obtained active peptides from insect brain extracts, and Gersch *et al.*[49] have reported on four active substances in the brain of the cockroach (*Periplaneta americana*), two of which seem also to be polypeptides. Which of the many active substances found in extracts of corpora cardiaca are neurosecretory products, stemming from the axons of the brain cells, has not been established. Three, at least, of the active constituents of the corpus cardiaca of the cockroach have been clearly defined as peptides[50], but a number of pharmacologically active 'small molecules' have also been found in extracts of this organ[51–53].

Crustaceans — Most of the known endocrine systems of crustaceans (sinus gland, postcommissural organs, pericardial organs) are neurosecretory. Only the chemistry of the hormones of the pericardial organs has, to some degree, been investigated. Some of these seem to be polypeptides[54–56].

While clearly only a beginning, these findings suggest that the situation in invertebrates may not be so very different from the 'chemical spectrum' of vertebrate neurosecretory systems: if, for example, the activities in mammalian hypothalamic extracts are considered as a whole, one finds a similar mixture of families of specific polypeptides and of small molecular substances of more general distribution (5-hydroxytryptamine, acetylcholine, etc.).

Returning now to the main theme, namely the characterization of neurosecretory systems, it would seem that any one criterion is insufficient. At present these systems may perhaps be best defined in the following terms: (1) neurosecretory fibres form complexes which can be histologically recognized; (2) ultrastructurally, they contain membrane-bound secretory granules; (3) they produce specific substances which may be mainly (or perhaps even exclusively) biologically active polypeptides.

A sharper definition is difficult — the distinction, for example, between 'true' neurosecretory systems and cholinergic and adrenergic neurones is not an entirely clear one. However, there can be little doubt that neurosecretory neurones are an essential link in the control of endocrine secretions.

Summary

Neuro-endocrine reflex arcs and the significance of neurosecretory neurones in such systems are discussed. The complexities of the afferent part of these arcs are illustrated by enumerating the regions of the central nervous system which may be concerned in the release of oxytocin by the stimulus of suckling. Another example given is the pathways leading to the secretion of antidiuretic hormone after an increase of blood osmoticity. The similarity between the latter mechanisms and the neuro-endocrine feedback of the hormones of the adenohypophysis is pointed out. The neurosecretory fibres are defined as part of the efferent arc, but it is stressed that it is not easy to give an unequivocal characterization of these units: Their electrophysiological properties are probably the same as those of 'ordinary' neurones and while ultrastructural studies show that they usually contain electron-dense inclusions of 1000-3000 A. diameter, secretory granules (perhaps with morphologically slightly different characteristics) are also found in other parts of the nervous system. The topographical relationships of neurosecretory fibres are of some help in defining them, but recent work has shown that the endings of neurosecretory fibres may also end in, or near, other structures than blood vessels. A recent suggestion is then discussed, namely that there are two different forms of neurosecretion, one concerned with the production of peptides and one with smaller molecular substances, and that these two types have ultrastructural correlates. A survey of the chemical nature of neurosecretory products in vertebrates and invertebrates is, on the whole, shown to favour this concept, although the important proviso must be made that the biochemistry of neurosecretory products is as yet in its infancy.

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Recent Advances in the Analysis of Lipids

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DURING the last decade significant progress has been made in understanding the chemistry and metabolism of lipids. These advances have been mainly due to the great emphasis laid on the etiology and the possible cure for atherosclerosis and other diseases connected with lipid metabolism. In spite of the difficulties often encountered by workers in this field, a remarkable progress has been made in developing improved procedures for the isolation, fractionation and chemical characterization of the biologically occurring lipids. With the development of gas-liquid chromatography, followed by the application of silicic acid adsorbents to column chromatography and later to thin layer chromatography, the biochemist has been able to separate efficiently the many components of complex lipid mixtures. The present review will discuss the recent methods employed in achieving the separation and identification of biologically occurring lipids.

Extraction and Isolation of Lipids from Tissues

The lipids in tissues exist largely bound to proteins as lipoproteins. Therefore, the various extraction procedures employed for isolating lipids from tissues involve the use of the solvent system, usually a mixture of polar and non-polar solvents, which rupture the protein to lipid bond and at the same time extract the lipid completely. Various solvent mixtures have been used for extracting lipids from tissues and body fluids, such as ethanol-ether, chloroform-methanol, etc.[1]. These solvent mixtures have inherent difficulties of achieving the desired extraction. One solvent system, e.g. ethanol-ether, may be efficient for lipid extraction from plasma but not from other tissues such as brain. The conditions of lipid extraction may also vary. For example, heat is required in the case of fatty tissues. Chloroform-methanol mixtures in varying proportions are the solvent systems of choice for extracting lipids from tissues and body fluids[2]. The extraction of total lipids is quantitative with this solvent mixture. The optimum solvent to tissue ratio for extraction of lipid may vary from tissue to tissue. Hanahan[2] has reported that three parts of solvent to one part of tissue are quite adequate for complete extraction. The extraction of lipids at 4°C. will avoid the possible activation of certain lipid degrading enzymes. Exposure to atmospheric oxygen and light (which induces peroxide formation) during extraction should be avoided as these will improve the quality of the final lipid extract. Sometimes a further check should be made for complete extraction by subjecting the solvent extracted residue to acid or alkaline hydrolysis at reflux temperature. If no fatty acids are recovered in the hydrolysates, it is reasonable to assume that the extracted material represent the 'total lipids'[2-5].

Chloroform-methanol solvent mixture with certain modification, e.g. addition of water, NaCl, 1*N* HCl, prior treatment of the tissues with acetone, etc., has also been used for the extraction of proteolipids[6-11], phosphatidopeptides[12], triphosphoinositides[13], gangliosides[14-18] and sulphatides[19-24].

Palmer and Rossiter[13] have recently described a method for the isolation of mono-, di- and triphosphoinositides from tissues. In this method monophosphoinositides are extracted with chloroform-methanol (2:1, vol./vol.) and di- and triphosphoinositides are extracted when one part of conc. HCl is added to 300 parts of chloroform-methanol (2:1, vol./vol.). The phosphoinositides are then separated chromatographically on formaldehyde treated paper.

Possible Alterations in Lipid Structure during Extraction

The optimum conditions for the extraction of lipids from tissues and body fluids vary from tissue to tissue. The nature of the sample, the purity of the solvents used, temperature, light and oxygen and the procedures employed for extraction can significantly influence the end results. Use of elevated temperature may alter the lipid composition of the mixture. The elevated temperatures along with solvents may activate certain lipolytic enzymes, e.g. activation of phosphalipases A, C and D by solvents[25-28]. The important considerations to be kept in view while attempting the isolation of lipids from biological sources are: (i) use of nitrogen atmosphere, (ii) use of purified and suitable solvents, (iii) rapid removal of the tissues after sacrificing the experimental animals to minimize enzymatic changes, (iv) quick maceration of the tissues, (v) use of proper solvent to tissue ratio, (vi) heat only when necessary, (vii) removal of non-lipid impurities without loss of lipid, and (viii) storage under conditions that minimize alterations in lipids.

Removal of Non-Lipid Impurities from Lipid Extract

Lipids tend to solubilize non-lipid impurities such as sugars, free amino acid, urea, etc. The following methods have been used to remove non-lipid impurities from lipid extracts.

Partition method — In this method the lipid extract is partitioned with 0·2 volume of water or salt solutions[2], viz. Na, K, Ca or Mg. The use of salt solutions avoids the interfacial fluffy layer and also decreases the loss of more acidic types of phospholipids, at the interface or in the water phase. The lower phase (chloroform layer) contains the pure lipids.

Dialysis — In this the lipid solution in petroleum ether is dialysed through a rubber membrane for 24 hr against petroleum ether. All the non-lipid contaminants are dialysed out. A routine check should always be made on the dialysate for lipid

components. If they appear, the fractions are recombined and dialysis repeated[29].

Other effective methods for the removal of non-lipid material from lipid extract have been reported using paper chromatography[30], electrodialysis[31], electrophoresis[32] and sephadex columns[33].

Storage of Lipid Extract

The conditions under which lipid extract is stored are quite important, for it has been reported that normal atmospheric conditions of storage and unfavourable temperatures significantly alter the compositions of lipid mixtures[34]. Lipid extracts, rich in polyunsaturated fatty acids, are more susceptible to these changes and may result in auto-oxidized and polymerized products. Exposure of lipid extract to light can modify its cholesterol content[35]. The browning and formation of viscous solutions of amino phospholipids have been reported in cases where adequate precautions for storage were not taken. In order to minimize these changes in lipid mixture, they should be stored in chloroform in a reasonably dilute solution at or under −8°C. in an atmosphere of nitrogen with minimum exposure to light.

Fractionation of Lipid Extract

In addition to the possible oxidative changes and enzymatic alterations occurring during the isolation of lipids from tissues, the presence of contaminants complicates the fractionation procedures. Several schemes involving complex formation[2-5], solvent fractionation[2-5], countercurrent distribution[2-5,36], salt fractionation[2-5], column chromatography[37-39], etc., have been employed to fractionate lipid mixtures and these have been reviewed recently[2-5]. While no one procedure can be recommended as the best, the column chromatographic techniques have proved very satisfactory and these will be discussed here.

Acetone Fractionation

Acetone has been widely used for the initial fractionation of phospholipids from the lipid mixture[2]. Phospholipids are insoluble in acetone whereas neutral lipids are soluble. Thus phospholipids of beef and rat liver have been obtained in 95 per cent yield by acetone treatment of the total lipid preparation at room temperature. The fractionation can also be carried out at 4°C., but in this case a significant amount of neutral lipids may be precipitated along with phospholipids. Acetone precipitates certain phospholipids adequately but in many instances it fails to precipitate all phospholipids. For example, phospholipids of erythrocytes and haddock flesh are not completely precipitated even at −20° or −25°C. In order to overcome this difficulty $MgCl_2$, which aids considerably in precipitating all phospholipids, is added to acetone. The addition of $MgCl_2$ may alter the cation composition of some of the more acidic phospholipids found in many tissues (i.e. phosphoinositides and phosphatidylserine). Trichloracetic acid has also been used to precipitate phospholipids, but this precipitant may cause some hydrolysis of plasmalogenic phospholipids even under mild conditions[40].

Column Chromatography

Column chromatography is of immense value in the isolation and purification of lipids in the study of their metabolism. Development of chromatographic methods has made most problems in lipid chemistry and biochemistry more practicable than they were previously. The problem of resolving lipid extracts from natural sources on a preparative scale into separate, intact, molecular species has been investigated most successfully by column chromatography on silicic acid[37,39]. Therefore, it is important to stress the necessity of complete separation of a lipid mixture. The technique of column chromatography is dependent on many factors and the investigator should choose the conditions giving maximum degree of separation of lipid mixture. Some of these factors are discussed below.

Adsorbants — The adsorbants which have been used in column chromatography for lipid separation are magnesium oxide[38,41,42], alumina[2,43-45], cellulose[12,38,46,47], silicic acid[39,48-67], florisil[68-73], diethylaminoethyl cellulose[38,60,74] and methylated sephadex G-25[75-78]. Among these, silicic acid has been most widely used. In most cases the selection of the adsorbant is the first problem. The capacity to adsorb the lipid strongly and allow the displacement without difficulty is a necessary requirement of an adsorbant. The adsorbant should have a preferential affinity for the lipid. The second important requirement is the particle size of the adsorbant. The particles should be small enough to give a maximum adsorptive surface, yet not so small that they tightly bind the solute and give extremely low flow rate resulting in the involvement of more time and other factors. The next thing to be considered is the availability and reproducibility of the adsorbant. This is particularly true of silicic acid.

Column — The selection of the column is governed to a great extent by the amount of adsorbant to be used. Long narrow glass columns usually give the best separations. However, more narrow columns with smaller particle size adsorbants give slower flow rates. A general practice is to keep the ratio of length to diameter of the column within a range of 5:1 to 15:1, with the final choice made on the basis of the amount of adsorbant needed to hold and separate the solute and the desired flow rate. Some preliminary experimentation will be necessary before a final selection can be made.

Elution — In practice, solvent pairs are often used and elution carried out with the mixture of the two. A solvent pair usually includes a polar and non-polar material, the selection of which is based on their ability to elute the most polar members of the lipid mixture to be resolved. The solvent pairs which have been used to elute neutral lipids from silicic acid columns are mixtures of petroleum ether-benzene, diethylether-hexane and diethylether-pentane and benzene-hexane. For eluting phospholipids, chloroform-methanol mixture has been used[38,39]. When neutral lipids are desired to be separated from phospholipids, they are eluted with chloroform and then phospholipids with methanol. Once the selection of solvent has been made, the lipids from the column may be eluted by (i) stepwise elution or (ii) gradient

elution[79]. In stepwise elution, the column is eluted with predetermined quantities of different specific mixtures of the solvent pairs. This type of elution is quite adequate when compounds of different polarities are to be separated. In gradient elution, the column is developed by the addition of a continuously changing proportion of one solvent to the other member of the pair. This method of elution is useful when it is desired to separate as many individual compounds as possible or to separate a mixture of closely related solutes on the basis of their polarity. In both stepwise and gradient elution, the usual practice is to start with the least polar mixture or a member of the pair and increase the polarity by the addition of the more polar material.

The elution of lipids from the column involves displacement analysis. The relative degree of solute and solvent affinity to adsorbant can be expressed in terms of the polarity, and this offers a convenient mode of expression of displacement development. If one considers a natural lipid mixture from the standpoint of polarity, the individual components may be rated with respect to degree of polarity beginning with the least polar hydrocarbons and concluding with the most polar phospholipids, i.e. lysolecithin.

Table 1 — Order of Elution of Lipids from Silicic Acid Columns[39]

Hydrocarbons
Esters other than steryl esters and diglycerides
Steryl esters
Fatty aldehydes (?)
Triglycerides
Long chain alcohols
Fatty acids
Quinones
Sterols
Diglycerides
Monoglycerides
Glycolipids
Lipoamino acids
Bile acids
Glycerophosphatidic acids
Inositol containing lipids
Phosphatidyl ethanolamines
Lysophosphatidyl ethanolamines
Lecithins
Sphingomyelins
Lysolecithin
Ethers
Miscellaneous lipids

Silicic Acid Column Chromatography

The use of silicic acid for separating lipid mixture was made by Trappe who separated glycerides from cholesterol esters[39]. Borgström[49] used silicic acid to separate neutral lipids from phospholipids. Since then, silicic acid has been used effectively to separate the lipid mixtures from various animal tissues and body fluids[49-67]. Silicic acid has the advantage of being commercially available in highly pure form and the results obtained with it are quite reproducible. The elution pattern of neutral lipids from silicic acid columns is of the following order: hydrocarbon, cholesterol, triglyceride, unesterified fatty acid, free fatty acid, free cholesterol, diglyceride and monoglyceride. In the class of phospholipids, more acidic type phospholipids are eluted first followed by inositides and phosphatidyl choline, sphingomyelin and lastly lysolecithin (Table 1).

Silicic acid columns have certain limitations, though they are widely used for separating lipid mixtures. The main difficulties are tailing of peaks and non-reproducibility of results. These difficulties arise because silicic acid is not an ideal adsorbant for all classes of lipids, especially phospholipids, where various phospholipids such as cardiolipin, phosphatidyl serine, inositides, sulphatides and gangliosides overlap in the elution scheme[80]. The difference in the binding of sphingomyelin with silicic acid as compared to lecithin is not great enough for the complete separation of these two substances[38,81,82]. These difficulties can be overcome to a great extent if certain factors discussed below are taken into consideration in using silicic acid as the adsorbant for lipid separation.

Water content — Water content of silicic acid determines its adsorption properties. The adsorption capacity decreases with increasing water content. For the separation of neutral lipids the water content of silicic acid has to be nil, whereas the presence of water in silicic acid favours the separation of phospholipids.

Silicate content — Most silicic acids contain a considerable quantity of silicate which binds water firmly resulting in increased retention time of the lipid on the column. Therefore, the purity of silicic acid is of considerable importance in reproducibility of results.

Particle size — Great care should be taken in choosing the particle size of the silicic acid. Particle size should be such that there is enough surface for lipid binding and desirable flow rates are obtained with least overlapping of fractions.

Loading factor — The amount of lipid applied on the column is of great importance. A convenient amount of lipid should be applied to achieve the best results. The reasonable amount of lipid which should be applied on the column is in the range of 6-10 mg./g. silicic acid. Overloading of the column may result in the overlapping of certain fractions and poor separation.

For a good fractionation of lipid mixture, fresh batches of suitably activated silicic acid should be used. This requirement is not so rigid in the case of phospholipids where the same column can be reused after adequate washing of the column with methanol followed by chloroform. The use of cotton plugs for supporting of column material should be avoided as this can adsorb some of the phosphoinositides[2]. Activation of silicic acid is achieved by heating it to 110°C. for 24 hr and sieved[2] to the desired particle size. The silicic acid can also be treated with solvents, e.g. diethylether and acetone and heated at 100°C. for 3 hr and sieved to the desired particle size[2]. The columns for separation are prepared by pouring a slurry of the activated silicic acid in suitable solvent into glass columns. After adequate washing of the column with solvents, the lipid is applied at the top and elution started. The column can be operated in a temperature controlled room, or fitted with a

water jacket to maintain a constant temperature during elution. The columns should be protected from light. The flow rate should be reasonable (10 ml./2-3 min.). If the flow rate is slow, the desired flow rates can be achieved by applying nitrogen pressure. Neutral lipids are eluted with increasing concentration of diethylether in hexane, or diethyl-ether-petroleum ether (b.p. 40-60°C.) or diethylether in pentane, or benzene in hexane. Phospholipids are eluted with increasing concentration of methanol in chloroform. The progress of elution of lipids from the columns can be followed either by weight assay or by chemical analysis of the particular lipid component in effluent[83]. For every unknown sample the amount of a particular solvent system to elute a particular component has to be predetermined. The lipid components eluted from the column are pooled according to the size of their peaks and evaporated to dryness *in vacuo*, preferably in a rotary evaporator under nitrogen at 40-45°C. The dried residue is dissolved quantitatively in a known volume of chloroform and stored under nitrogen at or under −8°C.

Silicic acid columns appear to function largely by ion exchange and hydrogen bonding[38]. The ion exchange reaction of silicic acid can be illustrated by the behaviour of phosphatidyl ethanolamine on silicic acid columns (Chart 1). A similar ion exchange reaction occurs with lecithin, resulting in its stronger binding on the column than the phosphatidyl ethanolamine.

The role of hydrogen bonding in silicic acid column chromatography is illustrated by the differences in the elution characteristics of a number of compounds. Lysolecithin is eluted from silicic acid columns after lecithin. The ion exchange reactions are presumably the same for both molecules, but the presence of hydroxyl group in lecithin creates an additional site for hydrogen bonding resulting in the stronger binding of lysolecithin on the column. The ion exchange reactions do not occur in the case of cholesterol. A substance such as lysolecithin which interacts by ion exchange and hydrogen bonding requires methanol for elution. Cholesterol with one OH group is rapidly eluted with ether, while cerebrosides containing several OH groups require chloroform-methanol mixture for elution.

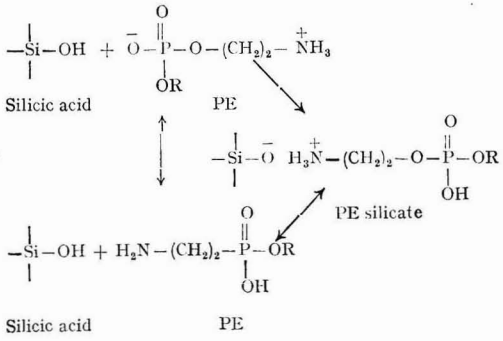

Chart 1 — Ion exchange reaction of phosphatidyl ethanolamine with silicic acid[38]

Diethylaminoethyl (DEAE)-Cellulose Column Chromatography

The major drawback of silicic acid is that the separation of acidic and non-acidic lipids is not clear. To overcome this difficulty cellulose ion exchangers have been used for fractionating lipid mixtures. Several ion exchange cellulose columns such as carboxymethyl cellulose, disodium phosphocellulose, Ecteola triethylaminoethyl (TEAE)-cellulose and DEAE-cellulose have been used for phospholipid separation[38,84]. These exchangers do not require the presence of water for the retention of lipids and reproducible results are obtained under careful conditions. Among these DEAE has been reported to be the most promising for lipid separation. Ecteola behaves essentially like DEAE and TEAE is similar to DEAE in many respects. The difference between DEAE and TEAE is that acetate and chloride forms of TEAE do not bind lipid, instead hydroxyl form binds lipids readily. Another difference between DEAE and TEAE is that DEAE in acetate form readily binds phosphatidyl ethanolamine in the absence of water, whereas the hydroxy form of TEAE does so only in the presence of water. DEAE-cellulose has been used in the acetate form because this form does not liberate strong acid during ion exchange reaction that would destroy acid labile lipids, e.g. plasmalogens. After a thorough prewashing with 1*N* HCl followed by water and 1*N* KOH, the DEAE-cellulose is put in the acetate form by treatment with glacial acetic acid. The acid is washed out with methanol and the column is prepared in a suitable chloroform-methanol mixture, before application of the sample. Phosphatidyl ethanolamine is obtained in pure form from DEAE-cellulose column and all the water-soluble non-lipid contaminants are eluted in a single fraction free of lipid (Table 2). The other fraction obtained, however, must be separated into individual lipid classes by other means, e.g. silicic acid column chromatography.

The zwitterion lipids, such as lecithin and sphingomyelin, show very little tendency to bind to DEAE-cellulose and are retained only very slightly by DEAE. They are eluted with non-ionic solvents

Table 2 — Elution Scheme of Lipids from DEAE-Cellulose (Acetate Form) Columns[38]

Eluting solvent (vol./vol.)	Substances eluted
Chloroform-methanol (7 : 1)	Lecithin, sphingomyelin, ceramide, cerebrosides, cholesterol, lysolecithin, sterol esters and glycerides
Chloroform-methanol (7 : 3)	Phosphatidyl ethanolamine
Methanol	Water-soluble non-lipids
Chloroform-glacial acetic acid (3 : 1) containing 0·001*M* potassium acetate	Free fatty acids
Glacial acetic acid	Phosphatidyl serine and gangliosides
Chloroform-methanol (4 : 1) containing 10 ml./litre conc. aqueous ammonia	Cerebroside sulphate, inositol phosphatides and cardiolipin

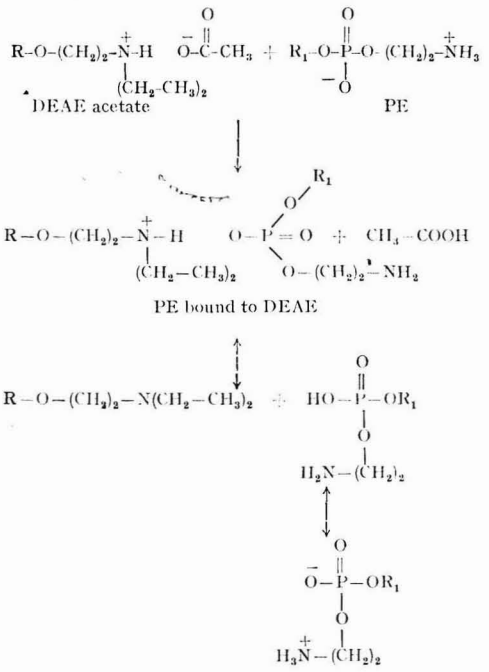

Chart 2 — Ion exchange reaction of phosphatidyl ethanolamine with DEAE-cellulose[38]

such as chloroform-methanol. The non-ionic lipids (cholesterol and cerebrosides), which do not have strong hydrogen bonding, pass through the ion exchange cellulose in the presence of polar solvents like methanol. Phosphatidyl ethanolamine is a proton donor in the zwitterion form and is retained more firmly by DEAE and is elutable from the column with a non-ionic solvent such as chloroform-methanol (7: 3, vol./vol.) mixture. This follows from the fact that DEAE is itself a proton donor. Escape from the positively charged site on the adsorbant is possible as the proton of the diethylaminoethyl group can be transferred to the phosphate group of phosphatidyl ethanolamine with the subsequent release of phosphatidyl ethanolamine molecule from the adsorbant. A proton transfer from the phosphate to $-NH_2$ to give $-NH_3^+$ would then restore the phosphatidyl ethanolamine to its original zwitterion form (Chart 2). Acidic lipids in the salt form exchange ions with DEAE. Phosphate or other acidic groups interact with the positively charged site of the adsorbant, with the production of potassium acetate, which is washed through the column. Acidic lipids are retained very firmly and are eluted from DEAE with acidic or basic solvents. The overloading of DEAE-cellulose columns results in the elution of acidic lipids with non-ionic solvents.

Cellulose Column Chromatography

Cellulose columns have been used for removing non-lipid materials from lipid mixtures[12,38,46,47]. Svennerholm and Svennerholm[18] have used cellulose columns for preparing gangliosides from brain. Rouser *et al.*[38] have used cellulose columns for separating lipid mixture. Whatman cellulose is washed with methanol, followed by water and then dried. The column is prepared by pouring a slurry of cellulose in chloroform. The column is then washed with chloroform-methanol-water (16:4:1, vol./vol.) mixture. All the lipids are eluted first with the above solvent mixture and then the gangliosides with chloroform-methanol-water (4:15:1, vol./vol.) mixture. A good separation of gangliosides is achieved by cellulose column chromatography[14].

Silicic Acid-Silicate-Water Column Chromatography

These types of columns have been successfully used for the fractionation of phosphatidyl ethanolamine (PE) from phosphatidyl serine (PS) from brain[85], liver and bile[86]. These columns are conveniently prepared by passing a mixture of chloroform-methanol-aqueous ammonia through a bed of silicic acid in a chromatographic column. When a mixture of PE and PS is applied on the column, PE is eluted with chloroform-methanol (4 : 1, vol./vol.) mixture and PS with methanol. Silicic acid-silicate-water columns appear to function in part as partition chromatography columns. A pure silicate column with a monovalent ion (such as ammonium ion) is of limited value for the separation of lecithin and sphingomyelin, as such columns have a very limited capacity. A mixture of silicic acid and silicate is advantageous because the ion exchange properties of silicic acid are retained and the system has a high capacity. Lipids are bound less firmly to the stationary partition phase and the peaks do not have the long tailing portions characteristic of pure absorption columns.

Magnesium Silicate (Florisil) Column Chromatography

Florisil has been used by various workers to separate faecal lipids[68], tocopherols[69], standard lipid mixtures[87], steroid hormones[70], cerebrosides[71] and fatty acid esters[72]. Recently, Carroll[73] has presented a scheme for the quantitative elution of lipids from florisil columns (Table 3). The order of elution of neutral lipid classes from florisil columns is the same as that observed with silicic acid columns, but florisil has the advantage of being in a coarse mesh size. Florisil seems to have as much adsorptive surface as the commonly used fine mesh preparation of silicic acid and lipids up to 10 mg. or more per gram

TABLE 3 — ELUTION SCHEME OF LIPIDS FROM FLORISIL COLUMN[87]

Eluting solvent	Lipid eluted
Hexane*	Hydrocarbons
Ether (5%) in hexane	Cholesterol esters
Ether (15%) in hexane	Triglycerides
Ether (25%) in hexane	Cholesterol
Ether (50%) in hexane	Diglycerides
Methanol (2%) in ether	Monoglycerides
Acetic acid (4%) in ether	Free fatty acids

*Purified skellysolve B

of adsorbant may be readily separated without evidence of overloading. Although florisil has proved very satisfactory for the separation of neutral lipid class, it cannot be used to separate and recover phospholipids satisfactorily. Free fatty acids like phospholipids are adsorbed more strongly on florisil, but it is possible to recover free fatty acids quantitatively by including acetic acid in the elution solvent mixtures. This is a further advantage of florisil over silicic acid, since the free fatty acids and triglyceride fractions found to overlap on silicic acid are easily separated on florisil. Recently, Carroll[73] has reported that if florisil is treated with conc. HCl, a separation of phospholipids similar to silicic acid columns can be achieved.

Magnesium oxide columns have been used to separate choline containing phospholipids from non-choline containing phospholipids, which are strongly adsorbed on the columns[38,41,42]. The results obtained with this adsorbant are not quite reproducible. Aluminium oxide columns have been used for the preparation of lecithin of high purity[2,43-45]. But recently it has been reported that aluminium oxide degrades some lecithin to lysolecithin[88]. On aluminium oxide columns non-choline containing phospholipids are firmly retained, whereas choline containing phospholipids are eluted with chloroform and methanol mixtures. For a successful fractionation of phospholipids on these columns, the *p*H of alumina should be slightly on basic side.

Recently, methylated and acetylated sephadex columns have been used for the separation of lipid mixtures[75-78]. The advantage of using methylated sephadex in lipid separation is that phospholipids are eluted ahead of triglycerides, cholesterol esters, cholesterol and fatty acids. Methylated sephadex appears to be of considerable potential value in the separation of lipid-soluble substances and may serve as a complement to partition and adsorption chromatographic procedures. The separation appears to be based partly on the differences in size and/or shape of the molecules and partly on liquid-liquid partition between a mobile phase and a stationary gel-solvent phase. By choosing different solvent mixtures, liquid-liquid partition chromatography of either the reversed phase or the straight phase type can be obtained. Recently, Hirsch[89] has described the column chromatographic separation of neutral lipids using factice, a hydrophobic polymer, as the stationary phase and aqueous acetone as the moving phase. The neutral lipids, i.e. cholesterol esters, triglycerides, diglycerides and monoglycerides, have thus been easily separated from each other. Separations of components with different fatty acid composition within the same lipid class can also be achieved with this liquid-gel system. Thus cholesterol esters with fatty acid chain length from 2 to 18 carbons are eluted as individual peaks. Similar separations are possible with glycerides or with mixtures of methyl esters of fatty acids. However, with the exception of cholesterol esters, lipids within a given class containing fatty acids of equivalent polarity are inseparable. Elution of lipids is performed with a single solvent mixture and detection of lipid in the effluent stream can be achieved by the use of automatic differential refractometry. Free fatty acids and phospholipids are poorly separated and must be removed from the lipid mixture prior to chromatography.

From the foregoing discussion on the column chromatographic separation of lipid mixtures, it is apparent that no adsorbant gives a clear cut and pure fractionation of lipid classes. Therefore, it is necessary that the eluted fractions be examined carefully for their purity prior to further analysis. This could be achieved by paper and thin layer chromatographic techniques. Both these chromatographic techniques are rapid and sensitive means of identifying and separating lipids. A brief discussion of these techniques is presented below.

Paper Chromatography

The intact lipids can be chromatographed on filter papers[90-98], formalin treated papers[99-102], acetylated papers[103], tetralin impregnated papers[104], silicic acid impregnated papers[105-107], phosphate impregnated papers[108], glass fibre papers[109-112], and on thin layers of silica gel coated on glass plates[113,114]. Each of these methods for the separation of lipid mixture into various constituents has its own advantages and disadvantages.

Silicic acid impregnated papers —Recently, excellent reviews have appeared on the preparation and use of silicic acid impregnated papers for the separation of neutral and phospholipids[115,116]. This method has been successfully used for both qualitative and quantitative separation of neutral and phospholipids. After applying suitable amounts of lipids (about 15 μg. phospholipid phosphorus and equivalent amount of neutral lipid) the papers are developed in solvent mixtures. Various solvent mixtures have been used for developing the chromatograms, but diisobutyl ketone-glacial acetic acid-water (40: 25: 5, vol./vol.; 40: 20: 5, vol./vol. at room temperature and 40: 20: 3, vol./vol. for 0-5°) mixtures have proved to be the best for phospholipid separation. For neutral lipid separation solvent systems *n*-heptane-diisobutyl ketone (96:6, vol./vol.), *n*-heptane-diisobutyl ketone-acetic acid (91: 9: 2, vol./vol.) have been used. The development of the papers should be carried out in a temperature controlled room. Recently, a method has been reported where aqueous systems such as plasma, serum, cytoplasm or finely dispersed particles can be chromatographed directly without prior extraction with organic solvents[116].

Identification of Spots on Chromatograms

The lipid spots on developed chromatograms are identified by their staining characteristics and mobility behaviour (R_f values). This identification, however, can by no means be interpreted as conclusive and should be further supported by chemical and physical data. The various staining reagents used are the following: Rhodamine-6-G (0·001 per cent aqueous solution) for all lipids; ninhydrin (0·25 per cent in acetone-lutidine, 9:1, vol./vol.) for amino-lipids, iodine vapours for unsaturation, phosphomolybdic acid (1 per cent aqueous solution), stannous chloride (1 per cent in 3*N* HCl) or Dragendorf reagent[117] for choline containing lipids, and 2,4-dinitrophenyl hydrazine (0·15 per cent in 3*N* HCl) or Schiff's reaction for plasmalogens. Other stains such as Tri-complex Brilliant[118,119], Biebrich Scarlet,

Nile Blue, Rhodamine B, acid fast violet green and protoporphyrin[120] have also been used for detection of lipid on the chromatograms. The labelled lipids can be visualized by exposing the chromatogram to a photographic emulsion in the conventional manner. The lipid spots on silicic acid impregnated papers can be eluted with suitable solvents and quantitized. The quantitative analysis of phospholipids on chromatograms can also be done by neutron activation of the phospholipid spots[121,122]. This method has limitations. The background may be high, the neutron flux may not convert the same amount of phosphorus in each spot to radioactive phosphorus, and the amount of activity may not be enough.

The separation of lipids has also been done on silicic acid impregnated glass fibre papers[109-112]. After applying the lipids the papers are developed in diisobutyl ketone-acetic acid-water-benzene (160: 50: 8: 7, vol./vol.) and the spots detected by charring (spraying with 20 per cent H_2SO_4), phosphomolybdic spray and 0·001 per cent aqueous solution of Rhodamine-6 G. The lipid spots can be eluted and quantitized.

The separation of lipids on silicic acid impregnated papers is affected by various factors such as the impregnation procedure, humidity, temperature, the amount and way of application of lipid, exposure of the paper to volatile basic solvents and the way of storage. In order to get reproducible results, all these factors have to be borne in mind. Increased humidity in the atmosphere results in higher mobilities of the lipids. In some cases, chromatography should be performed at a lower temperature to minimize degradation of labile compounds such as plasmalogens. Unless one is careful, wrong conclusions are drawn regarding the identity of one compound from paper chromatographic techniques. A single spot on a chromatogram may not be from a single compound. Improper use of colour tests for location of lipid spots can give rise to confusion. Auto-oxidation of lipids, especially of phosphatidyl ethanolamine, phosphatidyl serine and lecithin, can give rise to streaking, extra spot or failure to migrate. Small amounts of divalent cations may cause retention of phospholipids, particularly lecithin and sphingomyelin at the place of application. Several other artifacts may be introduced such as the use of a strongly acidic silicic acid paper or strongly acidic solvents for chromatography. The application of labile plasmalogen forms of phospholipids (particularly of PE) to acidic silicic acid paper may result in appreciable hydrolysis of α,β-unsaturated ether bond in the plasmalogen to give a lysophosphatide. Therefore, all the interpretations of a paper chromatogram must be confirmed by isolation, hydrolysis, infrared examination, etc.

Formalin treated paper has been reported to be good for the separation of inositolphosphatides[13,99-102], and three types of diphosphoinositides, and triphosphoinositides[13] have been separated employing it. Lecithin has been fractionated into five components on tetraline impregnated papers[104]. Papers impregnated with anionic and cationic resins, $ZnCO_3$, $CaCO_3$ and $BaSO_4$ and cellulose phosphate, carboxymethyl cellulose and DEAE-cellulose papers have also been used for lipid separation[115,116]. The resolving power of these papers is inferior to that of silicic acid impregnated paper, but good separation is achieved of amino phosphatides. Paper chromatography has also been used for analysis of fatty acids of lipid mixtures.

Thin Layer Chromatography

Stahl has demonstrated the potential usefulness of thin layer chromatography (TLC) in lipid separation[113]. Several excellent books and review articles have appeared on thin layer chromatography and its application[114,123,124]. The main advantage of TLC is its quickness for separation of the lipid mixtures. TLC has been applied for the separation of neutral and phospholipids from several biological sources. TLC can be used both for qualitative and quantitative separation of lipids. Certain important points such as coating material, its thickness on plate, activation of plate, solvent system, humidity and temperature should be kept in mind for achieving the best results.

Other methods such as nuclear magnetic resonance[125], mass spectrometry[126] and infrared[127] and near infrared[128] spectroscopy have also been used for the identification of isolated lipids.

Gas-Liquid Chromatography

Gas-liquid chromatography has lately attracted the interest of the lipid chemists and is a technique undergoing rapid development. The method was first introduced by James and Martin[129] when they separated the normal saturated carboxylic acids up to lauric acid, and was later extended up to behenic acid[130] and since then there has been a rapid expansion in the use of this method in the lipid field. The general theory and practice of the gas chromatographic methods are extensively treated in recent text-books and reviews[131-138] and the problem of presenting the increasing amount of data in a consistent way had led to the recommendation of the Amsterdam Committee[139-141]. Some developments in the field of separation of fatty acid methyl esters have been described by Farquahar *et al.*[142]. The emphasis in recent studies of GLC in lipid field has been in improving separation by finding more suitable stationary phases and in improving the instrumentation. It has thus been possible to extend the working range for methyl esters of fatty acids as far as 34 carbon atoms[143] and to detect and estimate components below 0·05 per cent in the original sample[142].

During the past few years a number of instruments for GLC have become available commercially. In order to reduce the working temperature, to speed up the development of the chromatograms and to improve the separation, it is a common practice to convert the substances to lower boiling derivatives. The fatty acids are usually separated as methyl esters although sometimes higher esters are used. A number of methods have been used for the preparation of methyl esters of fatty acids[131-138]. The stationary liquid phases used for fatty acid methyl esters and similar compounds are either of the non-polar type such as silicone products or of the polar type such as polyesters. With non-polar phases, unsaturated and branched components emerge before saturated ones, whereas with polar phases, unsaturated acids emerge

after their saturated analogues. Late-emerging components of one chain length group may be retained in the column so long that they overlap with components from the following chain length group[144].

The efficiency of separation in a column is the result of at least two factors: column efficiency and solvent efficiency. Column efficiency is often expressed as the highest equivalent of a theoretical plate and is measured by the spread of a component when it passes through the column. This is related to many factors associated with the packing and design of the column. The solvent efficiency or separation factor depends on the distribution coefficients of the different solutes between the liquid and the gas phase. It is expressed by the ratio of the retention volumes of two peaks. The choice of stationary phase depends in each case on the particular separation desired. The plate efficiency of polar column is usually less than that of a non-polar column[145-147]. The capillary columns used by Golay[148] have height equivalent of a theoretical plate of the same order as packed columns, but they give better separation because of the extreme length of the column and the very small charge used[149-151]. The carrier gas used for fatty acid esters have been argon, helium, hydrogen and nitrogen. Argon is used in instruments equipped with β-ray argon ionization detectors, while hydrogen and nitrogen are used with thermal conductivity detectors. Hydrogen, alone or mixed with another gas, is used with flame and flame ionization detectors.

The practical identification of fatty acid methyl esters on chromatograms is done by comparing their retention time with suitable standards. Members in a homologous group may be identified by plotting the logarithms of retention times, or retention volumes versus chain length or degree of unsaturation. James[146] has devised a method to determine the degree of unsaturation of straight chain and simpler branched chain compounds. By plotting the logarithm of the retention time measured on one stationary phase against similar values from a different stationary phase, saturated components lie on one straight line and monoenes, dienes, trienes, etc., lie each on a separate parallel line. The quantitative aspect of GLC has been studied by several workers. A serious problem arises from the fact that the detectors do not always give a response proportional to concentration. The detectors most frequently used are gas-density balance[152-159], the thermal conductivity cells[160-162], and β-ray argon ionization cell[163,164]. In spite of the difficulties in making quantitative measurements, variation between the lipid compositions of similar biological samples is often so great that the inaccuracies of the detector are not apparent, and are small compared to the inaccuracies involved when biological comparisions are made.

There are certain limitations of GLC analysis and the quantitative interpretation of the chromatograms. One of them is the stability of the substances inside the column[165]. The esters of polyunsaturated acids are very stable up to 200°C. (ref. 166). Transesterification of fatty acid in polyester packed columns has been reported[167]. Esters of some unsaturated hydroxy and hydroperoxy acids undergo dehydration to more highly unsaturated derivative and the conjugated polyunsaturated esters undergo *cis-trans* isomerization[165]. Another disadvantage is that some components of the samples may never emerge from the column and, therefore, will go undetected. This may cause significant errors in calculating the composition of the samples since this is often based on emerging components only.

GLC technique has also been used for the separation of fatty alcohols[168], fatty aldehydes[169-171], fatty nitriles[172], mono-, di- and triglyceride[173-175], glyceryl ethers[176] and steroids[177]. It is possible to collect microgram quantities[178] of substances from analytical gas chromatograph, and also connect it to a radioactivity counter[179,180]. For this specially designed columns and detectors are needed. One serious disadvantage of preparative gas liquid chromatography is the possible contamination of products due to bleeding of the stationary phase and some subsequent purification is often necessary.

From the foregoing discussion on the separation of lipids by column and impregnated paper chromatography, it is apparent that the elution pattern of lipids from the column and their mobilities on the paper should not be considered as conclusive proof of their identity. All the lipid components must be chemically characterized before conclusions about their identity are made. Some of analytical methods of these lipids are discussed below. An excellent compilation of these methods published in the last twenty years is available[181].

Cholesterol

Numerous methods have been used for the estimation of total free and esterified cholesterol[182-205]. The most popular reaction for cholesterol estimation is the Lieberman-Burchard (L-B) method[204]. The reaction mixture consists of a combination of H_2SO_4 and acetic anhydride, which when added to a solution of cholesterol in chloroform gives blue colour. The fact that the colour stability is affected by H_2SO_4 concentration, solvent, temperature and light and the question whether free or esterified form of cholesterol is measured by this reaction have given rise to great number of modifications for the proper use of this reagent[204]. The difference in reactivity between free and esterified cholesterol is greater at low temperature and low concentration of H_2SO_4. Higher temperatures and larger amounts of acid cause such differences to become smaller and eventually disappear. L-B reaction is not specific for cholesterol. Highly unsaturated compounds interfere with colour development. Since the colour formation depends on the combination of the sterol with a strong acid, many modifications using reagents other than H_2SO_4, viz. *p*-toluenesulphonic acid, sulphosalicylic acid and chlorosulphonic acid, have been used. The addition of an aldehyde, e.g. salicylaldehyde, has been reported to intensify the colour, but these reagents give a red colour in the blank and also react with estrone, estradiol, anhydrosterone, testosterone and corticosterone[204].

Tschugaeff reaction gives red colour when a solution of cholesterol in glacial acetic acid is treated with zinc chloride and acetyl chloride. In this method both free and esterified cholesterol give similar extinction coefficients on molar basis. Old solution

of zinc chloride and acetyl chloride do not yield quantitative colours. Acetyl chloride also tends to distil away from the reaction mixture. Because of this other reagents such as benzoyl chloride, *o*-nitrobenzoyl chloride, phenylacetyl chloride and *m*-nitrobenzyl chloride have been used. Hanel and Dam have modified the original Tschugaeff reagents for the estimation of cholesterol[205]. This method is very simple and quick, but does not give quantitative values for esterified cholesterol. Cholestenol, epicholestanol and cholic acid give a faint colour. Both cholesterol and lathosterol can be determined simultaneously with this method. This method has also been adopted on a ultramicroscale for the estimation of cholesterol[206].

One of the most popular methods of cholesterol estimation is that of Zlatkis *et al.*[188]. In this method cholesterol is measured directly by adding a fixed volume of conc. H_2SO_4, glacial acetic acid and ferric chloride solution to serum or plasma. On equimolar basis free and esterified cholesterol give equal colour intensity. The presence of bilirubin, proteins, tryptophan, haemoglobin, etc., interfere in the analysis. Therefore, this method has been modified in various respects such as varying the concentration of ferric chloride, preparation of stock solution in phosphoric acid, use of extraction procedures before colour development and use of other metal halides[204].

Other methods such as gravimetric[204], titrimetric[204], turbidimetric[207], fluorometric[208,209], spectroscopic[210], polarographic[211–213], haemolysis red blood cells[214] and the monolayer film techniques have also been used[213] for the estimation of cholesterol. These methods have been critically reviewed by Kabara[204].

Total Lipids

Numerous methods using gravimetric, titrimetric and colorimetric techniques have been used for estimation of lipids on micro- and macroscales[215–222]. In the commonly used gravimetric analysis one has to be very careful for the moisture which could easily be adsorbed by the dried lipid during weighing procedures. Quantitative estimation of lipids on microscales by GLC has also been reported[223,224]. The method is based on the difference in volatility between lipid and solvents.

Triglycerides

Triglycerides have been estimated by a number of methods[225–236] such as gravimetric, colorimetric and chromatographic. The most widely employed non-gravimetric methods have been (i) estimation by difference either of cholesterol ester and phospholipid fatty acids from total titrable fatty acids or of cholesterol and phospholipids from total lipids; (ii) determination of glyceride glycerol, after alkaline hydrolysis and periodate oxidation, by measurement at 570 mμ of the chromotropic acid-formaldehyde complex formed; and (iii) determination of glyceride fatty acids by formation of their hydroxamic acids. Recently, infrared spectroscopic methods have been used for the estimation of triglycerides after their extraction and separation from other lipids[237–239]. By this method simultaneous estimation of triglycerides and cholesterol esters has been achieved. This method has been claimed to be simple and quicker than glycerol oxidation method.

Fatty Acids

A number of methods[221,240–259] have been used for the estimation of fatty acids, viz. alkaline isomerization, iodine value, gas-liquid chromatography, ester distillation, thiocyanogen value, quantitative paper and thin layer chromatography, titrimetry and colorimetry. The oxidative methods using potassium dichromate have the disadvantage of being unspecific. The titrimetric methods of Gordon[240] and Dole[241] for unesterified fatty acids have been widely used for their simplicity, quickness and reproducibility. In the Gordon method the recoveries of palmitic, stearic and oleic acids are quantitative and the titration values are unaffected by acetoacetate, β-hydroxybutyrate, succinate, etc. The reproducibility and accuracy of Dole's method are as good as that of Gordon's. Values for plasma fatty acids tend to be somewhat higher by Dole's method, indicating that the exclusion of titratable compounds other than unesterified fatty acids may not be complete. These methods have been modified according to needs of the later investigators. Gas-liquid chromatography has been found to be the best for fatty acid analysis.

Lipid Phosphorus

Numerous methods are available for the estimation of lipid phosphorus and have been critically reviewed by Lindberg and Ernster[260]. In the estimation of organic phosphorus, advantage is taken of the property of orthophosphate to form a complex with molybdic acid which on reduction gives a blue colour. All the reducing agents reduce molybdic acid in the absence of phosphate. Phosphate accelerates the rate of reduction and this acceleration occurs over a wide acid range but decreases with increasing acidity. The rate of reduction of molybdic acid, the accelerating effect of phosphate and the end points reached in these reactions are all dependent on the concentration of molybdate and the concentration and nature of reducing agent. Phosphate is converted into phosphomolybdic acid in 0·5*M* H_2SO_4 and reduced with 1-amino-2-naphthol-4-sulphonic acid (Fiske-Subba Row's method). This method has been critically reviewed by Furukawa *et al.*[261]. A great number of substitutes have been introduced in place of amino-naphthol-sulphonic acid, viz. hydroquinone, 2,4-diaminophenol, monomethyl-*p*-aminophenol, ascorbic acid, thiosulphate, stannous chloride and ferrous sulphate. From the point of sensitivity and reproducibility, Fiske-Subba Row's method gives unsatisfactory results. The acidity remaining after sample incineration affects the end results. To avoid these effects perchloric acid (King's method) has been used in place of sulphuric acid. Some modifications of King's method have been suggested to increase its sensitivity[262]. The interference caused by acid labile organophosphate compounds, e.g. creatine phosphate, in Fiske-Subba Row's method has been eliminated by carrying out the formation and reduction of phosphomolybdate at *p*H 4, in acetate buffer and ascorbic acid as the reducing agent (Lowry and Lopez's method). In all these methods non-phosphate

compounds occurring in biological system interfere. These non-phosphate compounds have been divided into three groups: (i) those which alter the acidity of the medium, e.g. acids, alkali, buffers, etc.; (ii) those which form molybdenum complex, e.g. fluorides, citrates, oxalates, etc. and (iii) those which change the concentration of the reducing agent, e.g. nitrates, hypochlorites, etc. In later modified methods of Lowry and Lopez, the phosphomolybdic acid formed in an aqueous solution of sulphuric acid is extracted with isobutanol (Berenblum-Chain method)[260]. After separating the two phases, the colour intensity is determined in the isobutanol. Isobutanol has been substituted with a mixture of equal parts of isobutanol and benzene (Martin-Doty method). The reduction of phosphomolybdic acid is performed in an aliquot of the organic layer with $SnCl_2$. These modified methods are completely independent of interfering substances and the colour intensity is stable within a wide range of time. This method can be employed even when small amounts of proteins are present in the sample by including silicotungstic acid in the water phase. The method has been modified to determine 0·002 μg. (ref. 263) phosphorus. Some methods have been reported which are based on a colorimetric determination of phosphorus, without involving a reduction of phosphomolybdic acid[260]. Another procedure is based on the change in a solution of dye quinaldine red in the presence of phosphomolybdate[264]. The determination of phosphorus based upon Misson's reaction involving the determination of yellow phospho-vanadiomolybdate has also been reported[260]. Attempts have been made to determine phosphorus by flame spectroscopy, but this method is too sensitive to disturbances[265]. Recently Bartlett[266] has reported a heating method for the estimation of lipid phosphorus. This method is quick, simple, sensitive and reproducible and the colour developed is stable for long periods.

Glycerol

The estimation of free glycerol in hydrolysates involves the oxidation of glycerol with periodate to form 1 mole of formic acid and 2 moles of formaldehyde. Formaldehyde is then determined colorimetrically by formation of a complex with chromotropic acid[267,268]. Under the conditions of the periodate oxidation there is essentially no interference with the common sources of formaldehyde such as glucose. Alpha monoglycerides are oxidized and contribute partially to the glycerol values. To make the procedure quantitative, a standard solution of glycerol is similarly treated. During acid hydrolysis with 2*N* HCl about 2 per cent of the glycerol is lost[268]. Other methods, such as enzymatic[269–275], fluorimetric and chromatographic[276], have also been used for the estimation of glycerol in plasma and tissues. Wheeldon *et al.*[277] have described a method where a simultaneous analysis of glycerol, inositol, ethanolamine and serine can be quantitatively determined in a lipid sample.

Determination of Inositol, Ethanolamine and Serine in Lipids

Inositol — A number of chemical[278–287], microbiological[288–299] and enzymatic methods[288] (inositol dehydrogenase and pig heart diaphorase) are available for the estimation of lipid inositol[300]. The chemical methods in use are nearly all based on oxidation with periodic acid. Estimation of inositol is based on the amount of periodate consumed. The oxidation apparently does not result in the conversion of all carbon atoms to formic acid. This procedure requires complete liberation of lipid inositol by hydrolysis since all hydroxyl groups must be free for complete reaction with periodate. Further, inositol must be isolated from hydrolysate because glycerol, sugars, amino sugars, ethanolamine, serine and sphingosine, all react with periodate. The hydrolysis is carried out with strong acid, i.e. 4*N* or 6*N* HCl, in sealed tubes at elevated temperatures (110-20°C.) for prolonged periods (20-40 hr). The products of hydrolysis can be separated by paper chromatography and the individual components determined[301].

Ethanolamine and serine — Several methods have been used for the estimation of lipid ethanolamine and serine, e.g. manometric measurements of nitrogen gas after treatment with nitrous acid[302–304], manometric measurements of carbon dioxide after ninhydrin treatment[305–308], determination of ammonia after periodate oxidation[309–313] and colorimetric measurements using fluorodinitrobenzene[314–319], ninhydrin[320–330] and 1,2-naphthoquinone-4-sulphonate[300]. The methods used for the estimation of serine, with the exception of periodic acid oxidation, are not specific and determine other amino acids arising from proteolipids, glycoproteolipids, etc. The manometric methods measuring liberated nitrogen are excellent in their reproducibility and have been used as the standard methods for the evaluation of newer methods. The principal disadvantage is the inconvenience and the rather large amounts of lipid needed for estimation. Also, nitrous acid reacts with unsaturated fatty acid and releases extra nitrogen giving higher values for lipid nitrogen. The manometric method of determining carbon dioxide liberated from amino acids by ninhydrin has been widely used for the estimation of lipid serine. The main disadvantage of the method is its lack of sensitivity. Ethanolamine and serine have been estimated by periodic acid oxidation after their hydrolysis and separation[277]. But the drawback of this method is that ninhydrin does not quantitatively react with amino acids and the colour is affected by metal impurities in the paper. The liberated formaldehyde is complexed with chromotropic acid. Periodic acid is more specific for the determination of lipid ethanolamine and serine than the other amine reagents since it requires a hydroxyl group vicinal to an amino group.

In colorimetric methods of estimation of ethanolamine and serine the dinitrophenylhydrazine method is the most sensitive and accurate. Ammonia interferes with this method. Ninhydrin has been used to estimate amino nitrogen and has the advantage of being a stable reagent and the coloured products are the same for all primary amines. The disadvantage is that the colour of the reaction products is unstable. Lea and Rhodes have used ninhydrin reaction in buffered methyl cellosolve to estimate ethanolamine and serine in unhydrolysed

phospholipids[302,326]. The results with egg phospholipids showed considerable variation in molar colour yield while free ethanolamine and serine gave more consistent result with greater colour yield. A critical review of these methods has been given by McKibbin[300].

Choline — The quantitative estimation of choline in tissue extracts and hydrolysates is subject to the limitations inherent in the extraction procedures and in the specificity and sensitivity of the methods employed[331-338]. The hydrolytic procedures of phospholipids may influence the quantities of its nitrogenous constituents. The colorimetric reineckate method is generally used for routine analysis because of its simplicity and speed. The specificity of the method depends on the nature of the hydrolysis. A more sensitive method is based on the precipitation of choline as the periodide[339]. The method is quite sensitive, but is not very specific because many other bases, proteins, peptones, purines and alkaloids give positive colours. Appleton *et al.*[336] have developed a very sensitive method for the estimation of a choline-iodide complex. Choline in phospholipid extracts is precipitated as the iodine complex which is extracted with ethylene dichloride and its absorption was measured at 365 mμ. The excess of free iodine does not interfere and betaine, urea, creatine, creatinine, uric acid, cystine, histidin, guanidine, lysine and arginine are not precipitated by the iodine reagent. As little as 5 μg. of choline can be measured. The various methods for choline estimation have been critically reviewed by Engel *et al.*[339].

Fatty acid ester — A number of methods are available for the determination of fatty acid esters using hydroxamic acid formation coupled with ferric ion complex production[340-350]. However, none of the methods yields quantitative results for cholesterol esters of long chain fatty acids. Recently, Skidmore and Entenman[349] have modified the above methods to give quantitative estimation of long chain esters of cholesterol.

Plasmalogens — The methods most widely used for the determination of free and plasmalogen bound aldehydes in lipid fractions are the fuchsin-sulphurous acid methods[308,342,351-353], the *p*-nitrophenylhydrazine method[354,355] and iodometric titration[356-358]. The fuchsin-sulphurous acid method has the advantage of being highly sensitive but is not very specific. The *p*-nitrophenylhydrazine method is very specific but tends to give too low values owing to the low rate of reaction. The iodometric method is, on the one hand, too specific in that it determines only the unsaturated structures in which the aldehydes are bound (alkenyl ether structure); on the other hand, polyunsaturated acyl groups may give rise to too high values.

Identification of hydrolytic products of phospholipids — From the foregoing discussion on the separation of phospholipids by column and impregnated paper chromatography, it is apparent that elution pattern of phospholipid components from the column and the mobilities on the paper should not be considered as the conclusive proof of their identification. As stated earlier, all the lipid components must be chemically characterized before conclusions about their identity are made. This can be further substantiated by total or partial hydrolysis of the phosphatides.

Total acid hydrolysis of phospholipids — The phosphatides are hydrolysed with 2*N* HCl in sealed tubes at 110°C. for 48 hr. This hydrolysis will split phospholipids completely to yield glycerol, serine, ethanolamine, choline, inositol, fatty acids and aldehydes. The hydrolysis products can be estimated chemically. Recently, Wheeldon *et al.*[277] have reported a procedure of hydrolysis coupled with enzymatic hydrolysis to estimate glycerol, inositol, ethanolamine and serine in the same hydrolysate. It has been reported that 6*N* HCl hydrolysis destroys some of the glycerol amino acids, ethanolamine and serine[268,277,293]. In certain cases phosphatidyl ethanolamine and phosphatidyl serine are not completely hydrolysed[330]. The hydrolysis products yield important information on the chemical composition of phospholipid molecules.

Partial acid or alkaline hydrolysis — These reactions have been widely used for the identification of phospholipids. The phosphatides are hydrolysed under specified mild conditions with methanolic-NaOH or with trichloracetic or acetic acid[329]. In general mild alkaline hydrolysis splits ester bonds and liberates fatty acids. In methanol, the fatty acids are cleaved off as methyl esters. Acetic acid hydrolysis cleaves the vinyl ether bond of plasmalogen to yield fatty aldehydes. The resulting water-soluble phosphate esters are isolated and then separated by paper chromatography and/or ionophoresis. The quantitative analysis is carried out by elution of the spots and determination of phosphorus in each spot. Some phospholipids resist alkaline hydrolysis[329].

Mild alkaline hydrolysis should be carried out under carefully controlled conditions. Uncontrolled mild alkaline hydrolysis gives rise to cyclization, acetylation and changes in the mobilities of phosphate esters[116,329].

Marinetti has used methanolic sodium for the hydrolysis of neutral lipids and phospholipids[116]. The ester phosphatides are completely deacylated at room temperature within 10 min. Sphingomyelin and cerebrosides are stable towards this reagent and the plasmalogens of monovinyl ether are converted to their corresponding lysoplasmalogens. The water-soluble phosphate esters formed by the methoxide hydrolysis can be placed directly on filter paper and separated by Dawson method[329]. There is no need to neutralize the methoxide or to isolate the water-soluble phosphate esters before chromatographing them. It is important to keep the reaction time below 30 min., otherwise the N-base of the phosphate esters will be split. Marinetti has used this hydrolytic procedure for the preparation of lysophosphatides (35 per cent yield) and mono- and diglycerides by carrying out the reaction at 0°C.

Plasmalogens are estimated by hydrolysing with 90 per cent acetic acid containing one drop of saturated mercuric chloride for 30 min. at 90°C. (ref. 359). Lysophosphatides are produced, and they can be estimated chromatographically. Dawson estimated plasmalogens by hydrolysing ' lower phase ' obtained from alkaline hydrolysis (see above) with 1 per cent trichloracetic acid containing 5 millimoles of $HgCl_2$ for 30 min. at 37°C. The phosphate esters

produced were chromatographed and phosphorus estimated in each spot[329].

Haemolysis test — Lysophosphatides, i.e. lysolecithin and lysoPE, are known to be strong haemolytic substances of red blood cells. Recently, Gottfried and Rapport[360] have reported a method by which lysophosphatides could be estimated qualitatively.

General Comments

From the foregoing discussion it is apparent that no single method is complete by itself, but when supplemented with other methods such as silicic acid column chromatography, paper and thin layer chromatography, total and partial hydrolysis, infrared spectroscopy, etc., yield valuable information about the isolated lipids. The fractions obtained by silicic acid column chromatography can further be purified by rechromatography on silicic acid columns or other types of columns, e.g. PE and PS, which are not completely resolved on silicic acid columns can best be separated on ammonium silicate columns. The combination of DEAE-cellulose and silicic acid column chromatography can be used for isolation of purer lipid fractions. Cellulose columns are best for qualitative and quantitative estimation of lipids and for checking the purity of column fractions. Mild alkaline and acid hydrolysis can be used for identifying and quantitizing the alkali-labile, acid-labile and alkali-acid-stable phospholipids. Complete acid hydrolysis can be used for estimation of major amino acid moieties in the phospholipid fractions, and also to determine the molar ratios of their constituents. Fatty acid ester group determination can be utilized for determining the degree of esterification of glycerol and glycerophosphate moieties of glycerides and phospholipids. All these methods have limitations and the investigator should always be cognizant of these pitfalls. As many criteria as possible should be used to identify a naturally occurring lipid.

In spite of the encouraging progress made in the knowledge of lipids and the greatly improved methods developed for their isolation and characterization, many problems of lipid metabolism and function still remain to be solved. There are many new lipid classes and individual molecular species still to be isolated and characterized.

Summary

The various methods employed for isolation of intact lipids from biological sources have been reviewed and discussed. In spite of the limitations of silicic acid as an ideal adsorbent for all classes of lipids, it has been successfully used for obtaining lipids in intact form. Silicic acid column chromatography supplemented with other types of column chromatography, i.e. DEAE-cellulose, ammonium silicate, cellulose, aluminium oxide, florisil, etc., has proved of immense value in resolving complex lipid mixtures from biological sources. Silicic acid impregnated paper and glass fibre paper, thin layer, formaldehyde coated, tetralin impregnated paper chromatography are simple and speedy ways of isolation and tentative identification of various lipid components. These lipid components can further be identified and characterized by their chemical reactions and their physico-chemical properties, e.g. infrared spectroscopy, nuclear magnetic resonance mass spectroscopy, etc. Gas-liquid chromatography has proved of immense potentiality in resolving fatty acid mixtures, fatty aldehydes, fatty alcohols, steroids and mono-, di- and triglycerides. The review is by no means complete, because so many unsolved problems still exist in lipid chemistry and metabolism for which solutions are yet to be found.

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REVIEWS

THE THEORETICAL SIGNIFICANCE OF EXPERIMENTAL RELATIVITY by R. H. Dicke (Blackie & Sons Ltd, London), 1964. Pp. xii+153. Price 32*s*. 6*d*.

The volume under review represents the notes including a number of reprinted papers for a course of lectures at the Les Houches Summer School in July 1963, given by one of the leading authorities on both the theoretical and experimental aspects of relativity. The aim of the course was to present the rather meagre experimental material available on the subject and use it as a criterion for defining a class of relativistic theories of gravitation which is compatible with it.

The various experiments are classified into the following groups: (1) Null experiments of extreme precision and of ordinary precision. Under this heading the early experiment of Eotvos and the later precision experiment of Dicke and his collaborators are dealt with at length and their importance which rests on the fact that the null result obtained is a necessary condition to be satisfied in order that Einstein's general theory holds is pointed out. (2) Next, the experiment of Hughes *et al.* and Drever which demonstrates the isotropy of space is discussed. This experiment eliminates the possibility of introducing a second long-range tensor field (in addition to the ordinary gravitational field tensor) into the theory. A zero mass long-range scalar field is not, however, excluded. (3) The ether drift experiments, the prototype of which is the famous Michelson-Morley experiment, and the three tests of general relativity are next discussed. The poor accuracy of observation on the gravitational deflection of light and on the perihelion rotation of mercury (data which could be used to exclude gravitational theories incorporating both scalar and tensor fields) is pointed out.

There are twelve appendices, reproducing some of the papers of the author and his collaborators. The subjects range from Mach's principle and a new theory of gravitation based on a long-range scalar interaction to cosmology and its implications for terrestrial phenomena (such as the earth's expansion, the geomagnetic field and the surface temperature of the earth), stellar and galactic evolution rates and dating of the galaxy by uranium decay.

The subject matter of the volume forms delectable reading; but this is marred by the numerous printing errors (which indicate bad proof-reading). At one place the symbol for the cosmological constant changes from Λ to A and then to V within a few lines. At another place we are wafted to gastronomical regions by a reference to a ' supper limit ' ! These and similar mistakes are perhaps the price one has to pay for emphasizing speed of publication.

K. VENKATESAN

ADVANCES IN HEAT TRANSFER: Vol. 2, edited by J. P. Harnett & T. F. Irvine (Jr) (Academic Press Inc., New York), 1965. Pp. xi+465. Price $ 16.00

The first volume of *Advances in heat transfer* appeared in 1964 and covered six topics, viz. (1) The interaction of thermal radiation with conduction and convection heat transfer by R. D. Cess, (2) Application of integral methods to transient non-linear heat transfer by T. R. Goodman, (3) Heat and mass transfer in capillary-porous bodies by A. V. Luikov, (4) Boiling by G. Leppert and C. C. Pitts, (5) The influence of electric and magnetic fields on heat transfer to electrically conducting fluids by M. F. Romig, and (6) Fluid mechanics and heat transfer of two-phase annular-dispersed flow by M. Silvestri. Research in the field of heat transfer continues vigorously as the work is connected with important current fields of interest like atomic energy, aviation and astronautics. Because of advances in the field of instrumentation, high speed photography and high speed computation, experimental work on complex problems can now be tackled with great vigour. Because of the highly specialized nature of the developments taking place, it is difficult for research workers in the broad field of heat transfer to know what is happening in the field unless reviews are available as various topics compiled by specialists who could give adequate background information and point out the implications of hundreds of papers on each topic appearing in journals.

The present volume covers the following five important topics in heat transfer and each topic is reviewed by an eminent specialist in the respective field: (1) Turbulent boundary layer heat transfer from rapidly accelerating flow of rocket combustion gases and of heated air by D. R. Bartz, (2) Chemical reacting non-equilibrium boundary layers by P. M. Chung, (3) Low density heat transfer by F. M. Devienne, (4) Heat transfer in non-Newtonian fluids by A. B. Metzner, and (5) Radiant heat transfer between surfaces by E. M. Sparrow.

The review on heat transfer from rapidly accelerating flows by Bartz is a topic of great current interest and deals with high temperature, high pressure heat transfer connected with rocket engines on which considerable research is going on. However, more work is still required before we can say that the problem is solved. In this excellent review Bartz has discussed at length the background of the problems and the possible analytical approach for the solution of the turbulent boundary layer development and heat transfer in rapidly accelerating flows. The review also deals with air experiments and measurements in rocket thrust-chamber.

The next review on chemical reacting non-equilibrium boundary layer by Chung concerns with an old problem which classically received much attention in connection with the combustion of solid and liquid fuels and also in catalytic reactors. During the last 10 years the problem has received great attention because of the advent of higher velocity vehicles in the aerospace technology. The review deals with the governing equations, chemical kinetics, boundary layers with surface reactions and gas phase reactions.

The problem of low density heat transfer which is the next review has received a spurt of interest

because of the current problems of long-range missiles and satellites. F. M. Devienne has given an excellent review of the advances in heat transfer in rarefied gases stressing the results obtained recently and pointing out those problems not yet solved. Accommodation coefficient has received considerable attention in this review.

Prof. A. B. Metzner has written a review on heat transfer in non-Newtonian fluids. Being a prominent worker in this field, Prof. Metzner's review is authoritative and comprehensive. The whole field of heat transfer in non-Newtonian fluids is a recent one and most of the work done in the field was published during the last 25 years. The review covers rheological and thermal properties of non-Newtonian fluids, heat transfer in steady ducted flow fields, boundary layer problems and miscellaneous heat transfer problems.

The last review is on radiation heat transfer between surfaces by Prof. E. M. Sparrow. The subject of radiation heat transfer has received a renewed interest because of its importance in space flights and high temperature energy sources. Most of the review is devoted to recently developed computational methods and results for radiant interchange between surfaces which are separated by non-participating media. The analysis and solution of radiation problems involving participating media have been discussed by R. D. Cess in the first volume of the book.

The book is extremely valuable for research workers working in the broad field of heat transfer whether they are physicists, mechanical engineers or chemical engineers. To the non-specialist it serves to keep him up to date with what is happening in this rapidly expanding exciting field.

N. R. KULOOR

AEROSPACE RANGES: INSTRUMENTATION, PRINCIPLES OF GUIDED MISSILE DESIGN by Joseph J. Scavullo & Frederick J. Paul (D. Van Nostrand Co. Inc., New York), 1965. Pp. xv+457. Price $ 15.75

A modern aerospace range accomplishes the missions assigned to it with the help of range instruments. The mainline range instruments have to measure accurately the trajectories and internal performance of test vehicles, control the flight of targets and boosters, correlate the observations with range time and process the data gathered. The book *Aerospace ranges: Instrumentation* is one of a series entitled Principles of Guided Missile Design. It presents a comprehensive review of the instrumentation that goes into the make-up of an aerospace range, their functions and their limitations. The book begins with a chapter giving a general outline of the requirements of a test range and problems associated with range instrumentation. Subsequent five chapters treat in some detail individual systems, such as optical and photographic systems, telemetry and flight test instrumentation, electronic instruments for trajectory measurements, data processing instrumentation and systems necessary for test communication and time signal distribution. The concluding chapter deals with the nature, magnitude and causes of the main inaccuracies of measurements by range instrumentation, a necessary and useful chapter indeed.

The book is a source of information to students and to persons newly introduced to a test range. It provides answers to numerous questions that arise in their mind, answers that are not easy to obtain from men working on a busy range. The knowledge presented in the book helps one to get a comprehension of aerospace range instrumentation and the working of a test range as a whole. The book, however, falls short of the needs of a serious engineer interested in the design of systems and subsystems for a range. Detailed discussions concerning the design philosophy of systems, subsystems and instrumented payloads have been avoided and references to such material are not presented for the reason that they are not accessible to all. This vital information still eludes the engineer. It is either classified or remains the monopoly of the specialists.

The book is certainly of use to all connected with a range.

T. S. G. SASTRY

PHYSICAL PROPERTIES OF MAGNETICALLY ORDERED CRYSTALS by E. A. Turov (Academic Press Inc., New York), 1965. Pp. xx+222. Price $ 10.00

Some of the physical properties of magnetic crystals depend on the nature of the energy spectrum of the elementary excitations over the ground state of the system. While the available microscopic theories provide a basic understanding governing the nature of excitations, it is difficult to derive quantitative informations from them. It is in this context that phenomenological approaches prove to be more fruitful. This book is an excellent example of the application of phenomenological methods to the exposition of physical phenomena in ferro- and antiferromagnetic insulators. Since the major contribution in this direction has come from Russian workers, the fact that the author of the book belongs to the same school is an additional virtue. One gets a first hand account of their work. The description of the weak ferromagnetism is particularly appealing.

This is indeed a valuable book and it will be of great assistance to the workers in this field.

K. P. SINHA

OSCILLOSCOPE MEASURING TECHNIQUES by J. Czech (Centrex Publishing Co., Eindhoven), 1965. Pp. xviii+620. Price Rs 84.00

This is another valuable addition to the Philips Technical Library. It contains a wealth of information and is very useful as a reference book for senior students as well as engineers who are engaged in CRO design or its application and are seeking up-to-date knowledge on the subject.

The book is a revision of the book *Cathode ray oscilloscope* by the same author published in 1957 and has resulted in a completely new book. The book is well written and A. Smith Hardy has done a fine job of almost flawless translation from German. The book contains a fabulously rich and well-chosen bibliography, but unfortunately it contains a very large number of references in German which are not so easily available to English readers.

The book is divided into four parts. Part I describes the various building blocks which make the

instrument complete as a measuring tool. The information is up to date as it deals with latest techniques of storage and sampling type oscilloscopes. Part II deals with the general measuring techniques. It includes amplitude, phase and frequency measurements, including use of CRO as a null-indicator. In addition, the electronic switch and intensity modulation have been dealt with in detail. Part III deals with a very extensive field of practical oscilloscope measuring techniques from different fields of electrical and non-electrical instrumentation. Part IV deals in detail with photographic recording and large-picture projection of oscillograms which is of importance in investigation, training and industrial fields.

The book would have been more complete if it had devoted some space to (i) the solid state circuitry which is increasingly being applied in CRO techniques; (ii) plug-in type units and their applications which have revolutionized the CRO technology and have made it an extremely versatile instrument; and (iii) sampling units commercially available at present which make it possible to convert a medium performance CRO to behave as a sampling type CRO. Also more space should have been devoted to circuit examples from renowned manufacturers other than those adopted from N. V. Philips instruments.

Despite these shortcomings, this book could become a standard reference for CRO techniques.

Y. N. BAPAT

APPLICATIONS OF NMR SPECTROSCOPY IN ORGANIC CHEMISTRY: ILLUSTRATIONS FROM THE STEROID FIELD by N. S. Bhacca & D. H. Williams (Holden Day Inc., San Francisco), 1964. Pp. ix+198. Price $ 8.75

Nuclear magnetic resonance (NMR) has become an indispensable tool for the organic chemists and a number of books on the subject appeared during 1959-61. As can be expected, a large number of applications of this tool have appeared since then and the book under review provides a cogent account of these applications in the field of steroids. The authors refer the reader to other, earlier monographs on the subject for a detailed discussion of the theory of NMR and proceed straightaway with its applications. The book consists of eight chapters. The first one is introductory in character, while the next three chapters deal with the chemical shifts. A concise account of long-range spin-spin coupling is given in Chapter 5. The remaining three chapters discuss the determination of configuration and conformation, solvent effects and miscellaneous topics respectively. The usefulness of spin-spin decoupling technique is illustrated at various places. The writing is very clear and the arrangement of subject matter is excellent. The book should serve as a valuable introduction to the subject of NMR as well as a useful reference work for the steroid chemist.

SUKH DEV

ELECTROANALYTICAL METHODS IN BIOCHEMISTRY by William C. Purdy (McGraw-Hill Book Co. Inc., New York), 1965. Pp. xiv+354. Price $ 12.50

The book which is meant primarily for biochemists consists of nine chapters: Introduction, Conductivity and high frequency methods, Potentiometric methods, Potentiometric titration curves, Polarography — Principles of the method, Polarography — The shape of the polarographic wave, Solid electrode voltammetry and amperometry, Coulometry and coulometric titrations, Miscellaneous electroanalytical methods. All the chapters are written simply and clearly keeping a happy balance between theoretical aspects and applications of the techniques. The appendix includes a variety of useful electrochemical data. Polarography is treated in considerable detail in two chapters and these contain considerable up-to-date information. However, data on metal ions of biochemical importance have not been assembled in any logical order. It would have been more useful if this information had been classified into some sequence, such as polarographic behaviour of metal ions (1) in buffer systems commonly used in biochemical work; (2) in the presence of intermediary metabolites and related compounds; and (3) in the presence of macromolecules. A more detailed account of square-wave polarography and its possible applications in biochemistry would have been a welcome addition. It is surprising that in the chapter on potentiometric titration curves no mention is even made of titration curves of proteins and a study of metal-protein interactions with this technique.

The book is pleasantly free from printing errors. However, there are other errors such as (a) on page 19, in equation 2-25, the term on the left-hand side is wrongly given as C whereas it should be C_{MN}; (b) on page 188, the statement that " Tanford found that the polarographic data agree well with results obtained from spectrophotometric studies " should refer to copper-protein interaction and not to zinc-protein interaction as stated in the text; and (c) on page 189, the statement that " the diffusion current increases at a *p*H value greater than 6 in solutions buffered by protein " should read the diffusion current *decreases*. On page 183, there is a howler, egg serum albumin !

The book should serve as a ready reference for biochemists interested in using electroanalytical techniques.

M. S. NARASINGA RAO

MODERN METHODS OF CHEMICAL ANALYSIS by J. A. Barnard & R. Chayen (McGraw-Hill Book Co. Inc., New York), 1965. Pp. xiii+273. Price 42*s*. 6*d*.

This book is addressed to two types of readers, one who seeks information on some aspect of analytical chemistry and to the undergraduate who looks for an overall picture of analytical chemistry in its present state of development. It consists of six chapters. The first five are devoted to chemical and instrumental techniques, while the last somewhat longish chapter relates to separation techniques. The principle of each method is outlined in brief followed by assorted illustrative experiments. Adequate references to the originals are given at the end of each chapter. The fairly comprehensive index of organic chemical reagents for colorimetric analysis included in Chapter 3 is extremely useful.

While it is difficult to do justice to all aspects of modern analytical chemistry in a volume of this size,

the authors have spared no pains to make the text all-embracing. Even so, there are a few omissions. For instance, there is no mention of moisture determination by the Karl Fischer reagent. A.C. polarography, colorimetry and controlled potential electrolysis are not referred to. Nor does fluorescence X-ray spectroscopy and the related electron probe micro-analysis find a place. The separation of electrolytes from non-electrolytes by ion-exclusion through ion exchange resin is also not included.

On the whole the book is well conceived and well got-up and is a helpful guide to the aspiring student.

P. R. SUBBARAMAN

TETRACYCLIC TRITERPENES by Guy Ourisson, Pierre Crabbe & Oscer R. Rodig (Holden Day Inc., San Francisco), 1964. Pp. 237. Price $ 8.75

This book, which is an adaptation of the French edition (1961) by G. Ourisson and P. Crabbe, presents the subject matter of tetracyclic triterpenes with a refreshingly original approach. The subject matter has been divided into four chapters and a catalogue. The introductory Chapter 1 is followed by the main chapter, which is entitled reactions of tetracyclic triterpenes. These reactions are discussed in terms of functionalities ring-wise, and stereoelectronic factors are highlighted. Chapter 3 discusses syntheses of tetracyclic triterpenes, while Chapter 4 gives some aspects of tetracyclic triterpene biochemistry. The catalogue gives an up-to-date coverage of various naturally occurring tetracyclic triterpenes and their derivatives, in terms of physical constants, and ultraviolet, infrared and rotatory data.

The authors are to be congratulated for presenting the subject matter reaction-wise, which has resulted in a concise and lucid treatment of the subject. The book, which is very well produced, is recommended alike to students and the specialists.

SUKH DEV

HISTORY UNDER THE SEA by Mendel Peterson (Smithsonian Institute, Washington), 1965. Pp. 108 +56 plates. Price $ 3.00

Man's interest in the exploration of the seas has grown considerably in recent years. He is devoting his attention more and more towards developing techniques of underwater exploration so that he may see for himself the various treasures that Nature has strewn on the sea bed. Apart from his interest in the natural history of the sea bed as well as its geological history, he is also anxious to study what we may call the "man-made history that lies on the sea bed". In short, man has begun to carry his archaeological studies from land to the sea in order that he may 'unearth' the vast submerged treasures which the sea had claimed from the kingdoms that once existed on the coasts of continents and thereby reconstruct the past history.

This interesting book is a practical guide for the undersea archaeologist. In the words of the author himself, "there has not been a single volume so far to which a serious underwater explorer may turn for instruction on exploration, recovery, preservation techniques and identification of artifacts". This book fulfils this long-felt need to a great extent.

Beginning with the historical aspects and narration of the various undersea archaeological exploration in the Western Hemisphere, particularly those undertaken during the past ten years by the Smithsonian Institution in the region of the Gulf of Mexico, the author goes on to discuss the various search techniques, the condition of underwater sites, surveying underwater sites, recovery techniques, and preservation of recovered material in six chapters. The last chapter is devoted to the problem of identification of shipwreck sites, which apart from its archaeological aspects, has great significance from the naval point of view.

The presentation is simple and the various chapters include considerable practical details. The chapter on the preservation of recovered material contains information of great value to a museum technologist. The bibliography, although described as selective, is fairly comprehensive. The 56 plates showing photographs of underwater surveying and the recovered material add to the value of the publication.

R. JAYARAMAN

THE IDEAS OF BIOLOGY by John Tyler Bonner (Methuen & Co. Ltd, London), 1965. Pp. 240. Price 15*s*.

The book written as a supplementary reading with the biology text also serves a good purpose for an intelligent layman to acquaint himself with the concepts of biology.

As the title suggests the book deals more with ideas than facts. The book, spread over six chapters entitled Cell, Evolution, Genetics, Development, Simple to complex, and Man, includes 24 figures which facilitate understanding the ideas set forth in the text.

A reading list and an index are provided at the end of the book. The book is worth possessing by all interested in biology despite the unusually high price of this paperback edition.

KULDIP CHAND

SCIENTIFIC SOCIETIES IN THE UNITED STATES by Ralph S. Bates (Pergamon Press Ltd, Oxford), Third Edition, 1965. Pp. 326. Price 63*s*.

A book of more than ordinary interest, the *Scientific Societies in the United States* presents the history of science associations since the founding fathers established the American Philosophical Society in 1729. Prior to that date, the scientific societies of Europe, particularly the Royal Society of London, fulfilled the needs of American scientists and the more prominent among them, Cotton Mather, Roger Williams and Benjamin Franklin, for instance, were members of the Royal Society and contributed to its *Transactions*. The umbilical cord connecting American science with European learned societies was severed in the early years of the eighteenth century and from then on numerous regional and national societies, academies, associations and councils came into existence as research enterprise in America gained momentum and attained an impressive magnitude, and American scientists made increasingly important and significant contributions to the pool of knowledge.

The book under review brings out vividly the dynamism of scientific societies in the United States established by scientists who firmly subscribed to the 'principle of association' and believed in 'action by joint force'. Science is clearly the concern of scientists; and by virtue of their knowledge and understanding, scientists alone can speak for science. As associations of scientists, the scientific societies of America have exerted considerable influence on the progress of science in the country and on national science policies. Almost every conceivable activity germane to the development of science is covered by the societies. There are societies for the promotion of industrial and vocational education and for 'encouraging students on the threshold of a scientific career, by making scientific work seem more attractive to them'. Junior academies have been established for high school students and there are associations to foster improvements in teaching of science and to exchange ideas on teaching techniques. Societies have been established for the dissemination of science, for giving impetus to improved types of journals and for promoting excellence in the preparation, editing and publication of scientific and technical documents. The advent of the atomic and space age has seen the rise of new societies and international cooperation in science has been greatly strengthened in recent years.

The American Scientific Societies have been a potent force in the intellectual life of the people and at times of national crisis, like the Civil War, World War I and World War II, they responded ably to the call for mobilizing the nation's scientific resources. The societies are the accepted agencies for the publication of the scientific output of the nation and the publications issued by them not only serve the needs of contemporary science, but also constitute the intellectual heritage of generations to come. Their influence in diffusing scientific information among the people and in leavening scientific education is unique and impressive.

The fact that the book is in its third edition is an eloquent testimony to its popularity. It includes a valuable bibliography and a chronology of science and technology in the United States. The book is not altogether free from errors. It is stated, on page 3, that the Royal Society began the publication of *Philosophical Transactions* in 1756; the actual date is 1665. The hydrogen isotope, deuterium, is made synonymous with 'heavy water' (p. 139 last line and p. 140 first line); line 27, on page 184, should read 'collect mineral, botanical and zoological specimens'; the date of establishing the Society for Industrial Microbiology is given as 1949 on page 208 and as 1948 on page 321. It is hoped that all the errors would be corrected in the subsequent edition.

The book provides stimulating reading. It has a message for scientists in every country to form associations to examine objectively all issues which conduce to the advancement of science. The book should be studied by the executives of all scientific societies in India with a view to ascertaining how best the societies can be activated to higher levels of achievement. It is warmly recommended to all college libraries and to individuals interested in the history of science.

B. N. SASTRI

PUBLICATIONS RECEIVED

WET COMBUSTION AND CATALYTIC METHODS IN MICROANALYSIS: Vol. 2, edited by J. A. Kuck (Gordon & Breach Science Publishers, New York), 1965. Pp. xx+412. Price $ 21.00

INTRODUCTION TO ATOMIC AND NUCLEAR PHYSICS by Otto Oldenberg (McGraw-Hill Book Co. Inc., New York), Third Edition, 1961. Pp. xiii+380

A BIBLIOGRAPHY OF INDOLOGY: Vol. 2 — INDIAN BOTANY, Part II — K-Z, compiled by V. Narayanaswami (National Library, Calcutta), 1965. Pp. xxx+412. Price Rs 8.25

COMPLEX ANALYSIS by Lars V. Ahlfors (McGraw-Hill Book Co. Inc., New York), Second Edition, 1966. Pp. xiii+317. Price $ 8.95

SOUND CONTROL OF THERMAL INSULATION OF BUILDINGS by Paul Dunham Close (Reinhold Publishing Corp., New York), 1966. Pp. vii+502. Price $ 17.00

RELATIVITY AND THE NEW ENERGY MECHANICS by Jakob Mandelker (Philosophical Library, New York), 1966. Pp. xii+84. Price $ 4.00

BIOSYNTHETIC PATHWAYS IN HIGHER PLANTS by J. B. Pridhan (Academic Press Inc., New York), 1965. Pp. xi+212. Price 75*s*.

THE ENCYCLOPEDIA OF PHYSICS edited by R. M. Besancon (Reinhold Publishing Corp., New York), 1966. Pp. xii+832. Price $ 25.00

CONSTRUCTION, REGLAGE ET ESSAISDES INSTRUMENTS D'OPTIQUE by M. Lachenaud (Dunod, Paris), 1966. Pp. xxvii+599

NOTES & NEWS

'Transit time' diodes — A new type of semiconductor for microwave power generation

Three new types of semiconductors called the 'transit time' diodes developed at the Bell Telephone Laboratories have potentialities for being developed as the first solid state devices that can generate sufficient power for use in microwave systems. Till now the only practical sources of substantial microwave power are the vacuum tube devices which have the disadvantages of being bulky, expensive and having a limited life. The solid state devices though free from these defects could not produce the high power levels required in the microwave systems. Even though none of the 'transit time' devices so far developed has equalled the power handling capacity of vacuum tubes (i.e. above 1 kW.), still because of their promise of providing more power output (or higher frequency operation with a given power output) compared to transistors or tunnel diodes, these will be useful for the high gain, high power stages that follow a low noise preamplifier. However, because of their high noise output, these devices are not likely to be used in the sensitive, low noise input stages of a receiver.

All the three types of 'transit time' devices developed, viz. (1) bulk gallium arsenide wafer, (2) silicon avalanche diodes and (3) Read avalanche diodes, operate at the room temperature as self-excited generators or oscillators. A 'transit time' device gets its name from the fact that its operating frequency is determined by the time it takes the electrons to move or 'transit' through the material. The details and mechanism of working of these devices are as follows.

The bulk gallium arsenide has no junctions but operates due to the 'Gunn effect' when a suitable d.c. field is applied across a wafer of uniform gallium arsenide. The frequency of microwaves generated is a function of the time it takes for the moving domains of high electrical resistance accompanying the Gunn effect to travel through the semiconductor and is controlled by the length of the material along the applied electric field. While operating continuously, these devices amplified signals in the 2-10 gigacycle range with power outputs exceeding 60 mW.

The second type of transit device, viz. silicon avalanche diode, is made from a semiconductor junction which is reverse biased to produce avalanche breakdown. As amplifiers these devices achieve a gain of 20 db. with a bandwidth of 30 Mc/s. at power output of several milliwatts.

The Read avalanche diode, a complex electrical structure, is usually made of silicon with a PNIN structure whose impurity profiles are controlled by phosphorus and boron diffusion. Recently developed diodes of this type have produced 19 mW. of power at 5·2 gigacycles with an efficiency of about 1·5 per cent.

Used as oscillators, some of these 'transit time' devices have exhibited spectral purities better than any other self-excited microwave oscillator, generating frequencies that are relatively free of random modulation and hence may find wide use as general purpose microwave oscillators for receivers or low power transmitters [*Bell Lab. Rec.*, **43** (1965), 409].

Laboratory simulation and measurement of lunar topography

Characteristics of the moon's surface roughness have been determined at the Bell Telephone Laboratories, New Jersey, by bouncing a laser beam off materials prepared in the laboratory and comparing these findings with measurements of microwave reflections from the moon's surface. The statistical values determined by this method for the first time for the dimensions of height, length and slope of lunar irregularities compare well with the estimates based on photographs taken by the Ranger 7 moon probe. The values for these dimensions are: height of irregularities, 16 ± 4 in.; distance between irregularities, $9 \pm 2{\cdot}5$ ft; and slope, 8 ± 4 deg. This attempt, in addition to furnishing a basis for determining lunar terrain, has provided a method of deducing statistical values for the surface irregularities of planets.

A surface having random distribution of irregularities required for the investigation of backscatter characteristics during bombardment of the surface with laser beams was obtained by blasting blocks of soft aluminium with different size alumina grits. These rough surfaces were electropolished to remove burrs and sharp points since this process has an effect similar to the processes believed to be involved in the shaping of the surface of the moon, specifically the smoothing of crater edges through bombardment by small meteorites. To study the statistical properties of the prepared surfaces, a stylus was run over the samples and the tracings showing the contour of the surfaces were statistically analysed on a computer. Then, in controlled experiments with laser light scattered from these surfaces, the energy distribution was obtained as a function of angle of incidence for each surface. The curve for one of the surfaces studied was almost identical to the curve showing the variation of microwave energy backscattered from the moon. Hence, it was concluded that the particular aluminium sample has statistical properties similar to that of the moon. The dielectric constant of the lunar surface was also determined from a comparison of the energy level of backscattered laser radiations at normal incidence on the aluminium sample with the energy level backscattered from the moon. This value ($1{\cdot}9 \pm 0{\cdot}3$) was near the values of dielectric constant for porous volcanic glass or loose sand (*News from Bell Telephone Laboratories, New York, Release dated* 30 *September* 1965).

New type of semiconductor

During the course of systematic studies on thermistors, Hisaw Futaki of Hitachi Ltd reported a new type of semiconductor called critical temperature resistor (CTR). This semiconductor shows an abrupt resistance change of 2-4 orders of magnitude at 68°C. with negative temperature coefficient. CTR is prepared by sintering a mixture of V_2O_5 and an acidic oxide (B, Si or P oxide) or a basic oxide (W, Ca, Sr or Ba oxide) in a suitable reducing atmosphere. Subsequent experiments showed that CTR prepared using ternary systems of vanadium oxide, the acidic oxide and the basic oxide has better characteristics than that prepared from the binary system in such properties as the large magnitude of the abrupt resistance change, the large negative temperature coefficient of resistance, stability at higher temperature and the ease in its manufacture.

Life of the samples obtained under suitable conditions is theoretically estimated to be several tens of years in air at 200°C. The large temperature coefficient of resistance of this newly developed semiconductor — 30 times as large as that of the usual thermistor — offers many interesting applications. The following prospective applications of CTR are envisaged.

Based on the property of the resistance change by ambient temperature, it can be used in (a) thermometers and as a temperature controller, having sensitivity of $\pm 0{\cdot}5$°C.; (b) infrared detector in which output signal is 30 times as large as in the usual thermistor; and (c) thermal switches. By virtue of the large change in thermal dissipation constant it finds application in manometer, gas analyser, anemometer, etc. The property of thermal inertia enables it to be used in delay circuit of relay, low frequency oscillator. The use as thermal switch, automatic gain controller, high frequency oscillator and as logic element in a flip-flop circuit are other examples in which the CTR holds promising applications [*Japan J. appl. Phys.*, **4** (1) (1965), 28].

Induction and multisensitive end-product repression

In the classical example of 'sequential induction' mandelate is degraded by the following pathway:

$$\text{Mandelate } (S_1) \xrightarrow{E_1} \text{benzoyl formate } (S_2) \xrightarrow{E_2} \text{benzaldehyde } (S_3) \xrightarrow{E_3} \text{benzoate } (S_4) \xrightarrow{E_4} \text{catechol } (S_5) \xrightarrow{E_5} \beta\text{-oxoadipate} \xrightarrow{E_6} \text{succinyl CoA} + \text{acetyl CoA}$$

According to the original hypothesis such inductions are sequential, the product of each step acting as an inducer for the next enzyme in the sequence. It is now known that such induction is not sequential but is coordinated. E_1, E_2 and E_3 are induced by S_1, E_4 by benzoate, and E_5 and subsequent enzymes by catechol.

The enzymes E_1, E_2 and E_3 are related in function and are said to be under the control of a single 'regulon' (regulon is distinguished from the operon, in that it describes a group of genes not necessarily closely linked). J. Mandelstam and G. A. Jacoby [*Biochem. J.*, **94** (1965), 569] found that all the three, benzoate, catechol and succinate, repress E_1-E_3. They suggested three possible modes of action as due to (1) multisensitive repression, (2) repression by a common end-product, and (3) sequential repression. Using appropriate mutants, they ruled out the last two possibilities, showing thereby that the mechanism (1) is the most likely one.

Degradation of *p*-hydroxymandelate also follows a similar pathway. The intermediates of this pathway, *p*-OH benzoate, pyrocatechol and succinate, also repressed the enzymes E_1-E_3, which have been shown to be common to both the pathways. Benzaldehyde and *p*-OH benzaldehyde repressed E_1-E_3. This enigma of an enzyme being virtually completely repressed by its own substrate was solved when an alternate enzyme, oxidizing aromatic aldehyde, was detected. Multisensitive repression systems were also found in the second regulon in each pathway.

This coordinate induction has the advantage of reducing the lag phase in the production of enzymes. The 'multisensitive' control mechanism also overcomes the disadvantage in terms of protein economy that might occur in 'coordinate induction' in that when the end-product of a group of enzymes is present in excess, it represses the formation of all the preceding enzymes in the degradative pathway. It is also of interest that the points where the new regulons begin are those where either the degradative pathways of different aromatic compounds converge or where there is the likelihood of the concerned compounds to occur under natural conditions. So an enzyme is not synthesized unless its substrate is present and even then it is not synthesized if the end-products required for cell growth are already present in excess. The site of action of these repressors is, however, not known.— V. N. UMA

Antibody formation and the coding problem

Antibodies are typical globulins and hence their formation is essentially biosynthesis of proteins. According to the current concept of protein synthesis, proteins are formed on the ribosomes from a pool of free amino acids with *m*RNA which is coded from the chromosomal DNA. The conformation of the protein is believed to be determined by its amino acid sequence and no genetic information is needed.

In spite of the numerous theories of antibody formation there is no agreement about the selective role of the antigen and its role as the template. If the antigen has merely to select between different types of receptors, then it is not necessary to postulate interference of the antigen in the process of globulin formation or its modification. If the antigen interferes in this process in such a manner that antibodies are formed instead of normal globulins, the question naturally arises as to in which phase of protein synthesis this interference occurs and how the complementary conformation of the combination of the combining group of the antibody is accomplished?

The several possible ways in which a template molecule might interfere with any of the principal steps in protein synthesis are: (i) the replication of DNA, (ii) the

transcription of the genetic code into the nucleotide sequence in RNA molecules, (iii) the amino acid sequence, (iv) the folding of the peptide chain, and (v) in case of the antibody the combination of the two A-chains and the two B-chains of the globulin to give the antibody molecule of composition A_2B_2.

Any interference in step (i), replication of DNA, could be excluded as it would involve a mutagenic action of the antigen. Evidence is against such a supposition.

Modification of step (ii) or (iii) should lead to changes in the composition and sequence of amino acids in the antibody molecule. Occurrence of such changes is indicated first by small but significant differences in the peptide maps of antibodies against human and horse serum albumins and against different types of pneumococcal polysaccharides. Since the antigens contain a number of different determinant groups, the antibodies formed are heterogeneous mixtures directed against different groups. It is difficult to resolve whether the differences reflect differences in the antigenic determinants or merely reflect different distribution of the injected antigens in the organism and antibody formation in different organs or cells. This situation would be simplified by the isolation of hapten-specific antibodies and by comparison of their amino acid composition and sequence. Small but significant differences have been found in the amino acid composition of anti-Ars (cationic diazobenzene arsonate) and anti-R_4N (cationic diazobenzene N-trimethyl ammonium) [Koshaland, M. E. & Englberger, M. F., *Proc. nat. Acad. Sci., Wash.*, **50** (1963), 61; Groff, I. L. & Haurowitz, F., *Immunochemistry*, **1** (1964), 39]. Many of the peptide spots of the tryptic digests of anti-Ars and anti-R_4N were identical. The observed differences cannot be caused by genetic differences, because they were also found when the two azoproteins were injected into the same animal or when a double labelled azoprotein containing both Ars and R_4N was injected [Gold, E. F., Knight, K. L. & Haurowitz, F., *Biochem. biophys. Res. Commun.*, **18** (1965), 76]. The differences in the amino acid composition of hapten-specific antibodies seemed to indicate modification of step (ii) or (iii) of the protein synthesis system. It has been postulated that isotopically labelled antigen determinants are associated with *s*RNA or other RNAs, that antigenic determinants are present in the vicinity of the sites of amino acid incorporation and hence the modifying effect [Campbell, D. H. & Garvey, J. S., *Advanc. Immunol.*, **3** (1964), 262; Saha, A., Garvey, J. S. & Campbell, D. H., *Arch. Biochem. Biophys.*, **105** (1964), 179].

The conformation of the combining site of the antibody is determined principally by the three-dimensional shape of the antigenic determinant and much less by its charge and polarity. The questions that arise are: In which phase of the biosynthetic process is the information concerning the shape of the antigenic determinant transmitted to the sites of protein biosynthesis and how does this occur?

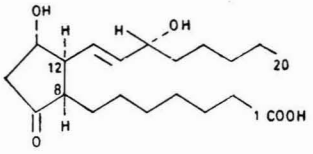
Since long-range forces do not exist in biological systems it must be postulated that the folding of the peptide chains, i.e. step (iv), occurs in the immediate vicinity of the antigenic determinant, although this seems to be incompatible with the observed differences in amino acid sequence and also with the claim that the conformation of the peptide chain is determined by its amino acid sequence. It could be said that conformation and amino acid sequence are interdependent and that changes in conformation caused by a template might secondarily lead to changes in amino acid sequence. This is quite contradictory to the original central dogma. However, external factors have been shown to modify the coding process [Commoner, B., *Nature, Lond.*, **203** (1964), 486]. The possibility of deviation from the central dogma in the biosynthesis of antibodies has been pointed out. F. Haurowitz [*Nature, Lond.*, **205** (1965), 847], on the basis of the above views, assumes that antigenic determinants are bound to some of the ribosomes or the *m*RNA molecules and the determinant group, which is usually rigid and polar, affects the biosynthesis system in two ways. Firstly, dipole induction and other intermolecular forces originating from the template induce a small portion of the newly formed peptide chain to fold over the surface of the antigenic determinant and thus to produce a complementary pattern. Secondly, while most of the aminoacyl residues are incorporated into the peptide chains of the antibody molecule according to *m*RNA, deviations from the code occur during the formation of the combining site. Only those aminoacyl residues are incorporated which allow the peptide chains to fold complementarily over the antigenic determinant, whereas other aminoacyl residues are rejected. This rejection may be considered as the inability of unsuitable aminoacyl-*s*RNA complex to displace the terminal *s*RNA residue, as the incorporation process has been described as displacement of the terminal *s*RNA residue of an *s*RNA peptide ester by the incoming aminoacyl-*s*RNA complex.

Antibody formation, according to this view, involves primarily direct interference of the antigenic template in step (iv) of protein synthesis by rejection of unsuitable aminoacyl residues, only by indirectly interfering in step (iii). The process most probably occurs in the same cell in which the normal globulins are formed and is controlled by the same genetic apparatus, the same types of DNA and RNA.

The last step (v) in protein synthesis (here the combination of the two sub-units) could be entirely excluded by any interference, as the four sub-units can combine *in vitro* spontaneously even in the absence of the antigen to yield biologically active antibody.— M. R. SAIRAM

Prostaglandins

von Euler and Goldblatt demonstrated independently the presence of blood pressure depressing and smooth muscle stimulating material in human seminal plasma and sheep vesicular gland extracts. The factor was provisionally named as prostaglandin and was



11α, 15-dihydroxy, 9-keto-prost-13enoic acid (PGE_1)

I

lipid soluble and had acidic properties. For nearly three decades this smooth muscle stimulating principle eluded isolation and a series of compounds, PGE_1, PGE_2, PGE_3, PGF_1, PGF_2 and PGF_3, having a basic structural formula (I) have been isolated from sheep vesicular gland extracts, prostrate glands, human semen, etc. The chemical structures of all these compounds have been elucidated; PGE_1 is 11α,15-dihydroxy-9-keto-prost-13-enoic acid; PGE_2 differs from PGE_1 by a Δ^5-double bond and PGE_3 has a third double bond between C_{17} and C_{18}. The PGF compounds have a hydroxy group at C_9 instead of a keto group found in the PGE compounds. The different prostaglandin compounds are present in many organs, the highest amount being found in human seminal plasma and sheep vesicular glands. Semen of bull, boar, dog, rabbit, pig and horse does not have prostaglandins. Semen from men with documented normal fertility contained more prostaglandin than semen from infertile men, where hardly any smooth muscle activity is seen.

D. A. Dorp, R. K. Beerthuis, D. H. Nugteren and H. Vonkeman [*Biochem. biophys. Acta*, **90** (1964), 204] and S. Bergstrom, D. Danielsson, D. Klenberg and B. Samuelsson [*J. biol. Chem.*, **239** (1964), 1135] suggest that prostaglandins in sheep and human semen are formed in the seminal vesicles and in the vesicular glands respectively. When γ-homolinolenic acid, arachidonic acid or an eicosa pentaenoic acid was incubated at 37° with homogenized vesicular gland from sheep, PGE_1, PGE_2 and PGE_3 respectively were formed.

All the prostaglandin compounds as well as the crude prostaglandin extract from human semen and vesicular gland of sheep strongly stimulate all the smooth muscle organs except the fallopian tubules of rabbit and man where prostaglandins and various PGE compounds inhibit the spontaneous motility. Blood pressure of rabbit, cat, dog and man is lowered. Most pronounced blood pressure reducing activity is seen with PGE compounds. On human myometrium all PGE compounds have almost the same activity, indicating that the number of double bonds appears to have little importance for their activity on this organ. This is not the case, however, for other smooth muscle organs (jejunum, duodenum and uterus of rabbits) where usually PGE_2 is the most potent and PGE_3 the least active of these substances. The keto group, however, seems to be essential for the inhibitory effect, since PGF_1 and PGF_2 have a tendency to stimulate the myometrium.

With regard to the proposed role of prostaglandin in normal fertility, pure prostaglandin compounds present in human semen were examined for their effect on the motility of human myometrium [Bygdeman, M., *Acta physiol. scand.*, **63** (1964), 242]. Normally all PGE compounds and HSF-PG inhibit the spontaneous motility of human myometrium. Contrary to PGE compounds PGF_1 and PGF_2 have a tendency to stimulate the spontaneous motility of human non-pregnant myometrium. An inhibitory effect on human myometrium of an autopharmacological active substance is uncommon, the only other substance with such an effect *in vitro* is bradekynin. The sensitivity of the myometrium to PGE and HSF-PG varies in a characteristic way during the different phases of menstrual cycle. The activity is pronounced around ovulation time and less during the other phases of the cycle.

The factors which regulate the reactivity of prostaglandin during the menstrual cycle, however, stress the importance of ion gradients, especially the intra- and extracellular relationship between cations, i.e. K, Na and Ca. Variations in the extracellular ion concentration profoundly change the reactivity pattern of the myometrium. A high extracellular potassium concentration, for instance, decreases the membrane potential, increases the frequency of the contractions and increases the sensitivity of the myometrium to different stimuli.

At low extracellular potassium concentrations the effect of PGE is enhanced at the same time as the stimulatory effect of PGF_1 is abolished. The change in sensitivity of human myometrium to PGE compounds when potassium concentration is varied seems to be specific. The effect of potassium concentration on spontaneous motility and ion distribution indicates that the membrane potential of human myometrium is charged simultaneously. With this assumption, the effect of prostaglandin is also dependent not only on the extracellular potassium concentration but also on the membrane potential. A low extracellular potassium concentration corresponds to a hyperpolarized membrane. At this time the sensitivity of myometrium to PGE_1 is enhanced and to PGF_1 depressed. A high extracellular potassium concentration and consequently a partly depolarized membrane depresses the effect of PGE_1, but increases the stimulatory effect of PGF_1. A low extracellular calcium concentration affects the sensitivity of myometrium to prostaglandin, especially at ovulation time.

The sensitivity of human myometrium to PGE compounds and varied effects during different phases of the menstrual cycle may also be due to the influence of estrogen and progesterone. The effect of estradiol and progesterone on the sensitivity of human myometrium to PGE_1 was also investigated. Hormones investigated affected the sensitivity of myometrium to PGE *in vitro*. The effect was most pronounced at ovulation time. Here progesterone and estradiol together with progestrone decreased the sensitivity of the myometrium to PGE. During early and middle proliferative phases very confusing effects were observed. No statistical differences were found between the ion content, either during the various phases of the menstrual cycle or under different hormonal treatments.— T. S. ANANTHA SAMY

Reactivities of histidine residues at the active site of ribonuclease

There is considerable evidence to indicate that the histidine residues at positions 12 and 119 in ribonuclease are at the active site of the enzyme. It was demonstrated that inactivation of RNase A by iodoacetate at *p*H 5·5 and 25°C. was due to the introduction of a single carboxymethyl substituent on either imidazole nitrogen 1 of histidine 119 or imidazole nitrogen 3 of histidine 12 under these conditions. The yield with substitution at N_1 of 119 histidine was found to be 8 times that of the product with substitution at N_3 of histidine 12. These two chromatographically distinct carboxymethylated derivatives were shown to be alternate products which are formed simultaneously. In order to explain these and other findings, including the behaviour of the aggregates of RNase the following mechanism has been suggested. In the simplest hypothesis, histidine 12 and 119 are both considered to form the active site and the protonated form of the one binds the anionic alkylating agent which then alkylates the unprotonated form of the other. In the other mechanism, the orientation is provided by lysine 41.

The difference in yields of the two alkylation products may be the result of greater steric hindrance at one site or the other. In order to test this hypothesis a study has been made of the reaction between ribonuclease and a series of halo acids [Heinrikson, R. L., Stein, W. H., Crestfield, M. A. & Moore, S., *J. biol. Chem.*, **240** (1965), 2921]. The results of this study indicate that the reaction with a series of unbranched α- and β-bromo acids ranging from 3 to 6 carbon atoms also leads to the alkylation at the same sites. Reaction may occur predominantly either at histidine 12 or at histidine 119, depending upon the chain length, the position of halogen atoms relative to the carboxyl group and the optical configuration of the α-carbon of the reagent employed. These findings confirm the earlier contention that both 12 and 119 carboxymethylated derivatives of ribonuclease arise from the same molecular species of the enzyme; the 12 derivatives are not produced by side reactions involving some minor contaminant of ribonuclease.

The most striking finding from the present study is the great difference in the reactivities and site of reaction of the D- and L-antipodes of α-bromopropionic acid and α-bromo-*n*-butyric acids. The D-antipodes of these acids form histidine 12 derivatives in 70-78 per cent yields. The L-antipodes form histidine 119 derivatives in yields of 78 and 74 per cent respectively. Moreover, the D-antipodes react at histidine 12, 20 times more rapidly and at histidine 119, 2-3 times more rapidly than do the L-antipodes.

These effects must reflect the stereochemistry of the active site itself, as such differences are not observed in the model reactions with histidine. Notable is the variation in the site of reactions with the nature of the alkylating agent. Only D-α-bromopropionic acid and D-α-bromo-*n*-butyric acids alkylate predominantly at histidine 12 while the others alkylate at histidine 119. With the α-bromo acids the rate of reaction decreases as the size of the substituent on the α-carbon atom increases. Part of this effect is intrinsic to the reagent itself. The 300-fold decrease in the reactivity at histidine 119 in passing from bromoacetate to L-α-bromopropionate is significantly larger than would be expected on the basis of the model reactions and must be caused in part by steric effect. The decrease in the rate of reaction at histidine 12 as one proceeds from bromoacetate to D-α-bromopropionate (less than 5-fold) is less than 30-fold that expected from the histidine reaction and may be a result of secondary non-polar interactions between the methyl group in the alkylating agent and some group or groups in the enzyme. Probably similar interactions operate in the case of D-α-bromo-*n*-butyric acid since its rate of reaction is nearly identical with that of the C_3 acid. These results indicate that the space into which these reagents fit when they are oriented in such a fashion as to permit alkylation at histidine 12 is big enough to accommodate both the C_3 and C_4 α-bromo acids easily and the C_5 acid with difficulty. It is not large enough to accommodate α-bromocaproate, however, for this reagent does not alkylate histidine 12.

One aspect that is not entirely clear on steric grounds is why the haloacetate, the least hindered of the reagents studied, reacts so predominantly at histidine 119. In the reaction with iodoacetate the formation of the 12 derivative is somewhat favoured as the temperature is raised. This suggests that there may be present an interfering group, whose position shifts slightly with change in temperature. It is likely that there are groups in the vicinity of imidazole nitrogen 3 of histidine 12 which get in the way of the hydrocarbon side chains of α-bromovalerate and α-bromocaproate, when these reagents are bound at histidine 119. One or more of these intefering groups must be arranged on one side of the axis between the histidine residues to provide hindrance so that reaction with L-enantiomorphs of α-bromopropionate and α-bromobutyrate cannot readily occur at histidine 12. Conversely, the reaction at this position with the D-isomers of C_3 and C_4 acids may actually be facilitated somewhat by a non-polar region between these histidine nitrogen atoms.

The differences in the rates and sites of alkylation with the reagents of systematically varying structures are reflections of the stereochemistry and conformation of the active site. A final point that bears emphasis concerns the possible use of these reagents. Because of their selectivity in terms of both rate of reaction and the site at which alkylation occurs, these reagents provide a potential sensitive method of detecting conformational changes in the active site of ribonuclease. — A. S. ACHARYA

Progress Reports

Fire Research in UK

The annual report for 1964 of the UK Fire Research Board, besides covering the activities of the Board relating to prevention and control of fire hazards, design of building and choice of building

materials to minimize chances of fire hazards, general characteristics of flames, and processes of combustion, presents the results of a statistical study regarding fire outbreaks on various aspects, such as cost of damage, industries affected, causes of fires, etc.

Studies made on spontaneous heating and ignition in miscellaneous materials have led to conclusions on methods of shipping carbon safely and economically. An equipment for studying the thermal explosion of organic peroxides in the paste form has been constructed and it has been found that a paste containing 65 per cent peroxide undergoes thermal explosion at a lower temperature than dry peroxide and in the case of dry benzoyl peroxide rust and some decomposition products of the peroxide added to the dry peroxide reduce the induction period of explosion. Measurements have been made on the speed of rise of lower flammability limit flames in vertical tubes using methane-air and propane-air mixtures. The results have been explained on the basis of the controlling effect of rising bubbles of hot gaseous products.

As a result of experiments on the factors that affect the rate of burning of cellulose materials the rate of charring has been found to be proportional to the density and the permeability along the grain. An equation connecting the densities of the flame and surrounding air and the entrainment velocity of air by flames has been developed and the experimentally determined values of flame heights have been found to agree with the values predicted on the basis of the equation. Studies made on radiative transfer of heat from flames have shown that the radiative transfer from alcohol flames is about five times that of convective transfer at the centre of the tray containing the flame. The burning rate of wood cribs is affected by heat received from some other source as, for example, radiative transfer in the fuel bed.

A theoretical expression derived on the basis of dimensional analysis for the conditions of merging of separate fuel bed flames to form a single flame has been tested by actual observation. The theory is found to give reasonable estimates of flame heights at merging. Another expression for the length of an advancing flame has been found to agree with an empirical formula derived for low rates of burning. A thorough study of the characteristics of fires in compartments is in progress and the observations made so far agree with the deductions made from an assumed theoretical model. Several important deductions regarding the effect of ceiling, ventilation, the corridor, etc., in the case of indoor fires have been arrived at. The studies have resulted in further deductions regarding several aspects such as effect of ambient atmosphere on burning wood, products of combustion with restricted ventilation, soot production in diffusion flames, etc. A theoretical expression for the conditions of equilibrium of flow of hot gases in vented single-storey buildings has been developed. An apparatus for testing small concrete beams in flexure at high temperatures under well-controlled conditions has been developed and a number of preliminary conclusions have been drawn as a result of investigations made on the spalling of concrete. Experiments have been made to compare under identical conditions the behaviour of ducts of various materials forming part of a ventilation system and the results will be of interest to industry, especially to the plastics industry.

A thorough study of fire resistance of different building materials has been made and deductions useful to the building industry have been made. From studies on various aspects of the problem of keeping escape routes in a building, methods of dealing with the smoke hazard have been developed. Several types of heat-sensitive and smoke-sensitive detectors have been developed and their sensitivity and operation characteristics are being studied. Studies have been made on the performance of sprinkler systems and the rates of application of water required to extinguish developing fires in three types of wood crib have been determined. From a study of the new techniques in extinguishing fires, recommendations have been made regarding such extinguishers as freezing point depressants, inert gas and foam generator, vaporizing liquid extinguishers and dry powders.

As a result of studies made on gas explosions and dust explosions, standards for keeping industrial equipment safe have been developed. Special hazards such as cyclones, fires due to electric lamps, fires in brickwork impregnated with domestic fuel oil, incidence of fires on newsprint, etc., have also been studied and recommendations have been made for reducing loss in such accidents.

New Periodical

Journal of Combinatorial Theory

This new journal, to be published quarterly commencing from spring 1966, is devoted to the publication of original mathematical articles dealing with theoretical and physical aspects of the study of finite and discrete structures in all branches of science. Manuscripts in English, French or German should be addressed to the Editor-in-Chief, *Journal of Combinatorial Theory*, Academic Press Inc., New York. Annual subscription for the journal is $ 15.00 for institutions and $ 9.00 for individuals.

Dr Vikram A. Sarabhai

A worthy successor to the late Dr H. J. Bhabha for the chairmanship of the Atomic Energy Commission has been found by the Government of India in Dr Vikram A. Sarabhai, whose personality represents a rare combination of the fine qualities of an outstanding scientist, a capable organizer and a connoisseur of fine arts. He has also been appointed Secretary to the Department of Atomic Energy, Government of India.

Born in August 1919 at Ahmedabad, Dr Sarabhai studied at the universities of Bombay and Cambridge, taking his Natural Science Tripos from the latter in 1939. After about six years of study as a research scholar in the field of cosmic rays under Sir C. V. Raman, he continued his investigations at the Cavendish Laboratory, Cambridge, from where he got his doctorate degree. Dr Sarabhai has been an excellent

organizer of scientific institutions and the rapid and brilliant progress of such institutions as the Physical Research Laboratory, Ahmedabad, the Textile Industry's Research Association, Ahmedabad, and the Indian Institute of Management, Ahmedabad, with which he has associations, bear testimony to this aspect of his capacity.

In recognition of his scientific contributions, Dr Sarabhai was awarded the Shanti Swarup Bhatnagar Memorial Award for 1962 and he was honoured by the Government of India by the award of Padma Bhushan early this year.

Dr Sarabhai is a fellow of several learned societies, both Indian and foreign. He has been an active member of the Scientific Advisory Committee to the cabinet, the National Planning Council of the Planning Commission, the Governing Body of the Council of Scientific & Industrial Research and the Central Advisory Board of Education, Government of India. He is a member of the Editorial Boards of the *Journal of Scientific & Industrial Research* and the *Indian Journal of Pure & Applied Physics* since its inception. Deeply interested in the problems of disarmament, Dr Sarabhai has devoted considerable time and effort in propagating the ideas behind the Pugwash movement and is the convener of the Indian Pugwash Committee.

One of his major interests in research centres around astrophysical implications of cosmic ray time variations and the investigations made so far from his school of study have led to a deeper understanding of the solar activity with respect to cosmic rays. Space research is another field of interest to Dr Sarabhai and it was under his direction that the Equatorial Rocket Launching Station at Thumba and the Experimental Satellite Communication Earth Station at Ahmedabad were set up.

FORTHCOMING INTERNATIONAL SCIENTIFIC CONFERENCES

Date	*Conference*	*Place*
3-10 August	Seventh International Congress on Nutrition	Hamburg
8-12 August	International Heat Transfer Conference	Chicago
14-20 August	Eleventh International Conference on Combustion	Berkeley (Calif.)
21-24 August	International Symposium on Free Radicals in Solution	Ann Arbour (Mich.)
22-26 August	Thirteenth International Symposium on Microscopy	Chicago
22-27 August	Second International Congress of Food and Technology	Warsaw
25-31 August	Symposium on Radio Astronomy and the Galactic System	Netherlands
28 August-4 September	Sixth International Congress of Electron Microscopy	Kyoto
29-31 August	Second International Congress on Instrumentation and Aerospace Simulation Facilities	Stanford (Calif.)
29 August-5 September	Second International Conference on Palynology	Utrecht
29 August-6 September	Tenth International Conference on Low Temperature Physics	Moscow
31 August-10 September	Thirteenth International Conference on High Energy Physics	Berkeley (Calif.)
3-7 September	International Conference on Solid State Science	Cairo
5-9 September	Second International Biophysics Congress	Vienna
5-9 September	Ninth International Conference on Coordination Chemistry	Moritz (Switz.)
5-10 September	Third International Congress of Human Genetics	Chicago
8-13 September	International Conference on the Physics of Semiconductors	Kyoto
9-10 September	High Energy Physics Instrumentation Conference	Stanford (Calif.)
12-16 September	International Conference on Nuclear Physics	Gatlinburg (Tenn.)
12-16 September	Sixth International Conference on Microwave and Optical Generation and Amplification	Cambridge
14-18 September	Fourth International Conference on Operational Research	Cambridge (Mass.)
17-24 September	Twenty-sixth International Congress of the Pharmaceutical Sciences	Madrid
21-23 September	International Conference on the Supramolecular Structure in Fibres	Boston
28 September-4 October	International Symposium on Macromolecular Chemistry	Tokyo
30 September-1 October	International Symposium on Neuroendocrinology	Paris
Fall 1966	Seventeenth International Astronautical Congress	Madrid
Fall 1966	International Heat Engineering Conference	Karl Marx-Stadt

Announcement

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Palynological Society of India

National Botanic Gardens, Post Box No. 36
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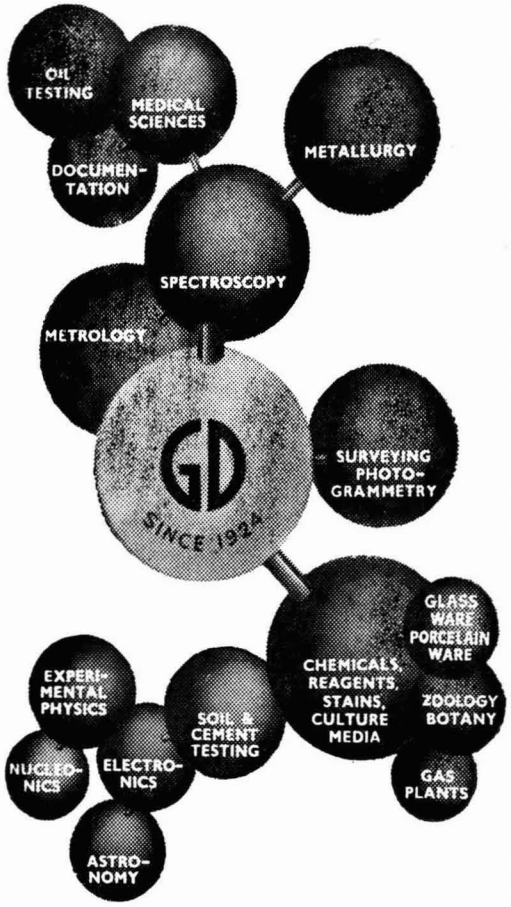

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