

Journal of Scientific & Industrial Research



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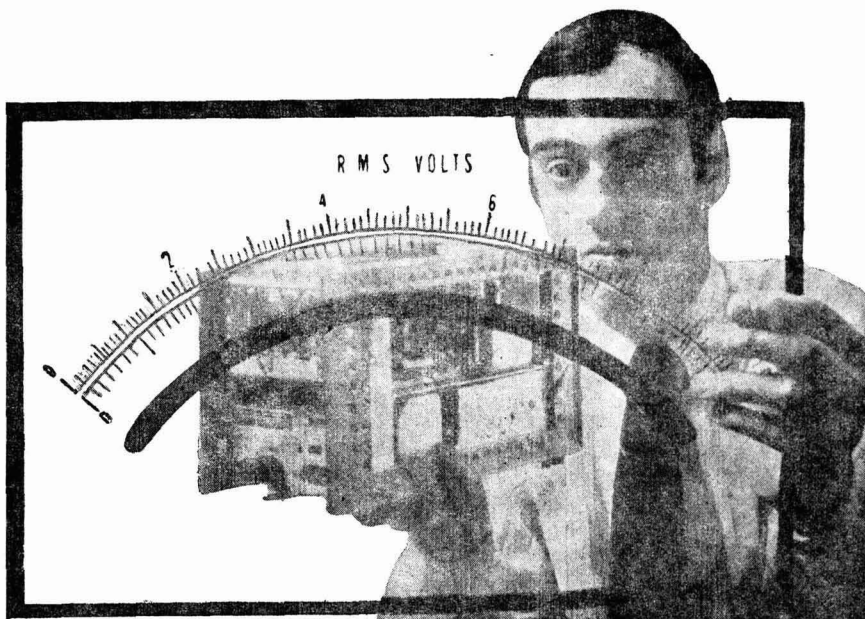
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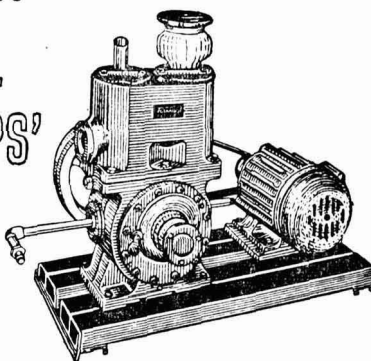
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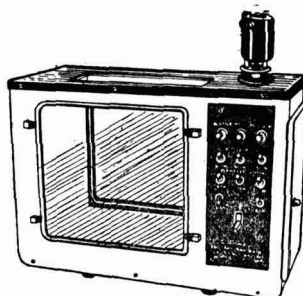
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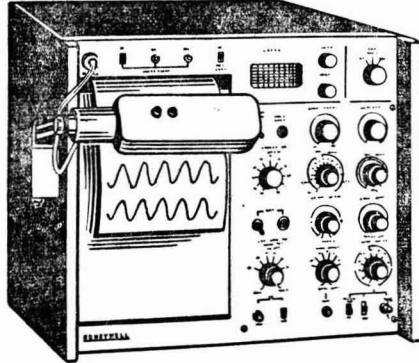
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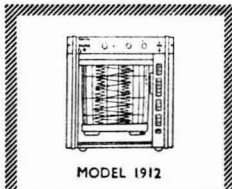
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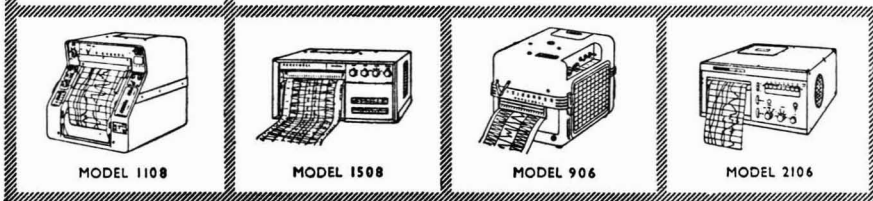
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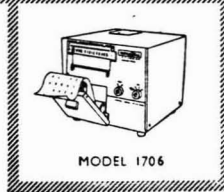
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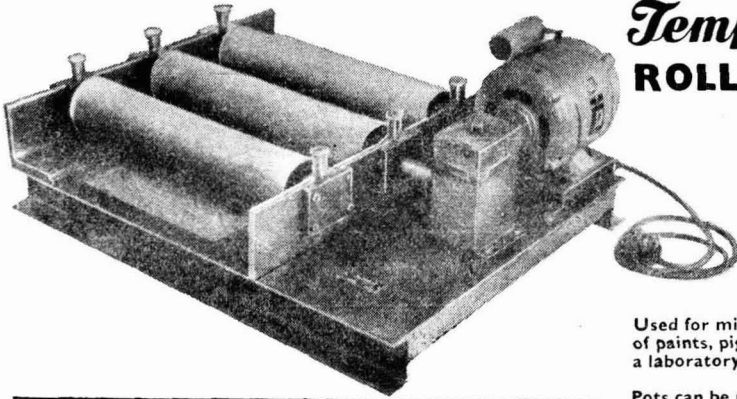
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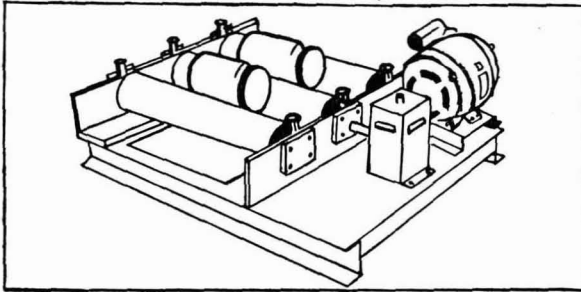
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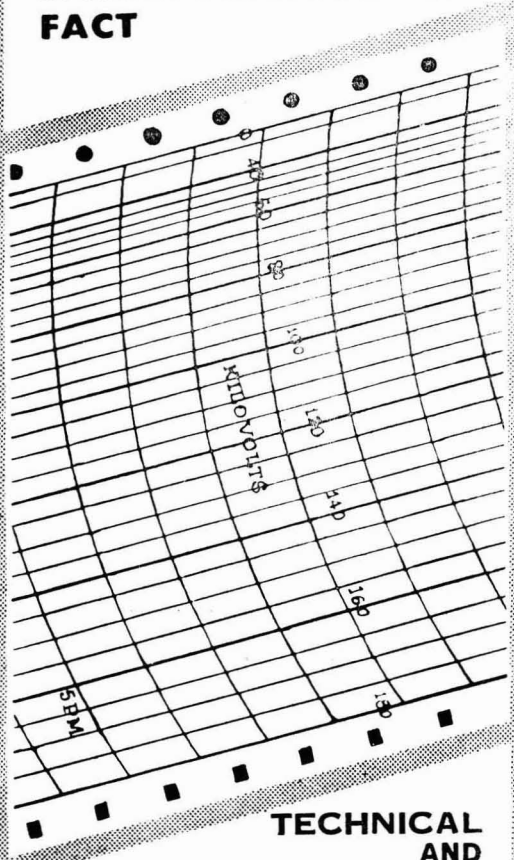
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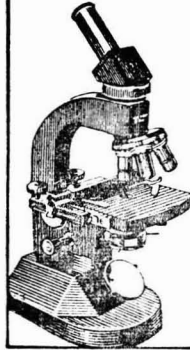


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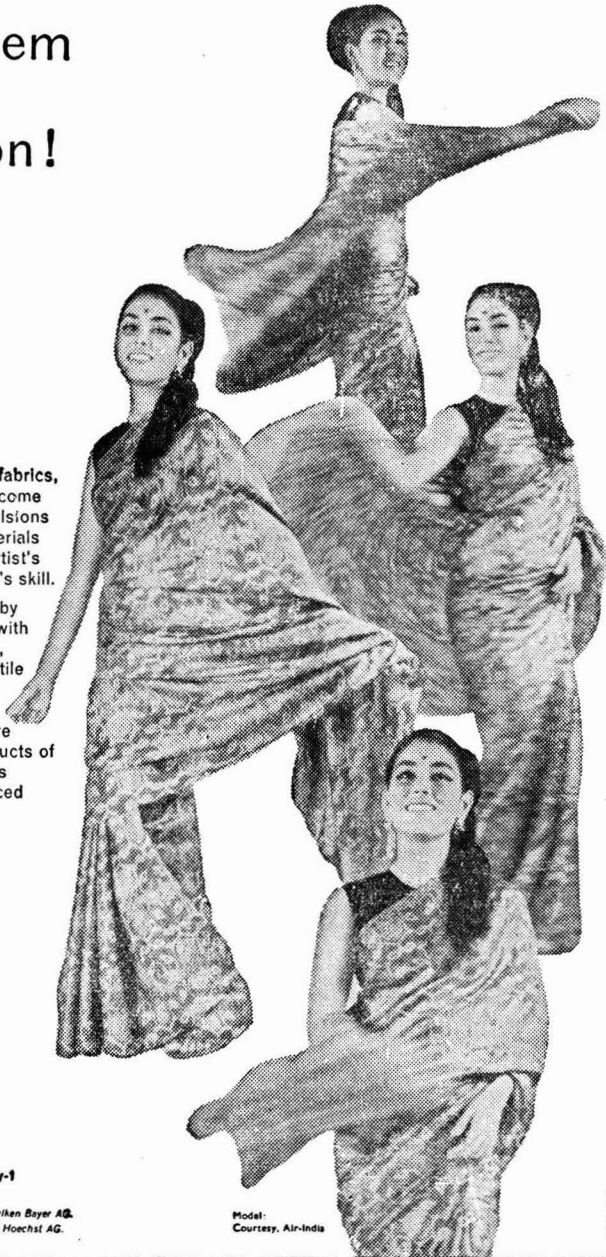
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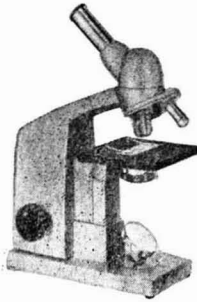
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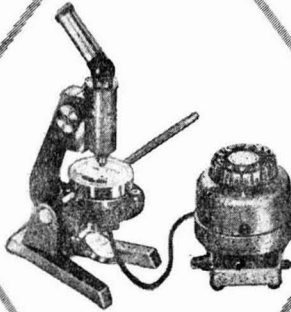
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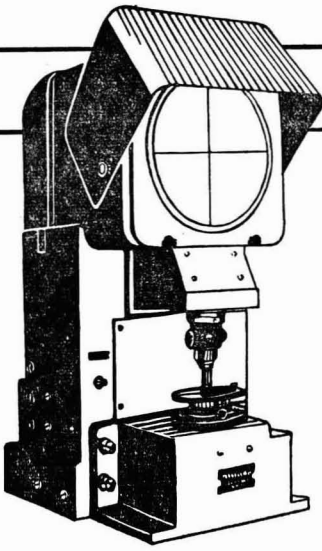
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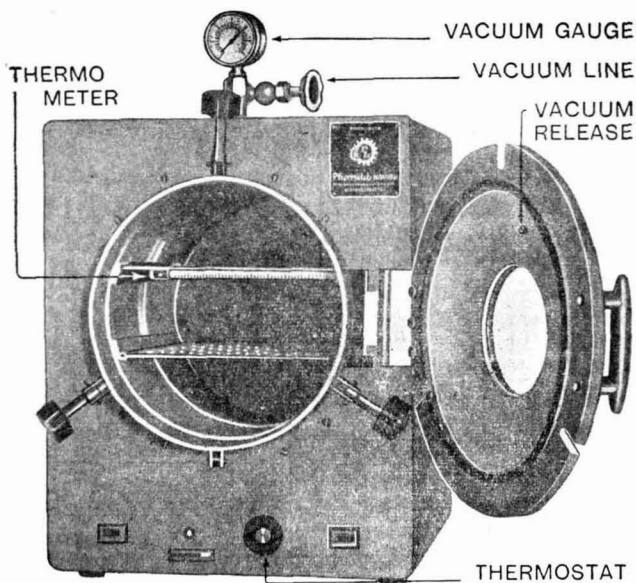
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Current Topics

A New Experiment in Science Talent Search at Postgraduate Level

AT present, search for talent in science among students is carried out every year on an all-India basis at the PUC level and talented students are selected from a large number of applicants who have passed PUC examination meritoriously. The selected scholars are paid stipends ranging from Rs 150 to Rs 300 per month to pursue their studies in the science subjects up to the Ph.D. level. This step is quite useful for keeping a sustained interest in science subjects among university students. Some of the students who are selected, however, do not finally choose a career in science, but move to professional studies like medicine and engineering. In order to spot out students who are motivated in the pursuit of science, it is essential to conduct tests for talent at various other levels, for example, at B.Sc. and M.Sc. levels. The search for talent at M.Sc. level should be to find those students who, in addition to academic excellence, possess such qualities as motivation, perseverance and patience, which make a successful Ph.D. student.

A new orientation programme at the M.Sc. level was conducted at the Biochemistry Department of the Indian Institute of Science, Bangalore, during June-July 1969 to attract talented scholars into research in biochemistry leading to Ph.D. degree. Applications were invited, through advertisement in the newspapers as well as by letters to heads of departments of chemistry and biochemistry in various universities, from candidates who had secured high marks at the high school, PUC, B.Sc. and M.Sc. Part I examinations and who had finished writing M.Sc. Part II final examination. Fifteen candidates were selected out of 193 applicants. Ten of the selected students joined the department and took part in the programme. They were provided free boarding and lodging and return train fare. Each student was assigned to a staff member who gave the candidate a small research project which could be completed in 2-3 weeks. The candidates also attended a lecture a day by staff members on general and research topics in biochemistry. During their stay at the Institute, they visited the different research sections of the department and got acquainted with the current topics of research. They also visited

the various science and engineering departments of the Indian Institute of Science.

All the students who attended the orientation programme were very much interested in it and were quite enthusiastic about attending the lectures and completing the research work assigned to them. Some of the chemistry graduates who had no earlier acquaintance with biochemistry got interested in the subject and preferred it to chemistry for a research career. All the scholars applied for the regular research programme of the biochemistry department and all of them were selected for admission to research leading to the Ph.D. degree. Six of the candidates opted to join the Indian Institute of Science and four elsewhere, the latter for personal reasons.

A similar programme on a much smaller scale has also been carried out at the Indian Institute of Science, Bangalore, for 'Science Talent' candidates deputed by the National Council of Education, Research and Training (NCERT), New Delhi, after their M.Sc. Part I examination and before joining the II year M.Sc. programme in their respective universities. A comparison of the merits and demerits of the two training programmes has been made by the faculty members in the department and it is generally felt that the orientation programme conducted for the students who finished sitting for their final M.Sc. examination was more successful and hence is to be preferred.

It is desirable to conduct a similar orientation course at the B.Sc. level in centres where they have adequate facilities and sufficient staff strength to carry out the programme. The selected candidates who may not exceed 30 in number and who have finished writing their B.Sc. final university examination may be given suitable projects to be carried out for a period of one month and those who are found suitable may be selected for admission to the M.Sc. degree class in chemistry or biochemistry.

In conclusion, it may be stated that it is of vital importance that early steps are taken by the authorities concerned to search for talented students both at the B.Sc. and M.Sc. levels. Such science talent search will have a significant impact on the improvement of all branches of chemical education and research in the country.— J. D. CHERAYIL, T. M. JACOB & P. S. SARMA, Department of Biochemistry, Indian Institute of Science, Bangalore 12

Alkoxides & Alkylalkoxides of Metals & Metalloids*

THE most noticeable development in science during the last 2-3 decades is the breakdown of the traditional dividing lines between various disciplines. Any number of examples come to mind, but one of the most dramatic, at least in the field of chemistry, is the remarkable development of a large variety of organic derivatives of metals and metalloids, which are inorganic compounds endowed with properties generally considered characteristic of covalent 'organic' species. The chemist has been led into these investigations in his continuing and unending search for better and more useful materials of construction. Starting from the use of lead tetraethyl as an antiknock additive in petrol in 1923, there are extensive applications today of these metal alkyls or true organometallics (compounds in which the metal is linked directly to the carbon atom of the organic radical) in industry, the most important being probably the use of aluminium alkyl-titanium tetrachloride combinations as catalysts in low pressure polymerization of olefins for the production of various plastic materials.

Apart from these truly organometallic compounds, other organic derivatives of metals also have been receiving considerable attention and the most important among these are those in which the metal atoms are linked to carbon through oxygen atoms. These can be divided into two broad classes: (i) alkoxides¹⁻³ or metal derivatives of alcohols; and (ii) carboxylates⁴, which are salts of metals with carboxylic acids. In chemical terminology, the alkoxides could be represented by the general formula $(RO)_nM$ and can be supposed to be formed by the replacement of hydrogen atoms in alcohols (ROH) by metal atoms (M).

Metal alkoxides can be prepared conveniently by the reactions of metals themselves, their oxides or chlorides with alcohols either alone or in the presence of bases like ammonia. Containing a high percentage of organic constituent, they possess the very useful property of being soluble in organic solvents. The lower alkoxides of metals, therefore, serve as very convenient starting materials for the preparation of derivatives with a number of reagents (glycols, glycerol and other polyhydroxy compounds, carboxylic acids, substituted carboxylic acids, ethanolic amines, β -diketones, keto-esters, phenols, thiols, etc.). These synthetic methods are of special importance in the case of those metals which are highly susceptible to hydrolysis in aqueous media. For example, the author was successful⁵ in 1953 in the preparation of aluminium tricarboxylates by the reactions of aluminium isopropoxide with carboxylic acids and removing the isopropanol produced azeotropically with benzene, whereas a number of earlier efforts for synthesizing

these derivatives had failed, leading to the general belief that these simple derivatives of aluminium were incapable of existence. Another notable success in the synthetic applications of alkoxides has been the development^{6,7} of a general method for the preparation of β -diketonate and mixed ligand derivatives of lanthanide elements—a class of compounds the synthesis of which had been attempted by numerous workers without much success. The work assumes some applied interest also in view of the laser activity exhibited by some of these derivatives.

The alkoxides (from normal and secondary alcohols) react readily with acetyl halides resulting in the stoichiometric replacement of the alkoxy groups by chloride radicals. The tertiary alkoxides also react initially in a similar manner. However, the tertiary alkyl acetate produced in the reactions begins to cause a side-decomposition in which the chloride radical on the metal is gradually replaced by the acetate group⁸.

Another group of compounds synthesized in the author's laboratories during the last two years, which deserve special mention, are a variety of double alkoxides^{9,10} like $\text{NaZr}_2(\text{OPr}^i)_6$, $\text{KTa}(\text{OPr}^i)_6$, $\text{ZrAl}_2(\text{OPr}^i)_{10}$, $\text{LnAl}_3(\text{OPr}^i)_{12}$. From a conventional point of view many of these can be said to be neutralization products of an alkali like NaOPr^i (in isopropanol) with a weak acid like $\text{Ta}(\text{OPr}^i)_5$. Some of these double alkoxides, however, depict sufficient covalent character and can be volatilized without decomposition. In the case of alkali metal derivatives, the above may be the first known examples of such covalent behaviour. Structural studies of these alkali and other double alkoxides are revealing extremely interesting and novel features, generally characteristic of coordination compounds.

Metal alkoxides are thus a group of compounds, the chemistry of which is arousing considerable interest from a variety of angles:

(i) From the inorganic chemist's point of view, these compounds provide an interesting comparison among the elements of the same group,

e.g. $\text{C-Si} \begin{matrix} \text{Ge-Sn-Pb} \\ \text{Ti-Zr-Hf-Th} \end{matrix}$. The physical and chemical characteristics (molecular association, volatility and reactivity) of a particular alkoxide are directly governed by the electronegativity and the atomic radius of the central metal concerned. These studies become easily feasible for most of the metal alkoxides due to their ready solubility in inert organic solvents and are proving of considerable importance in understanding the nature of M-O-C bonds.

(ii) From the organic chemist's point of view, these compounds provide an interesting gradation in properties with a change in the inductive and steric factors of the alkyl group. Studies in reaction mechanisms, mainly involving the alkyl groups, have in many cases revealed new facets to the conventional theories of organic chemistry and provide a wider perspective. The catalytic effects of many

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of these derivatives in a number of organic reactions have also proved of special importance and deserve more detailed study.

(iii) From the physical chemist's point of view, a study of entropies of vaporization for a series of derivatives [for example, $\text{Si}(\text{OC}_n\text{H}_{2n+1})_4$, where 'n' varies from 1 to 6 in a series of normal alkyl radicals] has thrown fresh light on the nature of the liquid state. The nature of intermolecular bonding, $\text{R} \begin{matrix} \text{R} \\ \text{M} \end{matrix} \text{O} \dots \text{M}$, has itself proved of considerable interest and appears to be mainly electrostatic. Special magnetic and structural studies of these derivatives, only recently initiated, are already yielding results of considerable theoretical interest.

(iv) From the industrial chemist's point of view, these derivatives are proving of great practical value in a number of industries^{11,12}, such as paints and varnishes, water-proofing, lubricants, resins and surface-coating. The most extensively developed range of compounds are those of titanium. Out of a large number of derivatives of this metal synthesized in the laboratory, fifteen are already in commercial production (a few even in tonnage quantities) and another five or six are available in developmental quantities. Aluminium isopropoxide and sodium methoxide are used as important catalytic agents for a variety of organic processes. Magnesium ethoxide is a cross-linking and condensation catalyst and serves for the coating of magnetic recording tapes and in the production of dielectric coatings. Antimony alkoxides are providing a convenient means of introducing the metalloid as a flame-proofing agent in various types of resins.

A rapid rate of progress in every field is the chief characteristic of the present age of science. It is reflected in this area also by extensive work on more than 30 new elements carried out in the past two decades. An estimate of the rapid growth of literature in the field can be made by a cursory look at the 315 references to academic literature cited at the end of a recent review article¹, of which more than two-thirds would belong to the post-1960 period.

In conclusion, it would be worth while to point out that a vast area of very interesting chemistry still remains untracked in this field. In view of the extremely hydrolysable nature of most of these alkoxides, their precise physico-chemical measurements require more carefully planned special techniques. It is a happy sign that these aspects of their chemistry are beginning to receive increasing attention during the last year or two and this may

be expected to lead to more intensive studies, in general, of these academically interesting and industrially useful compounds.

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Electron Transition in a Dodecahedral Crystal Field*

NOT long ago, the area of coordination chemistry of transition elements with coordination number 8 might have been described as obscure. Advances in synthesis and the classic structural investigations by Hoard and coworkers have sharply altered the picture in recent years. It is apparent now that the field of higher coordination compounds is large in scope and more interesting in physical and chemical characters than the 6- and 4-coordination compounds to which most of the experimental and theoretical investigations have been confined. In the present dissertation we will discuss 8-coordinated complexes of transition elements. Octa-coordination is rather common in complexes of the larger metal ions. The larger metal ions being about scandium, the span of octa-coordinate structure is really quite large.

A number of idealized geometries have been suggested for octa-coordination. The simplest and most symmetrical of these is the cube. Only seven *s*, *p* and *d* orbitals of metals possess proper symmetry for bonding to ligand atoms. Although an unfavourable model for metals entering into bonding through *d*-electrons, the cubic structure is not out of question for systems where *f*-bonding is possible, i.e. for lanthanides and actinides. We will, however, keep these systems out of our discussion. The next symmetrical structure of the octa-coordinate polyhedra is the square antiprism, having symmetry D_{4h} . Very close to anti-prism is the dodecahedral structure D_{2d} . The group theoretical considerations show that the three models differ electronically in that the lowest energy *d*-orbital is d_{z^2} in D_{4h} , $d_{x^2-y^2}$ in D_{2d} and d_{xy} in C_{2v} . A cubic structure conforms to the highest energy level, and is, therefore, the most unfavourable. Energetically D_{2d} and D_{4d} structures are equivalent. In D_{4d} all the ligand sites are equivalent, while in D_{2d} they are not. Intrinsic ligand non-equivalence will favour a dodecahedral geometry. The X-ray crystallographic measurements of Hoard and Nordsick have established the dodecahedral structure for 8-coordinated cyano and hydroxy-cyano complexes of molybdenum and tungsten. There is, however, no physical technique that will provide sufficient data for an unequivocal structure determination for these complexes in solution. There is, however, a possibility of establishing the structure of these complexes in solution from electronic spectral data, because the *d*-orbital level scheme for dodecahedron is completely different from that of an anti-prism. Further, even where the bonding scheme can be obtained from the geometry deduced from X-ray data, a crystal field calculation should lead to a more detailed understanding of the bonding and electronic configuration of these complexes.

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For a dodecahedron, the potential function, i.e. the potential function arising from a collection of point charges, can be written as a tesseral harmonic expansion:

$$V(r, \theta, \phi) = \sum_{k,m} \beta_{km} r^k Z_{km}(\theta, \phi)$$

where β is a constant. Let us put a single negative charge at the corners of the dodecahedron. Then relative to the crystal axes, the potential energy functions within the ligand dodecahedron become:

$$V(r, \theta, \phi) = 4r^4[\gamma_{40}^a P_4(\theta_a) + \gamma_{40}^b P_4(\theta_b)] Z_{40}^C(\theta, \phi) \\ + \frac{1}{2}(35)^{\frac{1}{2}} r^4 [\gamma_{40}^a \sin^4 \theta_a + \gamma_{40}^b \sin^4 \theta_b] Z_{44}^C(\theta, \phi) \\ + 4r^2 [\gamma_{20}^a P_2(\theta_a) + \gamma_{20}^b P_2(\theta_b)] Z_{20}^C(\theta, \phi)$$

where the origin is at the metal ion and where

$$\gamma_{40}^{a(b)} = -4c(4\pi)^{\frac{1}{2}}/3R^5 a(b)$$

$$\gamma_{20}^{a(b)} = -4c(4\pi)^{\frac{1}{2}}/5^{\frac{1}{2}}R^3 a(b)$$

$$Z_{40}^C(\theta, \phi) = 3/(4\pi)^{\frac{1}{2}} P_4(\theta)$$

$$Z_{44}^C(\theta, \phi) = \frac{3}{8} \left(\frac{35}{\pi}\right)^{\frac{1}{2}} \sin^4 \theta \cos^4 \phi$$

$$Z_{20}^C(\theta, \phi) = \left(\frac{5}{4\pi}\right)^{\frac{1}{2}} P_2(\theta)$$

where R is the metal-ligand distance and r is the coordinate of the metal *d*-electron. The matrix element of V can be obtained from the products of spherical harmonics and we get for one *d*-electron wave function the following matrix elements:

<i>d</i> -Orbital	Symmetry	Energy
x^2-y^2	B_1	$-0.515\Delta + 0.070\delta$
z^2	A_1	$-0.068\Delta - 0.070\delta$
xz, yz	E	$0.045\Delta + 0.035\delta$
xy	B_2	$0.492\Delta - 0.070\delta$

where

$$\Delta = \left[\frac{5}{(4\pi)^{\frac{1}{2}}} \right] \gamma_{40} \langle r^4 \rangle$$

and

$$\delta = \left[\left(\frac{5}{4\pi} \right)^{\frac{1}{2}} \right] \gamma_{20} \langle r^2 \rangle$$

Following Ballhausen and Jørgensen's prescription, $\delta = \frac{1}{3}\Delta$ and $\Delta = 10D_q$ we get the energy level scheme for a single *d*-electron in dodecahedral field as:

Orbital	Symmetry	Energy
$d_{x^2-y^2}$	B_1	$-4.92D_q$
d_{z^2}	A_1	-0.91
d_{xz}, d_{yz}	E	$+0.56$
d_{xy}	B_2	$+4.69$

For this scheme, three electron transitions, $d_{x^2-y^2} \rightarrow d_{z^2}$, $d_{x^2-y^2} \rightarrow d_{xz}, d_{yz}$ and $d_{x^2-y^2} \rightarrow d_{xy}$, are possible for a single *d*-electron. The D_q values calculated from one transition should reproduce D_q values for the other transitions, if the geometry is dodecahedral.

Similar calculation made on an anti-prism gives the level scheme as follows:

Orbital	Symmetry	Energy
x^2-y^2, xy	E_1	$+6.57D_q$
xz, yz	E_2	-3.11
z^2	A_1	-6.93

Only two transitions will take place in an anti-prism geometry and the second transition could be reproduced with the D_q calculated from the first.

The experimental data on the spectra of 8-co-ordinated cyano and hydroxy-cyano complexes of molybdenum show that they conform to dodecahedral geometry both in solution and in the crystalline state.

Development of Electronics in Bhabha Atomic Research Centre, Trombay, Bombay*

DURING the past two decades, the world has witnessed significant progress in a wide variety of disciplines. Two fields which have distinguished themselves even among these are the atomic energy and space sciences. The most important factor that has singularly contributed towards the accelerated progress of these disciplines is the rapidly growing field of electronics. Electronics has, more than any other single discipline, enabled man not only to probe into the unknown and vast expanses of infinite space but also into the equally uncharted and infinitesimal regions of the atom.

Electronics began as a systematic technology early this century with the invention of the radio-valve. Using the capabilities of the radio-valve to amplify, radio-communication was developed to world-wide application in 1922. Soon, broadcasting became a reality. It was gradually possible to control the cost and quality of broadcasting systems very closely, and by the thirties, electronics, though still it encompassed only the radio-technology, was a dependable and well-understood science.

Inventors were quite early to recognize that with electronics one could do things which were otherwise considered impossible. Using the known properties of radio-waves, magnetic fields and light sensitive materials, many useful applications of electronics in measurement, controls and processing were developed. They also developed 'transducers', which transform values of temperature, pressure, etc., into small electrical voltages. The technique of television was also developed to an advanced stage in the thirties. The use of electronics in nuclear research started in the early thirties. Thus it was evident that the potential of electronics was limitless and provided an area where inventors, scientists, en-

gineers, and manufacturers could find infinite scope for putting in fruitful efforts.

It was in this state of development that the Second World War broke out. The course of the war soon made it clear that technology was to play a major role in deciding its outcome. Owing to the impetus given by the war, electronics soon grew from its position of a very young and new science into a giant industry. New applications of electronics in communication, control, armaments and the well-known radar were developed speedily and electronic equipment worth thousands of crores of rupees was made for the armed services. In fact the Second World War with the growth that it provided created the modern version of electronics as we recognize it today—a vital branch of modern technology in its own right.

Soon after the war, these great inventions were applied to the benefit of industry and the home. Industrial heating, automatic control, television, computers and lasers are only a few examples of this. Electronics has opened up radically new methods of communication with the use of satellites and lasers. The use of satellites, apart from greatly increasing the number of channels for long distance communication, has made global television possible for the first time. The development and assimilation of electronic method was very rapid and advances in almost all areas of science and technology depended on electronics in a critical manner. The development of atomic energy in its present form would have been impossible but for the many contributions of electronics, particularly in radiation measurements and in control systems for nuclear reactors and ancillary plants. Electronics has changed the entire character of military operations, so much so that a good portion of defence expenditure of the developed countries is on the production of electronic equipment. Some of the operations such as reconnaissance, surveillance, counter-measures, detection, tracking, data processing and early warning and missile guidance are made possible by the radar and the computer. Electronics has thus come to play an

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ever increasing role in research, defence, industry, agriculture, medicine and even in the day-to-day household work.

It is, therefore, not surprising that most developed countries have spared no pains to aid the phenomenal growth of electronics. In almost all developed countries, electronics, along with its ancillary industries, accounts for a major portion of the country's investments. Thus, for example, in the United States, this industry, in spite of its relatively recent origin, runs fifth and is currently making steady progress bidding strong for a still higher place. During the year 1967, the United States marketed electronic equipment worth about \$ 22 billion, i.e. about Rs 16,500 crores. This is of the same order as India's gross national product for the same period. This comparison alone provides us with one of the prime reasons why India is still among the developing nations rather than among the developed nations.

It would be interesting to look at some of the recent electronics production figures for Japan and some other countries. Whereas in 1962 electronics production in Japan amounted to \$ 1.85 billion (Rs 1,350 crores), the figure rose to \$ 3.1 billion (Rs 2,325 crores) during the year 1966. During this year (1966), Japanese export earnings from electronics alone accounted for nearly Rs 660 crores. In the same year the figures for electronics production in France and UK were about Rs 1,300 crores and Rs 1,200 crores respectively. These figures bespeak the importance of electronics in the modern world, and its rapid development.

Electronics in India

It would be equally interesting to compare the above figures with the corresponding figures for India. During 1967, Rs 50 crores worth of electronics equipment was produced in India of which Rs 30 crores accounted for radio-receivers alone. This figure is indicative of the enormous headway India still has to make if she is to be in the vanguard of today's technological progress. That a developing country can leap-frog into sophisticated modern technologies, without the requisite industrial base, has been established by India during the past ten years, in the field of atomic energy and, to a certain extent, in the field of space technology. Part of this success can be attributed to the considerable amount of research and development effort in electronics that was concentrated on (a) the development of basic materials for the production of modern electronic components; (b) the development of the components themselves; and (c) the design and development of highly refined electronic instruments and equipment.

At this juncture it would be relevant to consider, in some detail, the present state of affairs in respect of the electronics industry in this country. This industry is still very much in its infancy and the work now being done, with exceptions such as the Bhabha Atomic Research Centre, the Electronics Corporation of India Limited, and the Indian Telephone Industries Ltd, mostly consists of assembly work and/or production, based on collaboration with foreign firms. It was only recently that the manufacture of radio-receivers, line-communication equip-

ment, nuclear electronic instruments and in a very small way radar equipment was undertaken. Though in the case of radio-receivers, line-communication equipment and nuclear electronic instruments, the indigenous designs are reasonably up to date, the design philosophy of radio-communication equipment and radar now being produced in the country is at least ten years out of date. Even the radio-receivers, which account for a large fraction of India's electronics production effort, are not yet completely indigenous. The manufacture of the components again is done mostly on the basis of foreign know-how and almost always with foreign collaboration. All this indicates that in the field of electronic components production, much ground remains to be covered. Most of the components of high specifications required for sophisticated electronics equipment, with a few notable exceptions, are yet to be indigenously developed and manufactured. In the case of instruments and more sophisticated units, most of the components as well as the know-how have, at present, to be imported. Thus, apart from the continuing drain on foreign exchange in the form of payment for components and sub-assemblies, there is a further continuing drain in the form of royalties to be paid for know-how, which is imported. Such a state of affairs does not generate an atmosphere, suitable for hard-nosed research and development which could profitably lead to indigenous know-how. Under these conditions a state of perpetual indebtedness in respect of know-how results and we are in the unenviable position of relying on foreign sources for even those concepts in the design of electronic equipment and components, which are already well established and are a few years old. It is obvious that the situation is worse still in respect of the development of entirely new concepts and designs.

Electronics at the Bhabha Atomic Research Centre

In realization of all these pitfalls, the Bhabha Atomic Research Centre decided to chart an independent course for itself, in the field of nuclear electronic instruments, electronic components and systems, design and development. Right from the year 1952, when a small unit consisting of a handful of electronic engineers and technicians was formed by the late Dr H. J. Bhabha, as the nucleus towards the accomplishment of this objective, the prime guiding motive has always been one of self-reliance. The ultimate aim of the exercise was to develop an independent electronic industry in which the design and fabrication of sophisticated electronic instruments and components would be done entirely on the basis of indigenous effort and know-how. Since making a modest start in 1952, this programme has grown steadily, both in size and in the range of activities covered, and during its last few years the erstwhile Electronics Production Division of the Bhabha Atomic Research Centre had attained annual production figure of about Rs 50 lakhs. This annual production represents a few thousand instruments of about forty different types, all of which have been developed, designed and fabricated indigenously and without any foreign collaboration.

In parallel with this, another group was established in 1953 with the responsibility to design, develop, fabricate, install and commission the entire control system of a nuclear reactor along with all the electronics, and electro-mechanical instruments, sub-assemblies, ionization chambers, and counters. At that time the know-how on reactor control systems available all over the world was extremely limited indeed. In spite of this and other difficulties this group completed the control system for India's first nuclear reactor 'Apsara' and followed it up by assuming responsibility for the installation and commissioning of the control system for the 'CIRUS' reactor. This group has also been responsible for the entire design, construction and commissioning of the control system for the reactor 'Zerlina' and the radiation monitoring system for the plutonium plant.

In realization of the importance of the role of computers in modern technology, the Bhabha Atomic Research Centre embarked on the design, development and fabrication of computers a few years ago. Computers cover a very wide range — from simple analogue and digital computers through special purpose analogue and digital computers to the most advanced high speed general purpose computer. A number of analogue computers for special purposes and a large sized general purpose analogue computer have been designed and fabricated in the Bhabha Atomic Research Centre and are now in regular use. A medium sized computer, which is intended to serve, depending upon its configuration, as a computer for real time application, on-line data collection and reduction or scientific computation, has been designed and fabricated and is undergoing final tests.

All the sophisticated developments described above have been accomplished without any foreign collaboration. The resultant accumulation of invaluable indigenous know-how has more than made up for the moderate price in foreign exchange that has had to be paid hitherto, for the import of components. In order to eliminate even this drain on foreign exchange, the development of components and materials has been afforded high priority by this Centre. A number of components, such as precision high stability resistors, Zener diodes, silicon rectifiers, germanium power transistors, thermoelectric modules and coolers, metal film resistors, multiturn potentiometers, tantalum capacitors and components for servo-instruments, such as motors, resolvers, synchros and tachos have been developed and produced on a pilot plant basis.

The Electronics Corporation of India Limited

The achievements listed above were accomplished by groups which, while functioning as almost independent units, have still had to operate within the framework of the Bhabha Atomic Research Centre, which is primarily a research and development organization. It was felt for quite some time that the occasion was opportune to embark on a commercial venture for the large-scale expansion and diversification of all the above-mentioned activities.

In 1963, it was decided that the work on the consolidation and expansion of the production facilities at the Bhabha Atomic Research Centre should be taken up expeditiously. In the meantime these

and other deliberations had given rise to the creation of the National Committee on Electronics chaired by the late Dr H. J. Bhabha. This committee which went into the problem of the consolidated needs of the country in the field of electronics, estimated that, within the next ten years' period, the electronics industry in India would have to develop exceedingly fast, if it has to satisfy the requirements of a fast expanding economy. The national requirements were such that by the year 1975, electronics production at a level of about Rs 300 crores per year was envisaged. The emergency a few years ago has only served to highlight the need for freeing ourselves from the shackles of foreign know-how in the entire gamut of the electronics industry, right from the production of basic components and hardware to the ultimate design and development of the most sophisticated instruments and systems.

The above considerations led to the establishment recently at Hyderabad of the Electronics Corporation of India Limited. This Corporation consists of the following six production divisions: (a) Nuclear and Allied Instruments Division; (b) Resistors and Capacitors Division; (c) Semiconductors Division; (d) Servo Components Division; (e) Power Reactor Instrumentation Division; and (f) Computer Division. The nuclei of these various divisions are composed of persons who were working at the Bhabha Atomic Research Centre and who have gone over to Hyderabad to continue and expand the work already initiated.

The production range of Nuclear and Allied Instruments Division at present scans nearly 60 different types of instruments. These instruments are used in the applications of atomic energy in a wide variety of fields, such as agriculture, industry, medicine and research. They include power supplies, radiation survey meters, scalars, special amplifiers, linear and log rate meters, electronic timers, multi-meters, β H meters, pulse generators, single and multi-channel analysers and oscilloscopes.

This Division is also producing Geiger counting systems, proportional counting set-ups, gamma ray spectrometers, medical spectrometers, non-contact thickness gauges, nucleonic moisture and density gauges, digital voltmeters, billion megohm meter and radiation detectors, radiation reference standard sources and accessories. It is estimated that the annual production of this Division will reach a figure of Rs 115 lakhs by the year 1970.

The Resistors and Capacitors Division will reach a production capacity of Rs 55 lakhs by 1970, comprising carbon/metaloxide film resistors, metal film resistors, multiturn potentiometers, and tantalum capacitors. All these components have fully met the rigid military specifications and the importance of indigenously producing these items for reasons of defence strategy is obvious. The Semiconductors Division is concentrating mainly on productionizing all the devices developed so far and the yearly output by the year 1970 will reach a value of Rs 85 lakhs. The range of production items covers silicon Zener diodes, low current silicon rectifiers, power transistors, lithium-doped germanium radiation detectors, thermoelectric cooling modules and coolers, and germanium and silicon single crystals.

The Servo Components Division is expected to produce servomotors, resolvers, synchros and tachos and motor assembly units, and resistance temperature detectors for nuclear power reactors worth Rs 30 lakhs per year by 1970.

The Power Reactor Instrumentation Division has already made considerable headway in the field of control instrumentation. It is confidently anticipated that this Division will be able to design and fabricate the entire automated control systems not only for India's future nuclear power reactors but also for the associated production and fabrication facilities. This Division is expected to reach an annual production capacity of Rs 80 lakhs by 1970.

The Electronics Committee set up under the Chairmanship of the late Dr H. J. Bhabha had estimated that India would require electronic computers of various sizes worth about Rs 100 crores during the next 10 years. The large computers will, at least in the immediate future, have to be imported or manufactured with foreign collaboration in view of their complexity and limited demand. A number of concerns have already undertaken the manufacture of small size computers. It is, therefore, in the field of medium size computers and special purpose analogue computers that the Computer Division is concentrating its main efforts and is expected to produce Rs 60 lakhs worth of computers by the year 1970.

The Electronics Corporation of India Limited is expected to reach an annual turnover of Rs 425 lakhs by the year 1970, resulting in considerable saving in foreign exchange to the country. Thus, one of the aims of the late Dr H. J. Bhabha, that of developing an independent electronics industry in which the design and fabrication of electronic instruments and components were to be done entirely on the basis of indigenous effort and know-how, is almost achieved.

Some of the more sophisticated equipment and systems which were recently developed and which will now be briefly described are 400-channel pulse height analyser, liquid scintillation spectrometer and digital computers.

The 400-Channel Pulse Height Analyser

The 400-channel pulse height analyser is fully transistorized, has a ferrite core memory and is a highly sophisticated facility for nuclear data processing.

The analyser system provides for 400 discrete levels for determining energy profiles, with a capacity of 10^5 counts per level/channel. These data are stored in a ferrite core memory for subsequent use. The analyser employs a 2 mc/s amplitude-to-digital converter with a variable dead time of $(\frac{1}{2}N+20)$ μ sec (where N is the channel number). It is possible to operate the analyser in the coincidence and anti-coincidence modes (prompt and delayed) with manual or automatic programming, using a crystal-controlled live timer. The data can be displayed dynamically on a cathode ray tube during the process of accumulation or statistically in a linear log fashion. Other novel features of the system are: add/subtract logic (for background subtraction), memory transfer facility and random access to the memory. The

analyser can also be used in the pulse height/multi-scaler mode. The data can be accumulated in two or four independent sub-groups with the help of a 4-channel input multiplexer. The system includes standard extra high tension supply for gamma ray spectrometry, with a suitable linear pulse amplifier. The accumulated data can be read out on a serial or parallel device such as an output typewriter or a serial/parallel printer. Analogue outputs are also provided for X-Y recorders.

This facility, if imported, would cost about Rs 1,50,000, whereas it is available for a price of Rs 1,20,000. The saving in foreign exchange effected in respect of each such unit is nearly Rs 50,000.

The Liquid Scintillation Spectrometer

The liquid scintillation spectrometer has been particularly designed for the assay of low activities of low energy beta emitters, such as ^3H , ^{14}C and ^{35}S . This system incorporates two matched photomultipliers cooled to $+10^\circ\text{C}$ and fast coincidence circuitry. This results in almost total elimination of the thermal noise contribution to background. Efficiencies of 45% for unquenched hydrocarbon samples of tritium and of 90% for carbon-14 at background levels of 50 and 40 cpm respectively are obtainable with this equipment.

Two identical linear amplifiers of maximum gain 1000 are provided and these are adjustable by three controls—input attenuator, fine gain and coarse gain. These amplifiers have excellent overload characteristics and have a rise time better than 100 nanosec.

The coincidence analyser consists of four discriminators—one for monitoring and three for analysis. There are also three coincidence anti-coincidence circuits. The resolving time is again 100 nanosec and three outputs are provided which are useful for applying the quench correction method of pulse height shift. The high voltage supply is capable of supplying continuously adjustable voltage from 500 to 2500 V at 1 ma. This supply provides two outputs for connection to the two phototubes.

Each scaler and timer is provided with a numerical Nixie decade readout. Only one scaler or timer may be selected for display at a time. Each scaler has six decades to cover a counting range of 10^6-1 . Preset time facilities are available in the form of nine time interval selections from 10 to 6000 sec. The counting automatically stops when any of the scalers records 10^6 counts before the preset time is reached. Only positive pulses from 0.2 to 10 V are accepted and the resolving time is less than 1 μ sec. The detection assembly is housed in a temperature chamber in which temperature can be maintained with an accuracy of 0.5% over the range 5-30°C.

An automatic sample changer with a sample capacity of 100 and a sample cycling time of 200 sec for a complete cycle of the conveyor belt excluding elevator up and down time and counting time has been designed and constructed. Numerical indicators are provided which display index numbers of the samples being counted. A programmer unit is also provided which coordinates all the functions, such as movement of the conveyor belt, sample selection and

indexing, up and down movement of the sample being counted and printout. This whole unit is available at a price of Rs 20,000 in which the foreign exchange component is Rs 8,000. The unit, if imported, would cost nearly Rs 40,000.

TDC-12 Computer

A computer which has already been designed at the Bhabha Atomic Research Centre is intended to serve, depending upon its configuration, as a computer for real time applications, on-line data collection and reduction of scientific computations. This computer is a single address, 12-bit (binary digit) fixed word length parallel computer using two's complement arithmetic. The machine has a 4096-word basic core memory with a cycle time of 2 microsec, and it can be expanded in modules of 4096 words if memory extension control is used. Standard features of the system include indirect addressing, self-indexing for address modification and facilities for instruction skipping and programme interruption as functions of input-output device conditions. Programme interrupt eliminates the need for timing programme loops, and permits devices to interrupt the operating programme and initiate a subroutine. The system also provides micro-programmable instructions which permit combining several shift, skip, or input/output transfer commands to be performed in one instruction. The TDC-12 can perform addition or subtraction in 4 μ sec, including instruction access and execution time. This provides a computation rate of 250,000 additions per second.

The machine has a tele-typewriter, high speed paper tape reader and punch and magnetic tape as standard input-output devices. It has provision for accepting a maximum of 31 devices.

The design is production oriented, using mostly indigenous components. The foreign exchange content is about 20% of the total cost. The cost of a basic system is about Rs 4 lakhs and that of a typical system including an additional 4096-word memory and magnetic tape would be about Rs 8 lakhs.

The machine is provided with the following software: (1) symbolic assembler which will translate programmes from symbolic language to binary machine language; (2) Fortran compiler; (3) hardware diagnostic programme; and (4) mathematical library. This type of computer is expected to have a wide demand in the next few years.

Research and Development Work

While the Electronics Corporation of India Limited has been set up as a separate commercial organiza-

tion, a considerable amount of research and development effort continues to be concentrated in the Bhabha Atomic Research Centre. The Electronics Division of the Bhabha Atomic Research Centre, which has confined its activities hitherto to research and development and the production of small numbers of prototype models, will continue its activities to develop new types of instruments. The highlights of the activities of this Division during the past two or three years include: (a) the completion of the second prototype model of a 400-channel ferrite core analyser; (b) finalization of three prototype samples of fast oscilloscope, which have successfully come through stringent environmental tests; (c) development of a feasibility model of radar simulator; (d) development of laser devices; and (e) development of a marine navigational radar.

Thus, the electronics programme of the Indian Atomic Energy Commission, which started off as a minor effort towards achieving limited objectives nearly 15 years ago, has now been transformed into a gigantic undertaking which will go a long way towards making India self-sufficient in the field of electronics. Work on the latest developments in electronics, such as nanosecond techniques, micro-miniaturization and space electronics is also well under way. Our efforts during the past 15 years have demonstrated unequivocally that Indian scientists and engineers, given the right opportunity and environment, can not only bring India up to date but also advance the frontiers of knowledge in this sophisticated field. It is to be hoped that the pioneering effort described above will lead to many similar efforts in the country not only in the field of electronics but also in other areas of science and technology. It is only then that India can take its rightful and deserving place in the vanguard of today's technology.

In closing, I wish to thank all of you for the great honour you have done to me in choosing me for the Shanti Swarup Bhatnagar Memorial Award.

Dr Bhatnagar's efforts to initiate a chain reaction in scientific progress are only too well known. The late Dr Bhabha initially participated in Dr Bhatnagar's efforts and later on continued the work, although he restricted himself to fields such as atomic energy and space technology. I realize that the conferment of this signal honour on me is a recognition of the success achieved by the bold and pioneering efforts of the late Dr Bhabha to put India on the scientific map of the world. It is no less a tribute to the hundreds of young scientists and engineers who have laboured hard and long to make the programme, I have described above, the success it has proved to be.

Choleraenic Toxin—A New Factor in the Causation of Cholera*

THE cardinal manifestation of human cholera is the copious outpouring of rice water stools and vomiting followed by dehydration, anuria (reduced output of urine) and collapse. Ever since the discovery of *Vibrio cholerae* as the pathogenic agent of Asiatic cholera, controversy has been going on to explain the mechanism of production of the clinical symptoms and lesions. Nearly a decade's work has revealed a new concept in the causation of the disease which may truly be called a breakthrough in our knowledge on cholera since Koch discovered the vibrio in 1884. Today, we are in a more fortunate position, because such an understanding is essential in formulating suitable measures for the prevention and cure of the disease.

Cholera endotoxin had traditionally been assigned an important role in the pathogenesis of the disease¹. This was based on the results of investigations carried out by numerous workers till 1955 using mice, rabbits, guinea-pigs, etc. They have used vastly different methods for the isolation from the vibrio of the moiety responsible for the pathogenic action. These workers, with the exception of a few, relied on the mortality in animals as the sole criterion in judging the factor responsible for the clinical symptoms. It will be presently seen that the toxin responsible for producing diarrhoea in man is the one which possesses a very characteristic property and till recently was not known. Perhaps this point might have eluded many more workers had it not been for the introduction of the infant rabbit model by Dutta and Habbu² in 1955 in which the human disease has been reproduced almost to its entirety.

The main handicap to isolate the moiety of the vibrio responsible for the pathogenic action was the paucity of laboratory models that respond specifically to diarrhoea following enteric challenge. The infant rabbit model is unique in that the symptoms of diarrhoea and dehydration similar to human disease are produced by intestinal inoculation of cholera vibrio. The disease could be reproduced consistently and quantitatively.

Following intrainestinal infection with *V. cholerae* in 10-day old rabbit, the diarrhoea begins approximately at the end of 18-24 hr, at which time the onset of copious yellow fluid is noted. As the disease progresses, the colour of the stool changes to typical 'rice water'. The animal then gradually collapses and dies by the 36th to 48th hr with severe dehydration as manifested by loss of 15% of body weight, loss of skin turgor, and dullness of all serosal surfaces^{2,3}. There is haemoconcentration evident from the significant rise in the plasma cell volume, non-protein nitrogen and blood urea. Inability to pass urine is a predominant feature in most of the infant rabbits.

*A dissertation by Dr N. K. Dutta, Director, Haffkine Institute, Bombay, on the occasion of the presentation of the Shanti Swarup Bhatnagar Memorial Award in Medical Sciences for the year 1965 at the National Physical Laboratory, New Delhi, 28 July 1969.

At no stage of the disease do the vibrios find their way into the blood stream except on rare occasions. Studies⁴ using fluorescent antibody technique and conventional histologic examinations in guinea-pigs indicate that cholera vibrios do not penetrate the mucosal epithelium in any region of the intestinal tract and are not found in the lamina propia of the villi. They exert deleterious effects by elaborating toxins in the intestine⁵.

The small intestine at the time of onset of diarrhoea is filled with fluid. The caecum and right colon are massively dilated with slight cloudy 'rice watery' fluid containing occasional flakes of mucus. There is also congestion of intestinal vessels.

Microscopically³, the small bowels of these animals demonstrate marked congestion of all villous and submucosal capillaries and venules and accumulation of acute inflammatory cells in the lamina propia. The mucosa lining remains intact. The crypt epithelium shows increased cytoplasmic basophilia and decreased height. After the diarrhoea has been present for several hours there is disappearance of the acute inflammatory infiltrate in the lamina propia, persistence of congestion, and accumulation of proteinaceous oedematous fluid in lamina propia. The latter picture persists until death.

The large intestine, on the other hand, reveals only minimal congestion and mucous discharge during the same time period. The remaining organs show no gross abnormality.

De and Chatterjee⁶ devised a method for the study of pathogenesis of cholera in which a small loop of intestine of adult rabbit is isolated from the rest of the gut by two ligatures. Following introduction of cholera vibrios into the ligated portion, large amounts of fluid having gross, microscopic and cultural similarity with the cholera stool accumulate after 24 hr.

Electron microscopic studies by Patnaik and Ghosh⁷ in isolated adult rabbit intestine indicate disruption of the plasma membrane of the microvilli. The mitochondria of the mucosal epithelial cells are swollen and the cytoplasm more opaque. There are demonstrable gaps between the endothelial cells of blood vessels in the lamina propia and fluid could leak through gaps into the perivascular spaces.

Our progress in understanding the pathogenesis of cholera took a step forward when it was shown by Dutta *et al.*⁸ that perioral administration of acid lysate of *V. cholerae* (whole toxin) produces diarrhoea and resulting dehydration in the infant rabbit. Grossly, the picture is identical as in the case of infected animals except that the signs and lesions appear earlier and the animals die sooner. In 1963, Oza and Dutta⁹ used ultrasonic method of disintegrating *V. cholerae*. This gives more potent whole toxin. When this toxin is administered orally in divided doses, the infant rabbit remains asymptomatic until shortly or after the last dose (6 hr after the initial dose). The explosive liquid motions begin at this stage, followed by copious and

almost continuous diarrhoea till death, which occurs between 12 and 18 hr from the initial dose of the toxin prepared from 5×10^{11} vibrios. The general signs and symptoms such as voiding of rice water stools, dehydration, and the significant rise in the concentration of red blood cells, non-protein nitrogen and urea in the blood and the changes in the large bowel reveal a picture identical to that from animals receiving viable vibrios.

Toxin is also elaborated by the vibrio cells during their growth in a liquid medium. Finkelstein *et al.*¹⁰ produced potent choleraenic toxin by growing the organisms in brain-heart infusion broth using shaken culture technique. These authors further thought that characterization and purification of choleraenic factor could be simplified even more, if the active product could be produced in chemically defined medium. When they used the basal medium of Finkelstein and Lankford¹¹ supplemented with 1% casamino acid, highly potent choleraenic toxin was obtained. The 'syncase' toxin, as it is called, elicits diarrhoea in infant rabbits very easily and with small quantities. This was confirmed recently by Pal *et al.*¹² in chicken isolated intestinal loop.

Sack *et al.*¹³ introduced canine (dog) model in which both infection and toxin administration result in signs and symptoms substantially similar to those in human infection. Using this model, Carpenter *et al.*¹⁴ administered syncase toxin which had produced cholera-like symptoms as observed in a human volunteer¹⁵.

De¹⁶ prepared 'enterotoxin' by culturing true cholera vibrio in 5% peptone water. The toxin appeared in 6 hr growth. When the toxin was injected into the ligated loop of the adult rabbit⁶, fluid accumulation and other changes occurred. The toxin is thermolabile and produces diarrhoeal symptoms in baby rabbits. It is precipitated by zinc sulphate and ammonium sulphate. It is rapidly inactivated at room temperature and remains active in the cold. It is non-dialysable and is labile to treatment with dilute acid¹⁷. Burrows *et al.*¹⁸ obtained the choleraenic toxin in peptone water supernatant (PSUP) which was prepared by centrifugation of 18 hr maximally aerated cultures in 3% Bacto peptone solution. The toxin obtained from the liquid medium during active growth of the vibrios suggests that it is an excretory product of the vibrios. Further support to this assumption (excretory product) came from the work of Chatterjee and Das¹⁹. Electron microscopic studies of thin sections of *V. cholerae*, harvested during the logarithmic phase of growth in alkaline peptone water or syncase medium, reveal an excretory process of the cell wall in the form of bulging out and pinching-off of portions. An identical phenomenon has also been seen in cells harvested after a short period of incubation (1-5 hr). Particles closely resembling the pinched-off cell wall structures have been detected in the bacteria-free filtrates of the log phase cultures of *V. cholerae*. No similar cell wall changes have been found in vibrios harvested from the stationary phase of growth. All the above studies indicate that choleraenic toxin is released from the vibrios either as a result of the disintegration of the cells or by a process of excretion. This is what may be

happening in the intestine of an individual infected with *V. cholerae*.

Whatever method is used for the preparation of the choleraenic toxin, its capacity to produce diarrhoea and other lesions in the infant rabbit referred to above remains the same. In the infant rabbit, when the diarrhoea sets in, the small bowel shows extreme congestion of the villous and sub-mucosal vessels³. Unlike the infected bowel, no acute cellular infiltrate is present. The mucosa shows no desquamation at any stage of the disease. Increased cytoplasmic basophilia and decrease in height occur in crypt epithelial cells. There is accumulation of proteinaceous fluid in lamina propria and persistence of the congestion. The large bowel containing massive amount of fluid shows mucous discharge and slight congestion. As before, the remaining organs show no significant changes. In short, the gross and microscopic studies of the small and large bowels reveal a picture identical to that from animals receiving viable vibrios, which in turn confirm with the picture presented by Gangarosa and his associates²⁰ that the cholera vibrio causes profuse diarrhoea in clinical cases of cholera through anatomically intact intestinal epithelium.

Once the vibrios enter the upper portion of the small bowel in man, and if the environmental conditions do not prevent their multiplication, then a bulk of the toxin is secreted during the log phase of their growth. Some quantities are also released as a result of the disintegration of the cells themselves. That such a phenomenon occurs is evident from the work of Panse and Dutta²¹ and later confirmed by others. They collected 30-40 ml of stools from each clinically diagnosed case of cholera. This was centrifuged and the supernatant filtered to make it free from organisms. When the stool filtrates were administered orally to infant rabbits, in 9 instances typical choleraic symptoms developed, similar to those seen in infant rabbits fed with sonicate or syncase toxins. De²² has also noted fluid reactions to the isolated adult rabbit loops similar to culture filtrate of *V. cholerae* when filtrates of watery stools from bacteriologically positive cases of cholera were injected in these loops.

Filtrates of cholera cultures increase capillary permeability when injected into the skin of rabbit or guinea-pig. This has been shown by Panse and Dutta²³. Basu Mallik and Ganguli²⁴ have demonstrated that intracutaneous administration of cholera stool filtrates evokes immediate increase in permeability of skin capillaries in the rabbit. Similar observation has been made by Craig²⁵. He showed that the stools of cholera patients and cultures of *V. cholerae* contain heat labile substances which exert identical reactions like erythema, induration, and prolong increase in capillary permeability in guinea-pig and rabbit skin. The stools of cholera patients evidently contain toxins which are derived from the vibrios which have found their temporary home within the gut of the patients.

What happens to the toxin? Is it absorbed in the system or acts locally? Finkelstein²⁶ showed that cholera toxin (syncase toxin) has no effect on the baby rabbit when administered parenterally, by intraperitoneal or intracardiac routes, in doses

greater than those required to produce fatal choleraic diarrhoea when given orally. The toxin has also no effect when given orally in the infant rabbit whose stomach has been separated from the rest of the intestinal canal by duodenal transection. This would mean that in the intact animal, toxin acts directly and locally on intestinal mucosa to cause intense fluid discharge, although some other manifestation of the disease may be of humoral mechanism. Vaughan Williams and Dohadwalla²⁷, working at the Haffkine Institute, separated off a small segment of jejunum of the infant rabbit, with one end blind and the other opening to the exterior as a fistula (Thiry 'loop') and restored the continuity of the remainder of the gastrointestinal tract by anastomosis. *V. cholerae* or the sonicate toxins were introduced in the separated segment which had blood supply intact. The results were that the animals suffered from typical choleraic symptoms and lesions, thereby implying that the toxins must have travelled by the only route available to them, the blood circulation. Therefore, the possibility of the local as well as the systemic effects operating simultaneously cannot be overlooked.

We have seen that infant rabbits respond uniformly with choleraic diarrhoea after the introduction of living cholera vibrios into the small intestine at laparotomy. Experimental cholera can also be produced in baby rabbits by administration of cell-free cholera toxin. These findings lead one to assume with some confidence that the cholera toxin is the pathogenic agent which causes the signs and lesions of the disease in man. However, for direct evidence one had to wait till Benyajati¹⁵ in 1966 dramatically announced that the syncase toxin upon administration to human volunteers by means of a catheter and Crosby capsule into the small intestine elicited diarrhoeal condition indistinguishable from naturally acquired cholera. The first volunteer who received the toxin had only one loose stool 12 hr after administration of the toxin. Nothing more happened. The second subject, who received four times the dosage as the first, started having loose motions, some nausea, and vomiting after 1½ hr following the administration of the toxin which was given over a period of approximately 3½ hr. The subject voided 14,800 ml of watery stools and in turn received 15,120 ml of fluids in replacement. This is a direct evidence of the fact that syncase toxin contains a factor responsible for diarrhoea in human. It also supports the validity of the infant rabbit as a laboratory model for studies in cholera.

Once it was shown that syncase toxin (unpurified) or sonicate lysate of vibrios produced cholera-like disease in man and in infant rabbits, an intense interest was created in the isolation, purification and identification of different cholera toxins. Each experimenter used his own method of isolation and assayed the fractions biologically with several available techniques. The commonly used ones are: (i) infant rabbit cholera which closely reproduces the human disease, (ii) isolated adult rabbit intestinal loop which stimulates the reactions of the small intestine in clinical cholera, (iii) intradermal reactivity in rabbit or guinea-pig skin which indicates the

existence of a factor affecting capillary permeability, (iv) mouse toxicity, (v) the toxicity in 10-day old chick embryo which is an indication of the presence of endotoxin, and (vi) anurian epithelium as a measure of the inhibition of net sodium flux in human intestine^{28,29}.

Burrows *et al.*¹⁸ fractionated vibrio cells by physical means eliminating possible denaturing effects of chemicals. The crude toxin (whole cell lysate, WCL) was prepared by ultrasonic disintegration of the cell, whereas cell wall (CW) and intracellular (IC) substances were prepared by fragmentation and separation with the help of Mickle disintegration apparatus. Subsequently, dialysates of IC and CW were prepared by dialysis through cellophane paper. For this purpose, IC and CW were homogenized in a Braun homogenizer.

The WCL which contained the entire vibrio substance was toxic to mice and to 10-day chick embryo, showed intradermal reactivity in rabbit skin, positive fluid reaction in isolated rabbits loop, diarrhoea in the infant rabbit and inhibition of sodium transport in anurian epithelium. Or, in other words, mixtures of several toxins which react differently in different assay methods constitute the WCL. The IC and its dialysand portion showed similar characteristics in the infant rabbit, but the latter did not show any positive reaction on anurian epithelium. The dialysate of IC substance, on the other hand, inhibited net sodium flux in frog's skin, but was devoid of any other activities, which the parent substance IC had shown. The CW substance produced fatality to mice and in 10-day chick embryo, but gave no intracutaneous reaction in the rabbit skin, no fluid accumulation in the isolated ileum and no diarrhoeal reaction in the infant rabbit. The dialysate of CW substance gave positive reaction to anurian epithelium, but was negative to all other assay methods. This will imply that the toxin responsible for sodium pump inhibition is present in the dialysates of IC or CW substances. The dialysand of CW was toxic to mice and embryonic egg, but gave negative results in other assay methods. This shows that the CW substance contained the conventional endotoxin but was devoid of choleraic toxin. Heating of different fractions for 30 min at 56-60°C removed positive reactions to ligated loop and infant rabbit diarrhoea wherever such tests were positive. Heating did not effect positive reaction to anurian epithelium.

From the foregoing results it seems that choleraic toxin is mainly located in the IC substance, is non-dialysable and heat labile. It is much easier to isolate choleraic toxin from a liquid medium in which vibrios are grown rather than from the whole cell lysate. The advantage is that the endotoxic materials are easily separated out. Burrows *et al.*¹⁸, therefore, prepared choleraic toxin in peptone water supernatant. This was done by centrifugation of 18 hr maximally aerated cultures in 3% Bacto peptone solution, followed by freeze drying. The peptone water supernatant was non-toxic to mice but slightly so in embryonated egg. But it produced diarrhoea in the infant rabbit, a positive intradermal reaction in adult rabbit skin, but failed to evoke reaction in the ligated loop of rabbit.

Finkelstein and his coworkers³⁰ introduced the most purified cholera toxin tested so far. They purified the syncase toxin described earlier by dialysis and gel filtration with Sefadex G200. Purified cholera toxin, as it is called, is stated to be almost free from endotoxin but still not pure, and gives a single zone precipitate with rabbit anticholera antibody absorbed with live vibrios. It causes experimental cholera when amounts as little as 6-12 µg/100 g body weight are administered orally to infant rabbits or lesser amounts injected intractably. It elicited rabbit skin reaction and did not have any haemagglutinating activity for chicken blood cells. It is heat labile, non-dialysable and proteinic in nature. It is stable under ordinary refrigeration but loses its potency at room temperature.

Dutta and Oza³¹ tried to ascertain the nature of the cholera toxin by subjecting unpurified toxins to the influence of a variety of enzymes. The toxin was prepared by the method of Gallutt³² or by sonication⁹. The toxin could be degraded by pure pancreatin or phosphorylase or lipase with the loss of cholera toxin property as tested by the infant rabbit method in each case. It became apparent that the lipid portion of the toxin could be hydrolysed by lipase, while the protein and polysaccharide portions remained intact.

In such circumstances, the cholera toxin property was lost. The toxins also failed to produce cholera in the rabbit when the polysaccharide part was degraded by phosphorylase, while the lipid and protein portions remained intact. The degradation of protein by trypsin did not affect the cholera toxin value, but when the more powerful proteolytic enzyme pronase was used, there was complete inactivation of the cholera toxin property of the toxin. The heat lability of the toxin was also consistent with participation of the protein moiety in the cholera toxin activity of the toxin molecule. The cholera toxin moiety, therefore, appears to be a complex molecule in which protein, lipid and polysaccharide take part. The heat lability of cholera toxin A and the neutralization of cholera toxin activity by antisera and formalin, no doubt, suggest that the protein moiety is important, but do not exclude the part played by lipid and polysaccharide as the enzymatic studies have shown. The work of Jasbir Kaur and Srivastav³³ is interesting. They found that the administration of cholera lipo-polysaccharide orally into the infant rabbit in a dose of 250 mg induced fatal enteric diarrhoea. It may be recalled here that in the assay of the sonicated toxin (WCL preparation) by the infant rabbit method², it was shown that 250 mg of the substance (WCL) which was equivalent to 5×10^{11} vibrios was necessary for fatal diarrhoeal effect. It appears that the cholera toxin is composed of protein, lipid and polysaccharide moieties.

It has now become clear that cholera vibrio produces several types of toxins. The most important of them is the cholera toxin (diarrhoea producing) toxin factor of Oza and Dutta⁹. This is responsible for diarrhoeal symptoms in the infant rabbits and in clinical cases of cholera. It is non-dialysable and heat labile. It is mainly elaborated

in the intracellular substance and excreted through the cell wall or released when cells are ruptured. It is relatively non-toxic to mice and 10-day old chick embryo. It is a complex molecule in which protein, lipid and polysaccharide constituents participate.

The next one in importance is the toxin which is more akin to conventional endotoxin of enteric pathogens. It is heat stable and non-dialysable too. It is mostly absent in liquid medium when the vibrios are grown in it. It initiates no diarrhoea in infant rabbits or stimulates fluid accumulation in the gut. Microscopically, no abnormality in the wall of small intestine is observed following its administration. It is toxic to mice and chick embryo.

The third toxin in this series is the one which inhibits 'sodium pump'. In 1960, Huber and Phillips²⁹ applied short circuit frog skin preparation of Ussing and Zerahn³⁴ to demonstrate its presence in the cholera stool and suggested its possible role in the outpouring of fluid in the gut of cholera cases. The material was heat labile. In the same year, Fuhrman and Fuhrman²⁸ working independently reported that a heat stable extract of cholera cultures inhibited active sodium transport of the frog's skin, which implied that the inhibitor might act on the access of sodium as well as on the pump in the intestine to cause diarrhoeal symptoms. Based on further animal experimentation, subsequent investigators could not associate the toxin as one responsible for causing diarrhoea.

The sodium transport inhibitor toxin differs from cholera toxin and endotoxins in that it is dialysable and when administered to infant rabbits initiates no diarrhoea or when introduced into the isolated intestines of adult rabbits evokes no accumulation of fluid or other characteristic reactions of infected gut³⁵.

The exact role of the sodium inhibitor toxin is yet to be understood. In the control as well as cholera infant rabbits following administration of syncase toxin, Finkelstein *et al.*³⁰ separated out about 2 in. of the small intestine by placing ligatures at both ends. ²⁴Na₂ in physiological saline was injected in the lumen of the loop. Groups of animals were sacrificed 5 min later and 1 hr later and the counts made on the radioactivity of the loops and carcasses of the rabbits. Sodium absorption during the first 5 min interval was significantly inhibited in the cholera animals. Otherwise there was essentially no difference between the control and experimental animals when examined at the end of 1 hr. Therefore, active transport of Na was inhibited in the first 5 min after administration of the isotope, but not after 1 hr. It now appears that the toxin which was said to reduce short circuit current in the frog's skin is probably due to ammonia which the toxin contained and was quite unrelated to the cholera toxin³⁶.

It seems that the former belief that the endotoxin of *V. cholerae* was the sole factor in the pathogenesis of cholera has given way. The vibrios' capacity to elaborate cholera toxin has now been recognized as the main factor in causing severe diarrhoeal symptoms.

Acknowledgement

I thank the Council of Scientific & Industrial Research for recognizing our contributions in the field of cholera by awarding the Shanti Swarup Bhatnagar Memorial Award for Medical Sciences. I do feel today greatly honoured.

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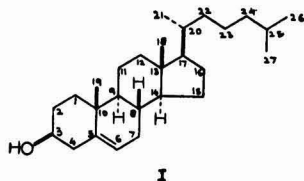
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Some Aspects of the Recent Advances in Plant Steroids

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STEROIDS are of wide natural occurrence. Possibly they are present in all living organisms. Some, which were previously known from animal sources only, are now found to be present in plant kingdom as well. Cholesterol (I), for example, until recently known primarily as an animal sterol, has been found to be distributed in plants also. The common sterols in plants have C_{28} and C_{29} compositions. The quest for knowing how biogenesis of sterols takes place in plants and to what extent the pathways are similar or different from the routes established in animal tissues has engaged the attention of the steroid chemists and biochemists. Interesting studies have been made in the biosynthetic field.



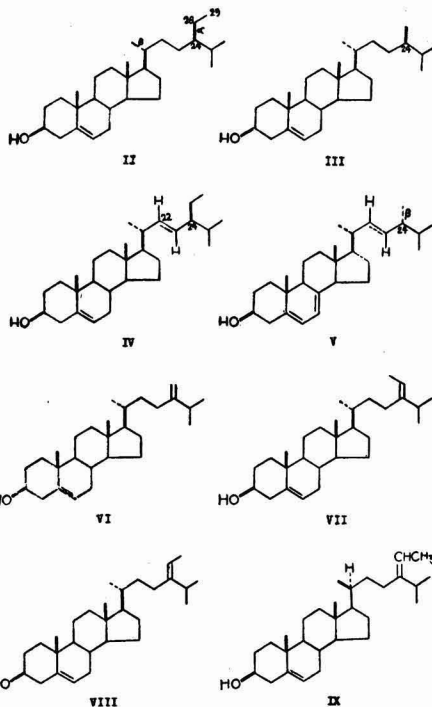
Insect moulting steroid substances, found previously as hormones in insects, are also widespread in plants. Some of them are common to both the sources, whereas others have certain structural variations. These are discussed in this review under the section on "Phytoecdysones".

The other plant steroids of interest are sapogenins, cardenolides, bufadienolides, alkaloids, and digitanols. The occurrence in plants of pregnanes (C_{21}) like pregnenolone and progesterone, androstane analogues (C_{19}), and (C_{18}) steroids like estrone and estriol has also been noted. How are these different plant constituents biogenetically related and what are the pathways through which they are synthesized in plants? There is an intensive research activity aimed at finding appropriate answers to these questions. Many new experimental evidences are available and a number of conclusions are now apparent. There has been rapid progress since the first comprehensive review on the biochemistry of the plant steroids by Heftmann¹ was published.

A survey of the studies pertaining to the above aspects of plant steroids constitutes this review. It has been attempted to project a unified picture of the biogenesis of plant steroids. The relevant literature published up to the end of 1968 has mostly been covered. It has been possible also to cite some references from early 1969 literature. More than half of the references are from the 1967 and 1968 research publications. Since submitting this review for publication, a recent review on the biosynthesis of plant steroids by Heftmann^{1a} has come to the authors' attention.

Phytosterols and Their Biosynthesis

The most common sterol among the higher plants is β -sitosterol (II)*, usually accompanied by varying amounts of campesterol (III). Stigmasterol (IV), having the *trans* 22-ene system, is also of common occurrence. Ergosterol (V) is the well-known yeast sterol. It has been recently isolated from the peel of the citrus Rangpur lime². Ergosterol differs from β -sitosterol, campesterol and stigmasterol in having 24β configuration, whereas the latter three have 24α disposition. Sterols having methylene or ethylidene groups at C-24 are also well known. 24-Methylenesterol (VI) has been isolated from red algae^{3,4}. Fucosterol (VII) is a typical sterol of the brown algae⁴. Gibbons *et al.*⁵ have identified the major sterol of the marine green algae *Enteromorpha intestinalis* and *Ulva lactuca* (Chlorophyceae) to be 28-isofucosterol (VIII). Gas chromatography and mass spectrometry identify the main sterols of the pollen from *Brassica napus* L. f. *annua* as (VI) and (VIII)⁶. Frost and



*Dr John W. Rowe suggests (personal communication) that it is no longer necessary to retain the prefix " β " to sitosterol (II). α -Sitosterol is merely citrostadienol (XXXIII)²⁰⁰ and what has been known as γ -sitosterol is a mixture of sitosterol and campesterol^{19,261}.

Ward⁷ have isolated 7,24(28)-stigmastadien-3 β -ol from *Vernonia anthelmintica* seed, having the 24(28)-double bond disposition corresponding to 28-isofucosterol, and the Δ^7 -avenasterol isolated from pumpkin seed⁸ appears to be the same. On the basis of nuclear magnetic resonance study, Frost and Ward⁷ also propose the 24(28)-double bond stereochemistry to be as shown for fucosterol (VII) and isofucosterol (VIII), which is contrary to that proposed by Dusza⁹.

The sterols listed above have 20 β configuration. Some sterols having 20 α stereochemistry are known, sargasterol (IX) isolated from marine brown algae *Sargassum ringoldianum* and *Eisenia bicyclis*⁴ is an example.

Goad¹⁰, in his review on phytosterol biosynthesis, lists some pertinent references to research papers and reviews concerning the structures and occurrence of the phytosterols.

Cholesterol in Plants

Tsuda and coworkers^{3,4,11} were the first to observe the occurrence of cholesterol in the plant kingdom. They investigated 15 species of red algae (Rhodophyta), growing in Japanese waters, and reported 12 of them to contain cholesterol. Three of the species were found to contain cholesterol and possibly chalinasterol (24-methylenecholesterol, VI). An investigation of red algae species, *Hypnea japonica* Tanaka., resulted in the isolation of only 22-dehydrocholesterol^{12,13}.

Earlier, Heilbron¹⁴ had studied various red algae species collected mainly around Aberystwyth, Wales, and found them to contain either 'sitosterols' or fucosterol (VII). Saito and Idler¹⁵ selected *Chondrus crispus* (Irish moss) out of this list and studied the moss harvested in Nova Scotia. The principal sterol was identified as cholesterol. Gibbons *et al.*¹⁶ identified cholesterol as the major sterol in several marine algae, including *C. crispus*, collected from Cardiganhire coast. In two species, *Dilsea carnosa* and *Polyides caprinus*, cholesterol was accompanied by a second sterol, believed to be 22-dehydrocholesterol. However, in *Rhodymenia palmata* and *Porphyra purpurea*, desmosterol (24-dehydrocholesterol) was the predominant sterol. That a variability in sterol composition may be due to seasonal or other causes is apparent from the work¹⁷ on red algae harvested from the east coast of Canada; for example, in three samples of *Rhodymenia palmata* desmosterol was found to be the major sterol, while cholesterol predominated in the fourth. It is of interest to note the recent reports^{17,18} indicating the occurrence in red algae of C₂₈ and C₂₉ sterols in addition to the predominant C₂₇ sterols.

From two species of blue-green algae, *Anacystis nidulans* and *Freyemyella diplosiphon*, cholesterol and β -sitosterol have been isolated¹⁹. The brown algae, *Laminaria saeoenensis* and *L. digitata*, are reported²⁰ to contain C₂₇, C₂₈ and C₂₉ sterols, with cholesterol as the minor component. Ikekawa *et al.*²¹ found Chlorophyta (green algae) to contain a large proportion of cholesterol, whereas Phaeophyta (brown algae) contained small amounts of the sterol.

Cholesterol has also been isolated from the fungus *Penicillium funiculosum* Thom. (PRL 1724)²². The

occurrence of cholesterol in *Streptomyces olivaceus*²³ and *Escherichia coli*²⁴ has also been reported.

The presence of cholesterol in higher plants was first demonstrated by Johnson *et al.*²⁵, who isolated the sterol from *Solanum tuberosum* and *Dioscorea spiculiflora*. Ardenne *et al.* have also demonstrated the presence of cholesterol in *S. tuberosum*²⁶, *S. demissum*²⁷, and *S. polyadenium*²⁷ by mass spectrometry of the isolated crude sterol fraction, which also contained triterpenoids and C₂₈ and C₂₉ sterols. The presence of cholesterol in various other species of *Dioscorea*^{28,29} and *Tamus communis*²⁹ has been reported.

The ' γ -sitosterol', isolated from *Digitalis canariensis* L. var. *isabelliana*, has been shown³⁰ by gas chromatography and mass spectrometry to be a mixture of β -sitosterol, stigmasterol and cholesterol. Gas chromatography indicates the presence of cholesterol, in addition to other C₂₇, C₂₈ and C₂₉ sterol entities, in the roots of the cactus *Wilcoxia viperina*³¹. The presence of cholesterol as a minor sterol constituent has been indicated in the seeds of *Phaseolus vulgaris*³²⁻³⁴, *P. chrysanthos*³³, and *Avena* spp.³⁵⁻³⁷. Cholesterol, along with other sterols, has been identified in a number of other seeds³⁸, for example, *Glycine max*³⁹, *Arachis hypogaea*⁴⁰, etc.

Cholesterol has been found to be a constituent of *Haplopappus heterophyllus* Blake⁴¹ and apple seeds⁴² by thin layer and gas liquid chromatography. The presence of cholesterol has also been revealed in *Euphorbia pepulus*⁴³, *Glossostelma carsoni*⁴⁴ and flue-cured tobacco⁴⁵. The sterols from the pine barks (*Pinus taeda* L. and *P. banksiana* Lamb.)⁴⁶ and rape seed oil and coconut fat⁴⁷ have also shown the presence of cholesterol in traces on gas chromatography.

Hügel *et al.*⁴⁸ inferred the presence of cholesterol, as well as other C₂₇, C₂₈ and C₂₉ sterols in the pollen from various species by mass spectral study. Devys and Barbier^{49,50} examined the pollen of *Hypochoeris radicata* and found it to contain cholesterol as the main sterol. It also occurs in the pollen from cottonwood (*Populus fremontii*)⁵¹. Cholesterol has been isolated along with estrone from pollen of *Phoenix dactylifera* L.⁵² and roots of *Clossostemon bruguieri*⁵³.

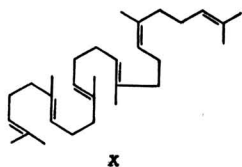
The presence of C₂₇, C₂₈ and C₂₉ sterols has been detected, through mass spectrometry, in the unsaponifiable fraction of the spores of the fern *Polystichum filix* L.⁵⁴.

Phytosterols Biosynthesis

Earlier, Clayton⁵⁵ and Goad¹⁰ have reviewed this topic. The following account on the subject is in a way complementary to the review by Goad¹⁰. The discussion is in the light of the more recent research publications.

The biosynthesis of sterols is a process of fundamental importance to the higher life forms. This has been operative on this planet for more than two billion years⁵⁶. It is well established that sterols and polycyclic triterpenoids are built in the early stages from acetic acid by stepwise conversion to squalene (X). This goes through the condensation of acetyl CoA (C₂) with acetoacetyl CoA (C₄), giving 3-hydroxy-3-methylglutaryl CoA (C₆). A reduction

follows to yield 3,5-dihydroxy-3-methylvaleric (mevalonic) acid, which then gets phosphorylated. Mevalonic acid pyrophosphate (C_6) undergoes decarboxylation and dehydration to isopentenyl pyrophosphate (C_5). Two of these C_5 units condense to produce geranyl pyrophosphate (C_{10}), and the addition of another C_5 unit gives farnesyl pyrophosphate (C_{15}). A tail-to-tail condensation of two C_{15} units then yields squalene (X).



Although Arigoni⁵⁷ suspected the presence of squalene in the radioactive fraction obtained from the germinating soya bean, the first demonstration of the biosynthesis of squalene in plants came from the work of Nes and his associates⁵⁸, who showed the synthesis of radioactive squalene from mevalonic acid-2-¹⁴C in peas (*Pisum sativum*). This was also observed later by Rees *et al.*⁵⁹, during their experiments on the biosynthesis of plant sterols and α - and β -amyrin. Nicholas⁶⁰ reported the biosynthesis of squalene from mevalonic acid-2-¹⁴C in *Ocimum basilicum*. A small but significant incorporation (<0.2%) of farnesol into squalene had also been observed⁶¹. Recently, Baisted⁶² demonstrated the incorporation of label from geraniol-¹⁴C into squalene, β -amyrin and sterols, principally β -sitosterol, in germinating seeds of *Pisum sativum*.

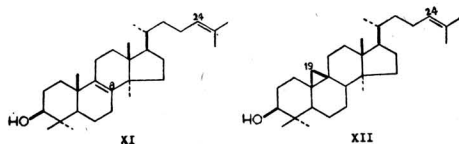
Cell-free systems of higher plants in which squalene has been identified include extracts from tomato and carrot plastids⁶³, *Echinocystis* endosperm⁶⁴, pea seeds⁶⁵ and seedlings⁶⁶. Graebe⁶⁷ has described a cell-free system from young pea fruits in which the hydrocarbons, kaurene, squalene, and phytoene are produced simultaneously from mevalonate-2-¹⁴C. van Aller and Nes⁶⁸ have demonstrated the incorporation of free geraniol into squalene with a homogenate from germinating pea seeds.

Biosynthetic studies have been reported demonstrating the incorporation of the carbons from mevalonic acid into β -sitosterol in soya beans^{69,70}, peas⁷¹, *Delphinium elatum*⁷², *Rauwolfia serpentina*⁷³, *Taraxacum officinale* L.⁷⁴, *Dioscorea spiculiflora*⁷⁵, *Salvia officinalis*⁷⁶, *S. sclarea*⁷⁷ and *Solanum tuberosum*⁷⁸; into stigmasterol in soya beans⁷⁰, *Dioscorea spiculiflora*⁷⁵, *Solanum tuberosum*⁷⁸ and tomatoes⁷⁹. Bennett and Heftmann⁸⁰ showed the conversion of squalene-¹⁴C to β -sitosterol by *Pharbitis nil* seedlings. Recently, Jacobsen and Frey⁸¹ have described the synthesis of radioactive cholesterol from mevalonic acid-2-¹⁴C by the seedlings of *Digitalis purpurea*. van Aller *et al.*⁸² have demonstrated the biosynthesis of 28-isofucosterol from mevalonate in *Pinus pinea*, its formation from 24-methylcholesterol and its conversion to β -sitosterol.

Some prominent features of phytosterol synthesis pertain to the cyclization steps in the conversion

of squalene to triterpenoid tetracyclic systems, the identity of these precursors of the sterols, the elaboration of the branching at C-24, and the introduction of C-22 double bond, and overall postulation of the biosynthetic sequence.

The role of lanosterol (XI) in the animal sterol biosynthesis is well established⁸⁵. It has also been shown to serve as a precursor of ergosterol in whole yeast⁸³. Lanosterol is of rare occurrence in plants and there are only a few cases of its detection^{28,84,85}. It has been suggested that cycloartenol (XII), which is more ubiquitous, may be playing an important role in phytosterol biosynthesis^{10,26,27,86-88}.

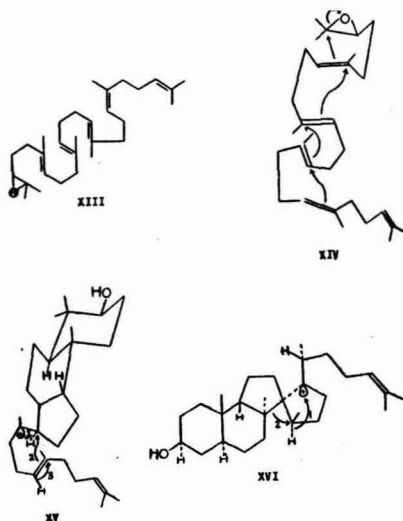


For cyclization of squalene, the initial stage is the formation of 2,3-oxidosqualene (XIII). In consonance with the demonstrations^{89,90} that (XIII) was an intermediate in sterol biosynthesis in a rat liver enzyme system, it is of interest to note that 2,3-oxidosqualene has been identified in tissue cultures of *Nicotiana tabacum*⁹¹. Accumulation of the oxide has been observed⁹² in *N. tabacum* during the inhibition of phytosterol biosynthesis with tris(2-diethylaminoethyl)phosphate hydrochloride. The biosynthesis of β -amyrin and 29,30-binoramyryl system has been achieved in pea seedlings from 2,3-oxidosqualene⁹³ and 2,3-oxido-29,30-binorsqualene⁹⁴ respectively. Of particular interest is the demonstration of the conversion of 2,3-oxidosqualene-¹⁴C into radioactive cycloartenol by cell-free system from bean leaves⁹⁵. This shows that the intermediate formation of 2,3-oxidosqualene is involved in the transformation of squalene to cycloartenol, as in the biosynthesis of lanosterol in animals and β -amyrin system in plants. The application of the oxido route to yeast sterols⁹⁶ and 3-oxygenated triterpenoids in a mould⁹⁷ has also been demonstrated.

There is proton initiated enzymic cyclization of folded 2,3-oxidosqualene (XIV), which has been pictured as proceeding through (XV) and (XVI)⁹⁸, followed by a series of hydrogen and methyl migrations, and ultimately lanosterol and/or cycloartenol can be formed.

The possibility of lanosterol being the biogenic precursor of cycloartenol has been discounted by Rees *et al.*⁹⁹ by their work on cycloartenol biosynthesis in potato leaves using mevalonic acid-2-¹⁴C-(4R)-4-³H₁. The ³H/¹⁴C atomic ratio in cycloartenol was 6:6, the same as that in squalene, indicating the occurrence of hydrogen migration from C-9 to C-8. The conversion of squalene labelled from mevalonic acid-2-¹⁴C-(4R)-4-³H₁ into an 8-ene system (e.g. lanosterol) would require the elimination of a tritium from C-9 (ref. 100).

Ehrhardt *et al.*¹⁰¹ demonstrated the labelling of cycloartenol after incubating the following plant materials with acetate-1-¹⁴C: tobacco leaf disks,

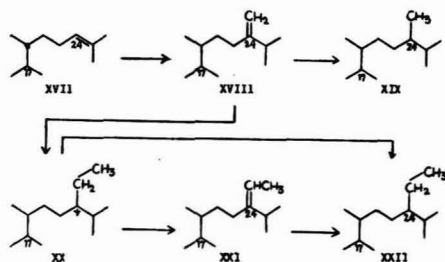


and tissue cultures of *Agave toumeyana*, *Dioscorea composita*, bramble, endive, and carrot. Lanosterol, lanostadienone and lanostadiene were not detectably labelled in these experiments.

However, Ponsinet and Ourisson^{101a} have shown that the latex of *Euphorbia lathyris*, which contains both lanosterol and cycloartenol, has the ability to convert cycloartenol to lanosterol, but not vice versa. Hence, it is likely that cycloartenol is a precursor of lanosterol which accumulates in many plants but not in animals and that lanosterol is a product which accumulates in many animals but not in plants.

The modifications taking place in the side chain, particularly the C-24 alkylation and introduction of C-22 unsaturation, are discussed next.

The C-24 unsaturated side chain (XVII) is probably first converted into the 24-methylene derivative (XVIII), which can be the precursor of 24-methyl analogues (XIX). The methylene derivative (XVIII) can undergo further methylation to give ethylidene derivative (XXI) and/or the ethyl analogue (XXII). This sequence is based on tracer studies¹⁰²⁻¹⁰⁵ using methionine labelled with ¹⁴C, and S-adenosyl methionine¹⁰⁶ may be the active form involved. Castle *et al.*¹⁰² considered the two alternate routes, say, for the formation of (XXII) from (XVIII). In most of the biogenetic schemes^{48,107-109}, the ethylidene derivatives (XXI), such as fucosterol, have generally been considered to be the precursors of ethyl derivatives (XXII), in analogy with methyl derivatives (XIX), which have been shown to originate by saturation of the intermediate methylene derivatives (XVIII)¹¹⁰⁻¹¹². However, Lenfant *et al.*¹¹³ by their experiments on the biosynthesis of the ethyl side chain of stigmast-erol derivatives by slime mould *Dictyostelium discoideum* using methionine-C³H₃ obtained stigmast-22-en-3 β -ol, with five deuterium atoms incorporated into the ethyl side chain. This indicates the conversion of (XX) to (XXII) either by reduction of (XX) by a hydride ion or by the elimination of

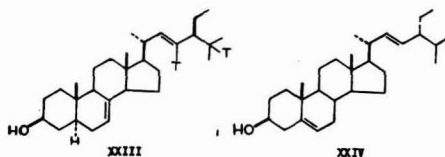


H⁺ (not through XXI) leading to unsaturated compounds which can then be reduced.

The C-24 alkylation may be taking place before the removal of the three extra methyl groups, since a number of naturally occurring 24-methylene and 24-ethylidene steroids and triterpenoids are known, which still contain one or more of these methyl groups.

A number of phytosterols are characterized by the presence of a 22-ene system in the side chain, but not much is known about the mechanism of its formation. Kemp *et al.*¹¹⁴ observed increase in the level of stigmast-erol and decrease in that of β -sitosterol in the root of maize seedling. This corroborates the biosynthetic relationship of these sterols suggested earlier by Bennett *et al.*⁷⁵ through their work on *Dioscorea spiculiflora*. Possibly stigmast-erol is formed by dehydrogenation of β -sitosterol in the side chain.

As would be expected, a hydrogen atom is eliminated from C-23 during the introduction of C-22 double bond in the formation of ergosterol¹¹⁵. Devys *et al.*¹¹⁶ applied 24-methylenedihydrolanosterol-23, 23,25-³H₂ to the leaves of spinach, *Spinacea oleracea*, and isolated thereafter a mixture of labelled sterols containing α -spinasterol (XXIII). Smith *et al.* have studied the stereochemistry of hydrogen eliminations at C-7 and C-22 (ref. 117) and C-6 and C-23 (ref. 118) during the biosynthesis of poriferasterol (XXIV) by *Ochromonas malhamensis*.

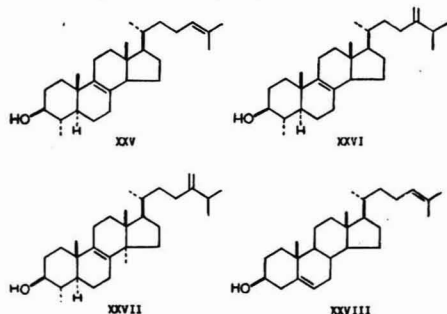


With regard to the intermediacy of lanosterol (XI) and/or cycloartenol (XII) in phytosterol biosynthesis, it may be mentioned that the conversion of lanosterol and cycloartenol to their corresponding 24-methylene derivatives has been shown to take place in pea seed homogenates¹¹⁹. The radioactive compound produced in peas after feeding with radioactive methionine has been identified as 24-methylenedihydrolanosterol¹²⁰.

Lanosterol being the precursor in ergosterol biosynthesis in yeast has been mentioned earlier⁸³. The isolation of 4 α -methylzymosterol (XXV)^{121,122} and 4 α -methyl-24-methylene-24,25-dihydrozymosterol

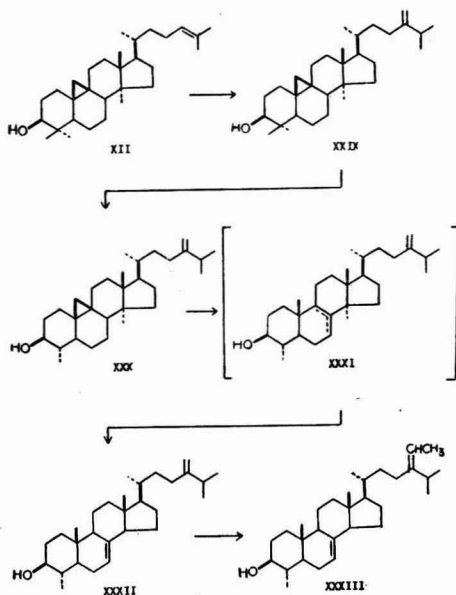
(XXVI)¹²² from yeast, and the observed incorporation of these into ergosterol¹²² point to the possibility of these compounds being involved in one or more biosynthetic routes to ergosterol. But the incorporation of obtusifoliol (XXVII)¹²² and 24-methylene-24,25-dihydrolanosterol^{110,111,115}, which have not yet been detected in yeast, is contrary to the postulated route involving 4 α -methylzymosterol (XXV). There can be possibilities of two or more biosynthetic pathways to ergosterol, or nonspecific enzyme systems which accept unnatural precursors.

Baisted *et al.*⁴³ administered lanosterol-¹⁴C to the leaves of *Euphorbia peplus* and noted the incorporation of radioactivity into the monounsaturated sterol fraction to the extent of 0.4%. This observation, however, does not settle the question of the intermediacy of lanosterol or cycloartenol to sterols. This kind of nonspecificity is apparent from the findings of Nes and coworkers that lanosterol (XI)^{119,120}, cycloartenol (XII)¹¹⁹ and desmosterol (XXVIII)¹¹⁹ can accept the methyl group of methionine in a cell-free system¹¹⁹ from peas and germinating peas¹²⁰. From this even the possibility about an essential requirement of 9,19-cyclopropane ring system for side chain alkylation is ruled out. There may be, however, two pathways functioning, one involving lanosterol (XI) as a precursor and the other cycloartenol (XII).

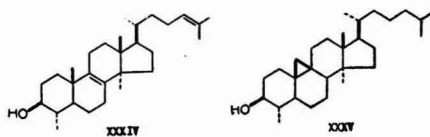


Alcaide *et al.*¹²³ demonstrated the incorporation of 24-methylenedihydrolanosterol-22,23-³H₂ into C₂₉ sterols, mainly β -sitosterol, in the leaves of *Nicotiana tabacum*. However, 24-methylenecholesterol-28-¹⁴C gave only the hydrogenated product, campesterol, and no second methylation step was observed.

Cycloartenol (XII), because of its relatively wider occurrence along with phytosterols, remains the focal point so far as biosynthesis of phytosterols in higher plants is concerned. It is postulated¹⁰ that the biosynthesis follows the sequence: cycloartenol (XII) \rightarrow 24-methylenecycloartanol (XXIX) \rightarrow cycloeucaleanol (XXX) \rightarrow an intermediate having 7-ene or 8-ene system (XXXI) \rightarrow 24-methylenelophenol (XXXII) \rightarrow 24-ethylidenelophenol (citrostadienol) (XXXIII), and that (XXXII) and (XXXIII) may be the precursors of the related C₂₈ and C₂₉ sterols respectively. It is speculated that the cyclopropane ring is opened to give obtusifoliol (XXVII)¹²⁴⁻¹²⁶ which has been isolated from different plants, and it can be further metabolized to phytosterols. It is of interest to note, however, the isolation from



potato leaves of a new sterol¹²⁷ which is tentatively identified as 4 α ,14 α -dimethylcholesta-8,24-dien-3 β -ol (XXXIV), and it is suggested that this may have a role in the biosynthesis of cholesterol in plants according to the sequence: cycloartenol \rightarrow 31-norcycloartenol \rightarrow 4 α ,14 α -dimethylcholesta-8,24-dien-3 β -ol (XXXIV) \rightarrow lophenol \rightarrow cholesterol. Although 31-norcycloartenol does not appear yet to have been reported in nature, the closely related 31-norcycloartanol (XXXV) has been isolated from *Polypodium vulgare*¹²⁸ and *Smilax aspera*¹²⁹. In maize leaves, the presence of cycloartenol (XII), 24-methylenecycloartanol (XXIX), cycloeucaleanol (XXX), obtusifoliol (XXVII), lophenol, 24-methylenelophenol (XXXII), 24-ethylidenelophenol (XXXIII), as well as cholesterol, campesterol, stigmasterol and β -sitosterol has been demonstrated¹³⁰.



Bernard and Reid¹³¹ mention the possibility of a phytosterol precursor other than cycloartenol from their observation of a trace of heavily labelled unidentified 4,4-dimethylsterol in the cycloartenol band obtained after feeding *Nicotiana tabacum* seedlings and tissue slices with mevalonic acid-2-¹⁴C.

In cholesterol biosynthesis in the animal tissues the transfer of 8(9)-double bond to 5(6)-position occurs¹³²⁻¹³⁵ through initial migration of the double bond from 8(9) to 7(8) by the 'addition-elimination' mechanism¹³⁶, followed by oxidative dehydrogenation^{133,134} to give 5,7-diene system, which by a NADPH-linked reduction of the 7(8) bond furnishes the 5(6)-double bond¹³⁵ of cholesterol. This kind

of route can be applicable to plant sterols as well, but there is no experimental verification.

In cholesterol biosynthesis, a 3-ketone is considered to be an obligatory intermediate¹³⁷, which could be formed at the step involving the removal of C-4 methyl groups. There is the possibility of a similar kind of precursor taking part in β -sitosterol biosynthesis⁵⁹. In this context, the reported isolation of cycloartanone from Spanish moss (*Tillandsia usneoides* L.)¹³⁸ and possibly cycloeculalenone from banana peel¹³⁹ are significant findings. These observations are contrary to the view that possibly at some stages esterified intermediates are involved in phytosterol biosynthesis. Sterols have been shown to exist as esters, for example, in birchwood¹⁴⁰, grape fruit peel¹²⁴, maize^{130,141,142}, and peas¹³⁰.

Cholesterol has been found to be the major esterified 4-desmethylsterol of the nuclear and chloroplastidic fractions of the maize shoot¹⁴², but only the nuclear fraction had an appreciable proportion of unesterified cholesterol. It has been shown that mevalonate-2-¹⁴C is rapidly incorporated into both free and esterified sterols in maize¹³⁰ and peas¹³⁰. Recently, Knapp and Nicholas¹³⁹ have found that the majority of the methylated sterols in banana peel are present as palmitate esters, the major component of which has been identified as 24-methylene-cycloartanol palmitate.

It is interesting to note the reported isolation of cholest-5-ene from *Euphorbia lateriflora*¹⁴³. This is a rare instance of the occurrence of non-oxygenated steroids in nature.

What is the function of sterols in plants? To this there is no definite answer. Maybe they have a structural role and form an integral part of the lipid layer of the cell membrane, as cholesterol in animal tissues. It has been suggested¹¹⁴ that unesterified sterols have a structural function in plant tissues. A considerable increase was observed¹¹⁴ in the level of unesterified sterol in rapidly developing shoot and root of maize seedling during the germination period, while the endosperm and scutellum had a virtually constant level of unesterified sterol. Duperon and Renaud¹⁴⁴ have proposed the participation of sterols in the building of new cells from their observed parallelism in the increase in weight of axial parts of germinating peas and increase in the sterol content.

Phytoecdysones

A principle, ecdysone (α -ecdysone, 20-desoxy-ecdysterone), which induces moulting was isolated¹⁴⁵ in 1954 from the pupae of the silkworm *Bombyx mori*. Its structure (XXXVI) has been elucidated by X-ray analysis¹⁴⁶ and synthesis¹⁴⁷⁻¹⁵⁰. The related hydroxy analogue ecdysterone (crustecdysone; 20-hydroxyecdysone) (XXXVII) which is more

active was isolated from silkworm¹⁵¹ and crayfish *Janus lalandei*¹⁵² and later shown to be the major moulting hormone of the insect *Antherea pernyi*¹⁵³. It has been synthesized by Hüppi and Siddall¹⁵⁴.

Kaplanis *et al.*¹⁵⁵ isolated ecdysones from the pupa of the tobacco hornworm *Manduca sexta*, and listed the other then known sources.

It has recently become generally recognized that insect moulting substances are widely distributed in the plant kingdom also. The phytoecdysones belong to the C₂₇, C₂₈ and C₂₉ series. An androstane analogue, rubrosterone, which is possibly a metabolite of phytoecdysone, is discussed later along with another plant androstane derivative.

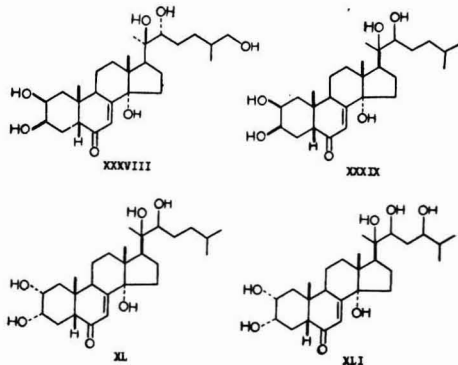
The known plant sources of ecdysone (XXXVI) are the ferns *Pteridium aquilinum* Kuhn¹⁵⁶, *Polypodium vulgare* Linné¹⁵⁷, *Lemmaphyllum microphyllum* Presl.¹⁵⁸, *Osmunda japonica* Thunberg¹⁵⁹, and *O. asiatica* Ohui¹⁵⁹. Ecdysterone (XXXVII) has been isolated, for example, from the conifer *Podocarpus elata*^{160,161} and a number of other species¹⁶², the yew *Taxus baccata*^{161,162}, *Bosea yervamora*¹⁶¹, *Achyranthes rubrofusca*¹⁶³, *A. longifolia*¹⁶³, *A. obtusifolia*^{163,164}, *Vitex megapotaamica*¹⁶⁵, *Polypodium vulgare*¹⁶⁶, etc. Along with ecdysterone (XXXVII), inokosterone (XXXVIII)¹⁶⁷ has been isolated from *Achyranthes radix*¹⁶⁸, *A. faurieri*¹⁶⁹, *A. japonica*¹⁷⁰, *A. bidentata*, etc.¹⁷⁰, *Cyathula prostrata*¹⁷⁰, and *Morus* sp.¹⁷¹.

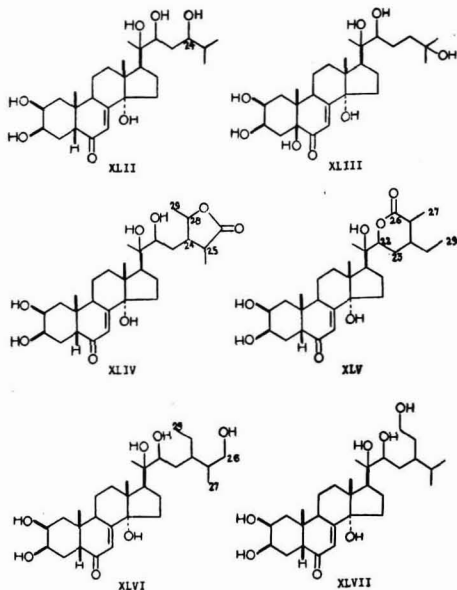
Nakanishi *et al.*¹⁷² isolated four active substances, ponasterones A (XXXIX)^{172,173}, B (XL)¹⁷⁴, C (XLI)¹⁷⁴ and D from *Podocarpus Nakaii*. The co-occurrence of 2 β ,3 β - and 2 α ,3 α -dihydroxy steroids in the same plant is of interest. Ponasterone A (XXXIX) along with ecdysone (XXXVI) and ecdysterone (XXXVII) have been isolated from *Osmunda japonica* and *O. asiatica*¹⁵⁹.

Shidasterone, an insect moulting substance from *Blechnum niponicum* Mahino (Blechnaceae), is considered to be a stereoisomer of ecdysterone¹⁷⁵.

For pteroesterone, isolated¹⁷⁶ from *Lastrea thelypteris* Bory and *Onoclea sensibilis* Linné (Aspidiaceae), the structure (XLII) has been proposed¹⁷⁷. A 5 β -hydroxylated product, 5 β -hydroxyecdysterone (XLIII), has been isolated from *Polypodium vulgare*^{178,179}.

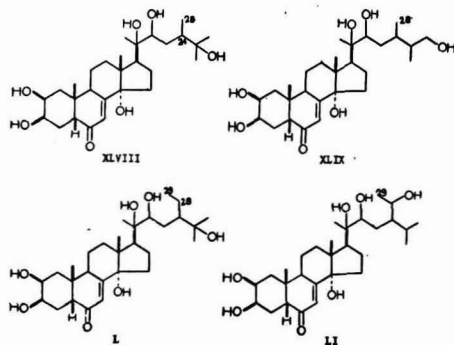
The first report on C₂₈ and C₂₉ insect moulting substances is that of Takemoto *et al.*¹⁸⁰ on the isolation of cyasterone from the roots of *Cyathula capitata* (Amaranthaceae) which has been found to possess the structure (XLIV)¹⁸¹. Another C₂₉





insect moulting substance isolated¹⁸² from *C. capitata* is designated as capitasterone and the available information indicates it to be (XLV). This may be the immediate precursor of cyasterone (XLIV). Two additional C_{29} steroids, amarasterone A (XLVI) and amarasterone B (XLVII), have been isolated from the roots of *C. capitata*¹⁸³. Amarasterone A (XLVI) may be the precursor of (XLV) and (XLIV).

Imai *et al.*¹⁸⁴ who had earlier isolated ecdysterone (XXXVII) and ponasterone A (XXXIX) from the leaves of *Podocarpus macrophyllus* and *Taxus cuspidata*¹⁸⁵ have now obtained¹⁸⁶ from *P. macrophyllus* four new phytoecdysones designated as makisterones A (XLVIII)¹⁸⁶, B (XLIX)¹⁸⁷, C (L)¹⁸⁷ and D (LI)¹⁸⁷. The fact that the phytoecdysones of *P. macrophyllus* have range from C_{27} to C_{29} is of interest from a biogenetic viewpoint. Makisterone C is probably identical with podedcdysone A, which has been isolated from *P. elatus*¹⁸⁸. Also lemmasterone isolated¹⁸⁹ from *Lemnaephyllum microphyllum* Presl. (Polypodiaceae) has been found to possess a struc-



ture¹⁵⁸ which appears to correspond with that of makisterone C (L).

The insect moulting steroids which occur in the plant kingdom may exist partly as glycosides. This possibility finds support from the insect moulting substance isolated from *Pteridium aquilinum* Kuhn var. *latiusculum* Undrew. (Pteridiaceae)¹⁹⁰ which has been characterized as ponasterone A (XXXIX) β -glucoside¹⁹¹.

It is interesting to note that the various insect moulting steroids have been shown to enhance the protein synthetic activity in the livers of mice, as well as does 4-chlorotestosterone, a potent anabolic steroid^{192,193}.

Biosynthetic Studies Pertaining to C_{27} Phytoecdysones, Sapogenins and C_{27} Alkaloids

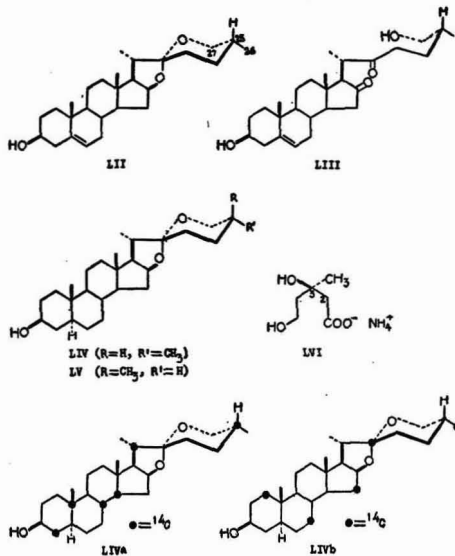
In the preceding section, C_{27} , also C_{28} and C_{29} , phytoecdysones were discussed. The other well-known C_{27} plant steroids are sapogenins and certain alkaloids. For all the three C_{27} steroid categories experimental evidences exist, which indicate a possible role of cholesterol as their precursor in the plants.

Knowing that Karlson and Hoffmeister¹⁹⁴ found *Calliphora erythrocephala* larvae to convert tritiated cholesterol to ecdysone (XXXVI), Heftmann *et al.*^{195,196} administered cholesterol-4-¹⁴C to *Podocarpus elata* seedlings and isolated ecdysterone (XXXVII) in radioactive form. However, when cholestenone-4-¹⁴C was administered¹⁹⁶ to the plant no significant incorporation of radioactivity into ecdysterone (XXXVII) was observed, suggesting that the transformation of 5-ene to 5 β -saturated system may not be going through the 4-en-3-one intermediate as is known to be the case in animals.

In analogy to the above observation, the possibility of the corresponding C_{28} and C_{29} sterols acting as precursors of C_{28} and C_{29} phytoecdysones can be postulated.

To study the mode of formation of sapogenins, Heftmann *et al.*¹⁹⁷ incubated tuber homogenates of *Dioscorea floribunda* with radioactive sodium acetate and radioactive mevalonic acid. Only the former was incorporated into diosgenin (LII). However, *in vivo* incorporation of acetate and mevalonate into *Dioscorea spiculiflora* was approximately equal. Estimation of radioactivity in the sterol and sapogenin fractions showed high specific activity of cholesterol, and its presence in small amounts suggested that the sterol may be the precursor of the sapogenins⁷⁵. A comparison of the specific activities of the sapogenins isomeric at C-25 showed that the 25 α isomer had a higher specific activity than the 25 β isomer.

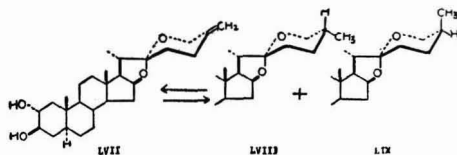
To test the hypothesis of cholesterol being a precursor of sapogenins, cholesterol-4-¹⁴C was administered to the seedlings of *D. spiculiflora*¹⁹⁸. The radioactive cholesterol got converted to the sapogenins diosgenin (LII) and kryptogenin (LIII). The biosynthesis of tigogenin (LIV), gitogenin, and diosgenin from cholesterol-4-¹⁴C-glucoside in *Digitalis lanata*¹⁹⁹ and neotigogenin (LV) from cholesterol-4-¹⁴C in *Lycopersicon pimpinellifolium*²⁰⁰ has since been demonstrated.



Joly and Tamm²⁰¹ applied radioactive ammonium mevalonate (LVI) to young plants of *Digitalis lanata* EHRH and observed the incorporation of radioactivity into tigogenin, gitogenin, and digitogenin. The use of ammonium mevalonate-3-¹⁴C led to the radioactivity pattern in tigogenin as shown in (LIVa), and the radioactivity distribution corresponded to (LIVb) when ammonium mevalonate-2-¹⁴C was applied. These observations conclusively exclude the possibility of the initially formed C₂₇ steroid unit being biosynthetically degraded and rebuilt into C₂₇ spirostanols. However, these findings do not fully corroborate the postulation of cholesterol being the precursor, since 26- and 27-methyl groups seem nonequivalent, as the equatorial 26-methyl group of tigogenin is derived from C-2 of mevalonate, and C-27 to which the oxygen bridge is attached corresponds to the methyl group of mevalonate. The essential intermediate was considered rather desmosterol (XXVIII) or an analogous compound, in which 24-double bond can lead to selective enzymatic oxygenation of C-27, giving ultimately the final spiroketal structure by stereospecific enzymic hydrogenation. Joly and Tamm²⁰¹ explain the incorporation of cholesterol^{198,200} and cholesterol glucoside¹⁹⁹ by their reconversion to the 24-double bond derivatives. However, Joly *et al.*^{201a} have now excluded the possibility that cholesterol is dehydrogenated to desmosterol prior to diosgenin formation. When cholesterol-4-¹⁴C-25-³H was administered to *Dioscorea floribunda*, the isolated diosgenin had the same ³H/¹⁴C ratio as the administered cholesterol. Tschesche *et al.*²⁰² have demonstrated that in *Digitalis* plants, desmosterol-3 α -³H did not give labelled spirostanols; instead it gave radioactive cholesterol. Cholest-4-en-3-one-4-¹⁴C formed radioactive tigogenin and gitogenin. These authors suggested that the biosynthesis of spirostanols has to pass through the 3-keto form. The same workers

have also found that labelled 22-oxocholesterol or 22 ξ -hydroxycholesterol is not converted in *Digitalis* to tigogenin or gitogenin. It has been further shown that tigogenin (LIV) is not a precursor of gitogenin (LVIII), and that they both may have a common precursor.

Takeda *et al.*²⁰³ from tracer studies have shown the interconversion of 25(26)-unsaturated and saturated sapogenins in the homogenates from the epigeous part of *Hosta kiyosumiensis* F. Mack. The plant enzymes were thus demonstrated to be capable of the transformations: 25(26)-dehydrogitogenin (LVII) \rightleftharpoons gitogenin (LVIII) and/or neogitogenin (LIX). This indicated that the 25(26)-unsaturated sapogenins are not artifacts but naturally occurring products.



Lastly, regarding sapogenins, another biochemical transformation may be mentioned. The conversion of gitogenin-26-¹⁴C into 12-oxygenated sapogenins on incubation with the homogenate from the hypogeous part of *H. kiyosumiensis* has been demonstrated²⁰⁴. This enzymatic conversion explained the observed increase of 12-ketosapogenins in the hypogeous part of the plant in winter, but contradicts the finding of Tschesche *et al.*²⁰² that sapogenins are not hydroxylated.

A group of C₂₇ steroidal alkaloids, of which tomatidine (LX) is an example, bears close structural relationship to steroidal sapogenins, and differs only in having a nitrogen atom in place of oxygen in the spiroketal system. It was, therefore, of interest to examine the role of cholesterol in their biosynthesis. The biosynthesis of radioactive tomatidine (LX) from cholesterol-4-¹⁴C has in fact been demonstrated in *Solanum lycopersicum*²⁰⁵ and *Lycopersicon pimpinellifolium*²⁰⁶. In the latter plant, the biosynthesis of structurally related neogitogenin (LV) has been mentioned earlier²⁰⁰. It may be noted that Tschesche *et al.*²⁰² found no conversion of gitogenin into tomatidine in tomato plants.

Earlier, Guseva and Paseshnikenko²⁰⁷ studied by degradative methods the labelling pattern of solasodine synthesized from acetate-1-¹⁴C, acetate-2-¹⁴C and mevalonic acid-2-¹⁴C; the compound (LXI) showed incorporation of the latter of the three.

The conversion of labelled cholesterol into solanidine (LXII) by *Solanum tuberosum* has also been reported²⁰⁹.

On the whole, the above account gives a prominent place to cholesterol in the biosynthesis of other C₂₇ steroids. Apparently no prior degradation to C₂₁ analogues and resynthesis of the C₂₇ unit is involved.

The biochemistry of steroidal saponins and glycoalkaloids, including various unsolved biosynthetic and metabolic problems, and physiological effects has been well reviewed by Heftmann²⁰⁸.

Biosynthesis of Cardenolides, Bufadienolides, C₂₁ Alkaloids and Digtanols

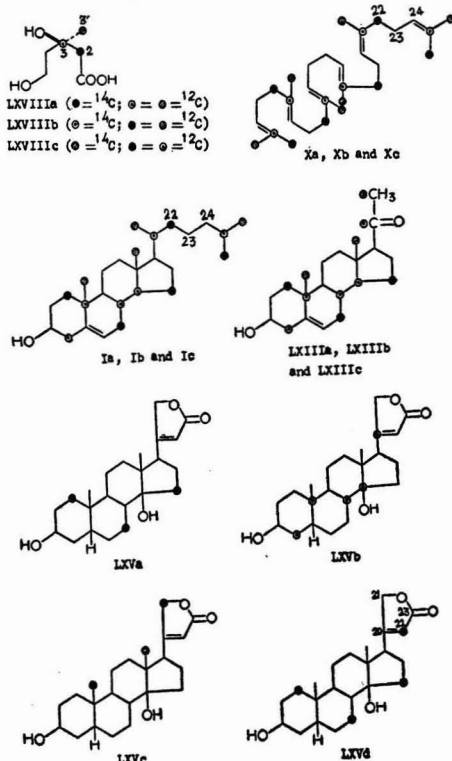
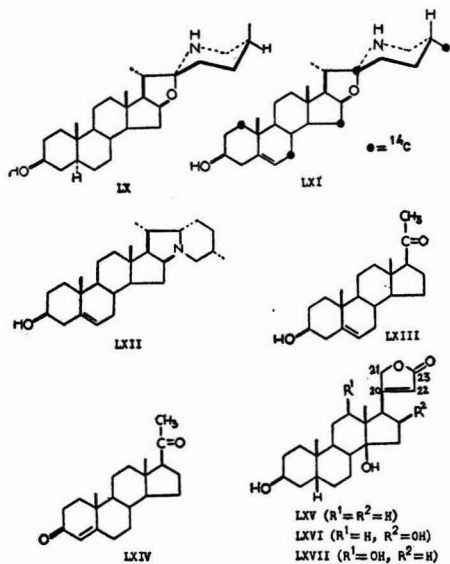
Pregn-5-en-3 β -ol-20-one (LXIII) seems to be of significance as a precursor of cardenolides, bufadienolides, C₂₁ alkaloids and other C₂₁ plant steroids. Tschesche²¹⁰ has given an earlier account of this aspect.

The occurrence of pregnenolone (LXIII) in plants is known. It has been isolated from *Xysmalolium undulatum*²¹¹ and *Trachycalychna fimbriatum*²¹². The possibility of cholesterol being the precursor of pregnenolone finds support from the observed conversion of cholesterol-4-¹⁴C into radioactive pregnenolone by *Haplopappus heterophyllus* Blake⁴¹ and *Digitalis purpurea*²¹⁴. In animals pregnenolone is the biogenetic precursor of progesterone (LXIV) and the same evidently holds true in plants, since, for example, the conversion of labelled pregnenolone into radioactive progesterone has been demonstrated in *Holarrhena floribunda*²¹⁵, *Digitalis lanata*^{216, 217}, and a range of dicotyledonous cultured plant cells²¹⁸. LeBoeuf *et al.*²¹⁹ discovered the natural presence of progesterone in *Holarrhena floribunda*, and recently the hormone along with cholesterol has been identified in apple seeds⁴².

There are various experimental evidences available showing the incorporation of pregnenolone into cardenolides. Tschesche *et al.* demonstrated the transformation of pregnenolone-21-¹⁴C-glucoside into digitoxigenin (LXV) by *Digitalis lanata*²²⁰ and *D. purpurea*²²¹. Caspi and Lewis²¹⁸ showed the incorporation of pregnenolone-7 α -³H into digitoxigenin (LXV), gitoxigenin (LXVI), and digoxigenin (LXVII) in isolated surviving leaves of *D. lanata*. Sauer *et al.*^{217, 222} administered pregnenolone-4-¹⁴C to a *D. lanata* plant and obtained digitoxigenin (LXV), gitoxigenin (LXVI), and other products discussed later. These experiments and the observed²¹⁴ conversion of cholesterol to pregnenolone

in *D. purpurea* would support the hypothesis that cholesterol or a closely related triterpene may be the precursor of butenolides, involving the initial cleavage between C-20 and C-22. This view bears corroboration from tracer studies using radioactive mevalonic acid.

Depending upon whether mevalonic acid-2-¹⁴C (LXVIIIa), mevalonic acid-3-¹⁴C (LXVIIIb), or mevalonic acid-3'-¹⁴C (LXVIIIc) is used as a precursor, according to well-documented evidences, one would get radioactive squalene (X) and in turn cholesterol (I) and pregnenolone (LXIII) in (a), (b) or (c) series respectively. The incorporation of mevalonic acid-2-¹⁴C (LXVIIIa) into digitoxigenin in *Digitalis* plants has been effected²²³⁻²²⁶; the digitoxigenin (LXVa) thus produced was found to be labelled at C-1, C-7, and C-15 (ref. 227, 228). No activity was detected in the butenolide ring of digitoxigenin. On the other hand, digitoxigenin obtained from plants which had been fed acetate-1-¹⁴C was labelled at C-20 and C-23 (ref. 226). Euw and Reichstein²¹³ found a significant amount of radioactivity at C-20 in digitoxigenin (LXVb), produced by the introduction of the ammonium salt of mevalonic acid-3-¹⁴C (LXVIIIb) into *D. lanata*. Leete *et al.*²²⁶ used mevalonic acid-3'-¹⁴C (LXVIIIc) and found the derived radioactive digitoxigenin (LXVc) to have one-third of its activity located at C-21 and the rest at C-18 and C-19. All these results are consistent with the hypothesis that pregnane derivatives



are derived from mevalonic acid via squalene through cleavage between C-20 and C-22 at an appropriate stage, followed by condensation with one molecule of acetic acid.

The observed conversion of pregnenolone-7 α -³H (ref. 216) and pregnenolone-4-¹⁴C (ref. 217) into radioactive progesterone and the demonstration of the incorporation of progesterone-7 α -³H (ref. 216) and progesterone-4-¹⁴C (ref. 229) into digitoxigenin, gitoxigenin and digoxigenin in *D. lanata* suggests that progesterone may be an intermediate in the biosynthesis of cardenolides from pregnenolone. The intermediacy of a 3-ketone, presumably progesterone, in the sequence of steps from pregnenolone to cardenolides was further shown by Caspi and Hornby²³⁰ by the study of the tritium distribution in labelled digitoxigenin produced by the administration of pregnenolone-3 α -³H and pregnenolone-4-¹⁴C to *D. lanata*. The digitoxigenin showed the total absence of tritium at C-3.

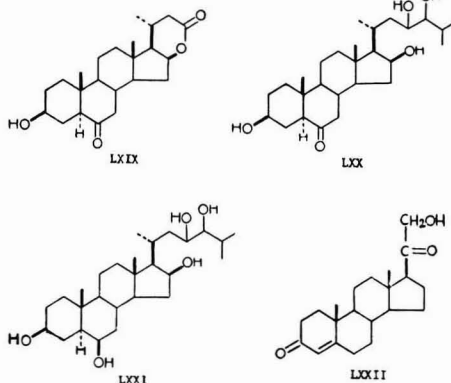
Wickramasinghe *et al.*²³¹ examined the significance of the biosynthetic pathway, cholesterol \rightarrow 20 α -hydroxycholesterol \rightarrow pregnenolone \rightarrow progesterone \rightarrow cardenolides. They simultaneously administered a mixture of 20 α -hydroxycholesterol-7-³H and cholesterol-4-¹⁴C (³H/¹⁴C ratio 8.68) to a *Digitalis lanata* plant. A considerable variation in the ³H/¹⁴C ratios of biosynthesized cardenolides, digitoxigenin (6.74), gitoxigenin (2.58), and digoxigenin (1.72), and also isolation of progesterone-7-³H totally devoid of ¹⁴C, lead one to speculate the operation of several biosynthetic routes. The sequence involving progesterone may not be the only pathway.

The question arises, whether there is a possibility of scission of the side chain of cholesterol between C-23 and C-24 and incorporation *in toto* of the resulting C₂₃ moiety into cardenolides. If that were the case, the digitoxigenin produced from mevalonic acid-2-¹⁴C (LXVIIIa) should have been radioactive at C-22 (LXVd), but this has not been observed. However, one cannot ignore the fact that there do exist systems in plants capable of cleaving the bond between C-23 and C-24. Investigations of the steroidal components of *Chionographis japonica* Maxim.²³² led to the isolation of chiogralactone^{233,234}, a C₂₃ steroidal lactone (LXIX), and sterols chiograsterol A (LXX) and chiograsterol B (LXXI)^{232,235}. Still the overwhelming experimental evidence remains in favour of the intermediacy of pregnenolone and progesterone in cardenolide biosynthesis, and the discussion which follows is based on this apparently significant pathway.

The studies carried out regarding the introduction of oxygen functions at C-14 and C-21 in the biosynthesis of cardenolides from the C₂₁ precursors pregnenolone and progesterone may be commented on next.

Tschesche *et al.*²³⁶ showed in the biosynthesis of cardenolides from pregnenolone that hydroxylation at C-14 is probably the first step, since the incorporation of 15,16-³H labelled 5 β -pregnane-3 β ,14 β -diol-20-one into digitoxigenin, gitoxigenin and digoxigenin was observed in experiments with leaves of *Digitalis lanata*, whereas 14-desoxy-14 α H-digitoxigenin-22-¹⁴C or β -anhydroidigitoxigenin-22-¹⁴C

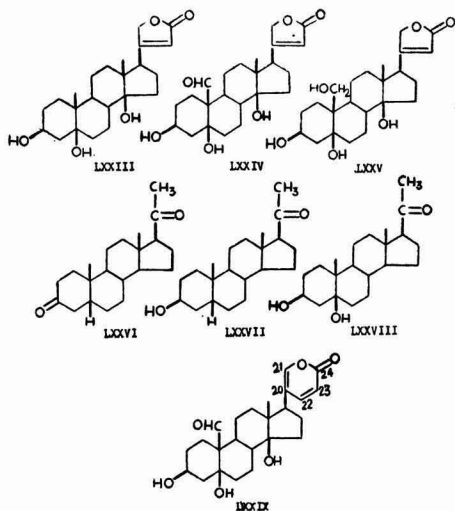
could not be similarly incorporated. Caspi and Lewis²³⁷ hypothesized that since 14 α -hydroxylated steroids occur in plants (for example, *see* Phytoecdysones), it could be possible that 14 α -hydroxy steroids can act as intermediates for the 14 β -hydroxy aglycones. It was visualized that such a change could take place by a free radical process or via an olefin (8,14 or 14,15), the latter may give the corresponding epoxide which upon reductive opening will yield the 14-alcohol. But it was shown²³⁷ that when 14 α -hydroxyprogesterone-1-³H and progesterone-4-¹⁴C were administered to *D. lanata*, the former was not incorporated into digitoxigenin. However, progesterone-4-¹⁴C was incorporated into the cardenolide in nearly normal amounts.



Visualizing that the C-21-hydroxy steroid deoxycorticosterone (LXXII) may have a role in the biosynthesis of cardenolides, Caspi *et al.*²³⁸ administered a mixture of progesterone-4-¹⁴C and deoxycorticosterone-1,2-³H₂ to a *Digitalis lanata* plant and evaluated the relative extents of incorporation of the two into digitoxigenin. It was shown that the transformation of progesterone into deoxycorticosterone *in vivo* did take place, but the route from progesterone via deoxycorticosterone to cardenolides proved to be of little importance. There was a greater incorporation of progesterone-4-¹⁴C into digitoxigenin as compared to deoxycorticosterone-1,2-³H₂. The evident conclusion is that in the sequence of events leading from progesterone to cardenolides other transformations precede the introduction of a C-21 oxygen function.

One has to consider not only the sequence of introduction of oxygen functions at C-14 and C-21 but also at various other positions of the cardenolides. For instance, Sauer *et al.*²³⁹ have recently isolated radioactive periplogenin (LXXIII), strophanthidin (LXXIV), and strophanthidol (LXXV) after the administration of progesterone-4-¹⁴C to *Strophanthus kombé* plants. It may be thought from a consideration of the structures of the three *Strophanthus* cardenolides that a biosynthetic sequence between them may follow the order: periplogenin (LXXIII) \rightarrow strophanthidol (LXXV) \rightarrow strophanthidin (LXXIV). But an interesting point

to be considered, with the intermediacy of progesterone indicated in *Strophanthus* as in *Digitalis* cardenolides, is the respective sequence of 5 β -OH, 3 β -OH and 5 β -H,3 β -OH systems formation. In this connection, approaches have been made by looking for possible intermediates among the metabolites of pregnenolone-4-¹⁴C (ref. 217) and progesterone-4-¹⁴C (ref. 229) in *Digitalis lanata* plants and of progesterone-4-¹⁴C in *Strophanthus kombé* plants²⁴⁰. Radioactive 5 β -pregnanedione (LXXVI) was isolated as a metabolite in both the plants, while 5 β -pregnanolone (LXXVII) and 5 β -hydroxypregnanolone (LXXVIII) were found only in *S. kombé*. In *D. lanata* 5 β -pregnanolone may be having a rapid turnover. As no radioactive 5 β -hydroxypregnanedione was detected in *S. kombé*, Sauer *et al.*²⁴⁰ have suggested the following biosynthetic sequence for *Strophanthus* cardenolides: progesterone \rightarrow 5 β -pregnanedione (LXXVI) \rightarrow 5 β -pregnanolone (LXXVII) \rightarrow 5 β -hydroxypregnanolone (LXXVIII) \rightarrow cardenolides.



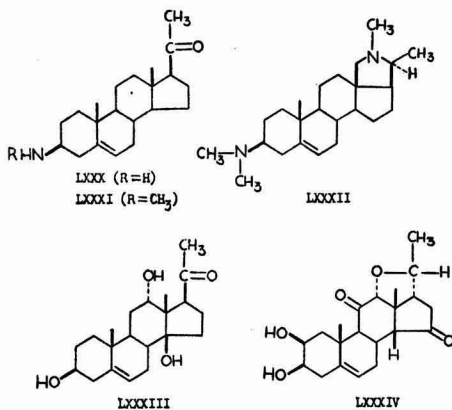
An observation worthy of notice is the finding in *Digitalis lanata*²²⁹ and *Strophanthus kombé*²⁴⁰ of the unusual 5 α -C₂₁ steroids, 5 α -pregnanedione and 5 α -pregnanolone, as metabolites of progesterone-4-¹⁴C. Previously, Graves and Smith²¹⁸ found these compounds in tissue cultures of eight other species of dicotyledons.

There is a solitary report in literature on a plant bufadienolide biosynthesis. Tschesche and Brassat²⁴¹ obtained radioactive hellebrin [the aglycone is hellebrigenin (LXXIX)] by administering pregnenolone-21-¹⁴C into the stem of *Helloborus atrorutens*. It is suggested that oxaloacetic acid may be a precursor in completing the six-membered ring of the bufadienolides, and malonyl CoA in the build-up of the lactone ring in the cardenolides²¹⁰. The possibility of a cleavage between C-24 and C-25 of a C₂₇ precursor and the remaining C₂₄ cholane moiety getting transformed to a bufadienolide may look highly speculative. As such, no cholane analogue seems to have been detected in plants.

Pregnenolone may also have a role as a precursor of alkaloids having C₂₁ steroid skeleton. A study²⁴² aimed at locating the precursors of the alkaloids present in *Holarrhena floribunda* showed that pregnenolone-4-¹⁴C, but not progesterone-4-¹⁴C, was converted to the alkaloids halophyllamine (LXXX) and halophylline (LXXXI) by the leaves. This would possibly involve the replacement of a hydroxyl by an amino group or, alternatively, the transamination of pregn-5-ene-3,20-dione, which could be a precursor of both the alkaloids and progesterone. The occurrence of pregnenolone in *H. floribunda* has been indicated indirectly by the isolation of radioactive pregnenolone after the administration of halophyllamine-4-¹⁴C to the leaves²⁴³. It was interesting to observe²⁴⁴, however, that after the administration of cholesterol-4-¹⁴C to the leaves of the plant, only the alkaloids (LXXX) and (LXXXI) were labelled, but no radioactive pregnenolone could be detected. Some alternate pathways may be at work in the plant.

Recently, the biosynthesis of conessine (LXXXII) from pregnenolone in *Holarrhena antidiysenterica* has been shown by Tschesche and Hulpke²⁴⁵. Regarding the building up of ring E, the possibility of its being derived from a Schiff's base from the aldehyde group at C-18 and an amino group at C-20 is postulated²¹⁰.

Lastly, under this section a reference may be made to the biosynthetic studies on digitanols, nitrogen-free pregnane derivatives, which occur in plants, such as *Digitalis* spp. Earlier, Tschesche and Brassat²²¹ were not able to demonstrate any radioactivity in digipurpurogenin (LXXXIII), which was isolated from *D. purpurea* leaves kept in a solution of pregnenolone-21-¹⁴C-glucoside for 11 days. An application of pregnenolone-4-¹⁴C to the leaves of the plant, *D. purpurea*, for 10 days showed the incorporation of radioactivity into digipurpurogenins²⁴⁶. Sauer *et al.*^{217,222} demonstrated the incorporation of radioactivity from pregnenolone-4-¹⁴C in *D. lanata* into digifologenin (LXXXIV), but not from progesterone-4-¹⁴C (ref. 229). Digifologenin has the 5,6-double bond intact as in pregnenolone, and it is likely that the 4-en-3-one system, progesterone, would not be acceptable as



a precursor. The same kind of observation has been noted²⁴² regarding progesterone not being converted into alkaloids (LXXX) and (LXXXI) in *Holarrhena floribunda*.

The above account establishes that pregnenolone has a conspicuous role in the biosynthesis of various plant constituents. It is interesting that a C₂₇ moiety, cholesterol, is degraded to C₂₁ units, pregnenolone and in turn progesterone, in plants as it happens in animals. One would expect that in plants further transformations to androstanes, C₁₉, and estranes, C₁₈, should also be possible. Though no experimental evidences have been provided as yet for such biosynthetic pathways, but the expectations are borne out by the reported presence of androstane and estrane analogues in the plant kingdom.

Occurrence of Androstanes and Estranes in Plants

The first report about the presence of androstanes in plants came from Zalkow *et al.*²⁴⁷. They reported the isolation of 5 α -androstane-3 β ,16 α ,17 α -triol (LXXXV) from the unsaponifiable fraction of the methanolic extract of the plant rayless goldenrod, *Aplopappus heterophyllus* Blake. However, Bennett and Heftmann⁴¹ were unable to detect (LXXXV) in the specimen of *Aplopappus heterophyllus* investigated by them. They mention that possibly the plant investigated by Zalkow *et al.*²⁴⁷ might have been *H. tenuisectus* (Greene) Blake, which has also been called 'rayless goldenrod'. However, *H. tenuisectus* did not appear to contain the androstanetriol ether^{247a}.

Another androstane, rubrosterone, isolated from *Achyranthes rubrofusca* Wight²⁴⁸ and *A. fauriei*²⁴⁹, has been shown to have structure (LXXXVI)²⁴⁹⁻²⁵¹. This possibly is a metabolite of the phytoecdysones. Rubrosterone possesses little insect moulting activity, while it still exhibits high stimulating effect on protein synthesis in mouse^{193,249}.

In 1933, Butenandt and Jacobi²⁵² reported the successful isolation of 18 mg of crystalline estrone

(LXXXVII) from an extract prepared from 50 kg of palm kernel residue. This was the first report indicating that steroid estrogens may occur as natural constituents of plants. In the same year, Skarzynski²⁵³ isolated from willow catkins a crystalline material, the physical properties of which coincided with those of estriol (LXXXVIII). Wafa *et al.*^{254,255} pointed to the presence of estradiol (LXXXIX) in the pollen of the date palm.

Jacobsohn *et al.*²⁵⁶ investigated various samples of African oil palm, *Elaeis guineensis* Jaq., but their results were negative and gave no evidence for the presence of estrone in the palm kernel residue. A simultaneous study by Heftmann *et al.*²⁵⁷ indicated the presence of estrone in the seeds of the date palm, *Phoenix dactylifera* L. (var. Khadrawy), and subsequently⁵² crystalline estrone was isolated from both the seeds and pollen of the date palm. Amin *et al.*⁵³ have recently isolated estrone from the pollen grains of *P. dactylifera* (var. Samani) and the roots of moghat (*Clossostemon brugueri*, Sterculiaceae).

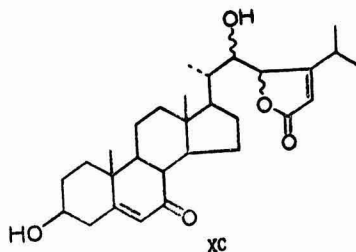
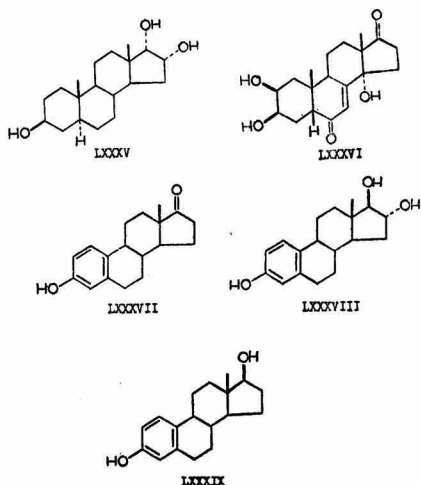
Heftmann *et al.*²⁵⁸ have discovered estrone in pomegranate seeds, *Punica granatum* var. *nana*. The seeds represent the richest source of estrogens yet found, containing 17 mg of estrone per kg. Estrone has been recently identified in apple seeds by Gawienowski and Gibbs^{258a}.

Cholesterol has also been isolated, along with estrone, from the pollen of *Phoenix dactylifera*⁵² and the roots of *Clossostemon brugueri*⁵³. The occurrence of cholesterol and estrone together is suggestive of their biogenetic relationship.

Note added in proof

Antheridiol — A Fungal Sex Hormone

Antheridiol, secreted by the female plant of the aquatic fungus *Achlya bisexualis*, induces the growth of antheridial hyphae in the male plant, and it thus initiates sexual reproduction in this species²⁶². The structure of antheridiol (XC), earlier deduced from spectroscopic evidence²⁶³, has been confirmed by Edwards *et al.*²⁶⁴ through synthesis. Two of the four possible 22,23 isomers of (XC) have been synthesized. One of these has shown physical and biological properties in good agreement with natural antheridiol. Antheridiol is indicated to possess the 22R,23S or 22S,23R configurations at these centres.



Antheridiol is the first specific functioning steroidal sex hormone to be identified in the plant kingdom.

Summary

The recent advances in the biogenesis of plant steroids have been surveyed. The literature regarding isolation from plants of steroids like cholesterol, phytoecdysones (insect moulting substances from plants), pregnenolone, progesterone, androstanes, and estranes like estrone and estriol has also been reviewed.

The biogenesis of plant sterols has a great many points of resemblance with sterol biosynthesis in animals. Cholesterol (C_{27}) appears to be the precursor of other C_{27} steroids, as C_{27} phytoecdysones, sapogenins and C_{27} alkaloids. Pregnenolone apparently is the precursor of cardenolides, bufadienolides, C_{27} alkaloids and digitanols. With the observed derivation of pregnenolone and progesterone from cholesterol in plants, a transformation analogous to animal systems, it is speculated that further degradation to androstanes (C_{19}) and estranes (C_{18}) should also be going on in plants.

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Cesium-137 & Potassium in Indian Whole Milk

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CESIUM-137 is present in the environment as a result of fallout from the testing of nuclear weapons. Continuing measurements of its levels in milk and other diet are useful in estimating long-term radiation dose to man from cesium-137 in the environment from man-made sources.

Stable cesium is geologically rare and it is not established that it is essential to life as a trace element. Since cesium is metabolically similar to the essential element potassium, the bomb-produced cesium-137 finds its way to plants, animals and to man. Cesium and potassium are well absorbed from food and get deposited in the soft tissues of the body. The biological half-life of cesium-137 in man ranges from 70 to 120 days. In the case of children, it is shorter.

Fallout, in the form of fine dust, gets deposited on the surface of the earth, on soil and the leaves of the plants. Cesium-137 has a tendency to be bound to the clay and may not be available for the plants through their roots. But it is partly absorbed through the leaves and gets into the circulatory system of the plants. If organic matter is present in large quantities in the soil, cesium-137 deposited on the soil also may become available through the

roots. The amount of cesium-137 in plants depends mainly on the rate of fallout rather than the cumulative deposit.

Through the plants, the isotope finds a direct passage to cattle and a part of it appears very soon in milk. The fallout deposited on the plants is rapidly removed by the physical agents like wind and rain. So the amount of cesium-137 found in milk varies with the rate of fallout (This is true only if the cattle are fed with fresh grass or leaves).

The levels of cesium-137 in milk can provide a useful index of its hazard to human population, because milk constitutes the major diet of growing children and an important constituent of food for adults^{1,2}.

This report gives the summary of the data on cesium-137 and potassium in liquid whole milk from Bombay and other countrywide stations in India, from 1962 in continuation of our earlier publication³.

Sampling and Measurement

Ten litres samples of liquid milk are collected from each station. Bombay and Anand samples are collected once a month and from other countrywide stations once in 4 months (approximately).

These samples are ashed below 400°C (to minimize the loss of cesium-137) and the ash, put in standard containers, is directly counted for cesium-137 and potassium-40 using a 3×3 in. NaI(Tl) crystal coupled to a 10-channel pulse height analyser. The measurement procedures are essentially the same as described earlier¹⁻⁴.

Results and Discussion

The levels of cesium-137/g potassium and potassium/litre in liquid milk samples from Bombay and Anand after 1962 are given in Table 1.

The yearly average values of cesium-137/g potassium and potassium/litre and the average yearly rainfall for countrywide stations are given in Table 2.

The interesting conclusions emerging from the data given in Tables 1 and 2 are: (1) the highest value of cesium-137 in Bombay milk for any individual sample was in July 1963 and the highest yearly average value was in 1964; for Anand samples the peak activity was in August 1963 and the highest annual average was in 1963; (2) there is an increase in cesium-137 content during monsoon months.

The values of cesium-137/g potassium contents for the countrywide milk samples show large variations. The maximum activity recorded for any station was in 1963. The variations in cesium-137/g potassium content with time for all these stations are, to some extent, similar to those in the case of Bombay and Anand samples (Fig. 1).

TABLE 1—CESIUM-137 AND POTASSIUM LEVELS IN BOMBAY AND ANAND MILK

Period of collection	Bombay		Anand		Period of collection	Bombay		ANAND	
	Cs-137 μμc/g K	K g/litre milk	Cs-137 μμc/g K	K g/litre milk		Cs-137 μμc/g K	K g/litre milk	Cs-137 μμc/g K	K g/litre milk
1962					1965				
Jan.	4.9	0.64	—	—	Oct.	15.2	1.14	2.1	1.10
Feb.	7.5	0.42	—	—	Nov.	10.9	1.12	4.7	1.03
May	5.7	1.06	10.0	1.07	Dec.	—	1.22	1.5	1.38
Sept.	14.7	1.04	6.8	1.05	Av.	14.8	1.24	5.0	1.15
Oct.	10.4	1.29	5.7	1.47	1966				
Nov.	9.6	1.10	3.8	0.76	Jan.	2.1	1.02	7.0	1.18
Dec.	10.9	1.46	4.0	1.42	Feb.	11.5	0.96	8.6	0.84
Av.	9.1	1.00	6.1	1.15	March	—	—	11.9	1.00
1963					April	12.8	1.28	4.0	1.21
Jan.	12.2	1.18	6.6	1.37	May	—	—	1.1	1.05
Feb.	12.8	1.33	6.0	1.22	June	10.9	1.03	1.2	1.21
March	11.9	1.10	6.1	1.31	July	13.6	0.82	2.0	1.09
April	12.5	1.21	8.3	1.07	Aug.	12.5	1.28	3.9	1.07
May	11.6	1.18	6.4	1.69	Sept.	10.2	1.11	1.0	1.14
June	22.9	1.38	7.6	1.26	Oct.	10.7	1.21	3.1	1.15
July	46.5	1.61	16.8	1.67	Nov.	8.1	1.26	1.0	1.09
Aug.	43.3	1.96	29.0	1.30	Dec.	10.0	1.01	1.0	1.06
Sept.	37.7	1.06	—	—	Av.	10.2	1.10	4.7	1.09
Oct.	30.8	1.57	17.2	1.29	1967				
Nov.	27.7	1.15	11.3	1.29	Jan.	8.7	1.21	1.45	1.28
Dec.	11.7	2.54	—	—	Feb.	—	—	—	—
Av.	23.5	1.44	11.5	1.35	March	7.04	1.15	2.31	1.04
1964					April	5.55	1.10	5.25	1.40
Jan.	—	—	13.9	1.07	May	5.91	1.19	1.0	1.41
Feb.	26.4	1.08	—	—	June	1.20	1.25	3.81	1.28
March	22.1	0.89	14.7	1.29	July	0.88	1.28	2.64	1.25
April	18.3	1.55	28.4	1.18	Aug.	7.52	1.16	3.45	1.49
May	23.3	1.30	13.0	1.18	Sept.	3.67	1.14	0.81	1.33
June	27.1	1.29	11.3	1.30	Oct.	5.18	1.22	1.0	1.42
July	29.2	1.29	11.1	1.31	Nov.	3.23	1.25	0.67	1.03
Aug.	45.2	1.11	11.1	1.31	Dec.	—	—	0.24	1.30
Sept.	28.0	0.93	9.9	1.39	Av.	4.89	1.20	2.06	1.29
Oct.	23.6	0.92	4.5	1.39	1968				
Nov.	43.2	0.97	3.6	1.64	Jan.	3.75	0.95	2.75	1.17
Dec.	—	—	7.0	0.62	Feb.	4.08	1.07	0.66	1.25
Av.	28.6	1.13	11.7	1.24	March	—	—	1.32	1.21
1965					April	3.18	1.42	0.91	1.23
Jan.	12.8	1.09	5.9	1.37	May	—	—	—	—
Feb.	17.7	1.25	—	—	June	1.85	1.25	2.55	1.28
March	14.4	1.23	5.2	1.25	July	3.86	1.40	—	—
April	14.7	1.31	5.2	0.85	Aug.	—	—	—	—
May	14.4	1.12	6.5	1.22	Sept.	4.96	1.32	2.37	1.26
June	18.0	1.12	11.3	1.16	Oct.	4.95	1.24	3.34	1.42
July	17.9	1.21	5.8	1.11	Nov.	3.23	1.25	1.31	1.27
Aug.	11.1	1.65	3.7	1.07	Dec.	3.14	1.33	4.7	1.35
Sept.	15.7	1.42	3.6	—	Av.	3.67	1.25	2.21	1.27

TABLE 2—CESIUM-137 AND POTASSIUM LEVELS IN INDIAN MILK

Station	Location	Year	Cs-137 μμc/g K	K g/litre milk	Av. annual* rain- fall cm	Station	Location	Year	Cs-137 μμc/g K	K g/litre milk	Av. annual* rain- fall cm
Adoni	15-5°N, 77-0°E	1962	10.8	0.92		Kodaikanal	10-0°N, 78-0°E	1962	15.2	1.45	167.2
		1964	13.8	1.05				1963	30.8	1.11	
Allahabad	25-2°N, 81-5°E	1963	10.5	0.93	102.7			1964	11.8	1.38	
		1965	6.7	1.34				1965	13.1	1.58	
Bangalore	12-5°N, 77-3°E	1962	0.9	1.53	92.4	Lucknow	26-5°N, 80-5°E	1966	10.6	0.98	
		1963	7.4	1.28				1963	11.1	1.40	99.2
Bhavnagar	22-0°N, 72-0°E	1965	3.7	1.89	60.1	Mercara	12-5°N, 75-6°E	1963	10.9	1.45	334.5
		1966	6.2	1.08				1966	1.2	1.05	
Bikaner	28-0°N, 73-3°E	1963	6.9	1.65	30.5	Muzaffarpur	26-2°N, 85-5°E	1967	1.1	1.3	
		1964	4.8	1.58				1962	5.3	0.95	118.4
		1965	3.8	1.90				1963	9.3	0.89	
Calcutta	22-3°N, 88-3°E	1962	3.0	1.17	157.0			1964	4.1	0.79	
		1964	7.9	1.54				1965	3.3	1.09	
		1966	3.4	1.16				1966	4.1	1.35	
		1967	1.0	1.14				1967	1.0	0.84	
Coimbatore	11-0°N, 77-0°E	1963	2.0	1.70	61.2	New Delhi	28-3°N, 77-1°E	1962	7.5	1.58	71.4
		1964	7.2	1.56				1963	35.1	1.33	
Cuttack	22-3°N, 86-0°E	1962	7.0	1.07	155.7			1965	6.4	1.61	
		1963	12.5	1.48				1966	1.3	1.49	
		1964	7.2	1.56		Patna	25-3°N, 85-2°E	1962	6.5	0.91	111.0
		1965	3.4	1.33				1966	2.2	1.06	
Gangtok	27-2°N, 88-4°E	1966	4.7	1.17		Poona	18-3°N, 74-0°E	1967	1.0	0.85	
		1963	197.7	1.18	311.3			1962	4.4	1.09	71.5
		1966	70.0	1.01				1963	25.0	1.28	
		1966	70.0	1.01				1964	12.6	1.25	
Jabalpur	23-1°N, 80-0°E	1962	3.6	1.21	144.8	Port Blair	11-5°N, 92-5°E	1966	1.5	1.34	318.1
		1963	8.3	1.46				Shillong	25-6°N, 91-9°E	1962	8.9
		1964	2.7	1.22		1963	90.5			1.29	
		1965	8.7	1.60		1964	13.0	1.92			
		1966	3.4	1.36		1966	4.3	2.27			
Kanpur	26-3°N, 80-2°E	1962	9.7	1.11	87.9	Srinagar	34-1°N, 74-9°E	1963	78.3	1.55	56.4
		1963	9.9	1.18				Trivandrum	8-5°N, 77-0°E	1962	17.3
		1964	2.1	1.38		1964	23.6			1.51	
		1965	2.8	1.03		1965	7.6	1.47			
		1966	8.4	1.51	76.1	1966	8.0	1.53			
Karnal	29-7°N, 77-0°E	1963	18.5	1.50		1967	5.3	1.28			
		1964	7.2	1.52		Varanasi	24-3°N, 83-2°E	1963	7.0	1.16	111.3
		1965	5.0	1.29				Visakhapatnam	17-5°N, 83-3°E	1962	10.0
		1966	6.2	1.01		1963	12.5			1.14	
		1967	6.2	1.01		1965	18.3	1.16			

*The average annual rainfall figures (except for Gangtok and Muzaffarpur) have been taken from India Meteorological Department publication, *Climatological tables of observatories in India* (1931-1960).

Higher levels of cesium-137 in Bombay and Anand milk in 1963 and 1964 are due to the increased rate of fallout from high yield nuclear weapon tests during 1961 and 1962. The levels depend mainly on surface deposition and the peaks seen in monsoon months can be due to the increased surface deposition due to rains. The peaks during monsoon months disappeared for Anand samples from 1964 and for Bombay samples from 1965. This effect is possibly due to low atmospheric content of fission debris, and so there is little increase in the surface deposition rate.

It was found that the feeding practices in different dairies in India vary widely. In Bombay dairy, the cattle are fed mainly on fresh green grass throughout the year. So the pattern of seasonal variations of cesium-137 in Bombay milk is representative of the fallout rate. For most of the countrywide stations, the main fodders are hay, oil cakes and grains, even though fresh grass feeding is done occasionally. Fodders like grains and oil cakes are transported from place to place, and so

the local effects of the cesium-137 concentration in milk produced at a particular place may be masked. Similarly, the seasonal variations of fallout deposition at such places will not be seen in milk.

It was shown by Brooker⁵ and others that the level of cesium-137 in milk is proportional to the rainfall. The annual average rainfall (averaged for the last several years) is given in Table 2, along with the annual average cesium-137 content in milk. For the stations in India, the levels in milk are not proportional to the rainfall as can be seen in Table 2. This can be attributed to the reasons stated in the previous paragraph. Similarly, there is no relation between the latitude and the levels of cesium-137 in milk for the stations in India.

Among the countrywide stations, Srinagar and Gangtok (Sikkim) samples show much higher concentrations of cesium-137 in milk compared to the values at other stations.

Deposition of radioactivity in India is mainly during summer due to monsoon rains originating near the equatorial regions and, therefore, having

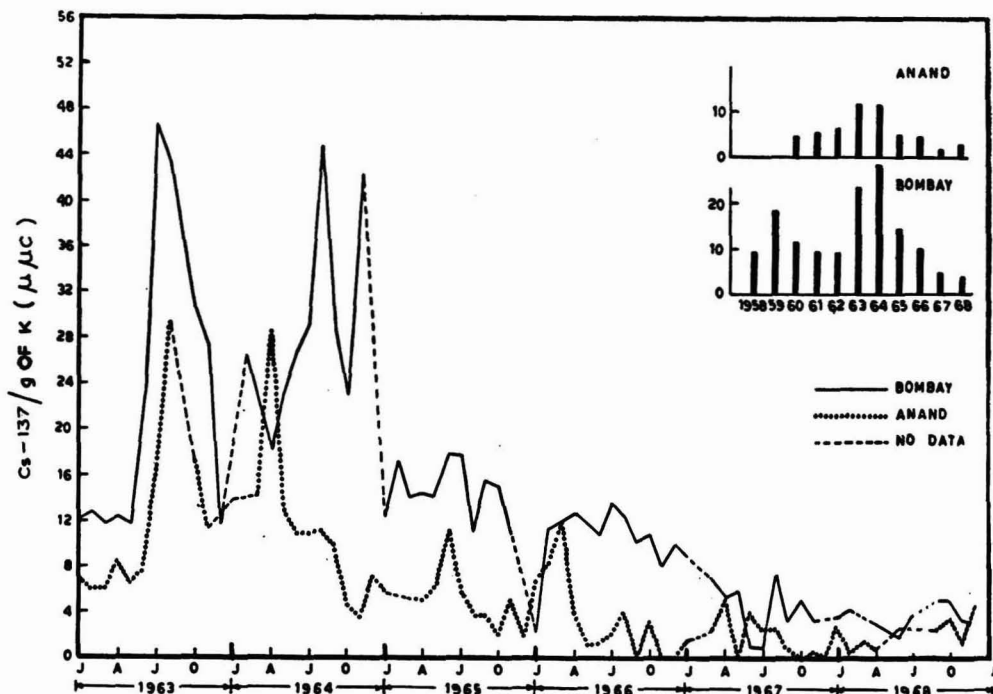


Fig. 1 — Cesium-137 level in Bombay and Anand milk samples

low specific activity. At Srinagar and Gangtok, the rains are distributed more or less throughout the year, with higher rate during winter and spring, when the air activity is maximum. Even during summer, the monsoon currents do not seem to be very effective at Srinagar, as has been shown by Vohra *et al.*⁶. Also, winter rains at Srinagar and in some parts of northern India are due to the movement of low pressure originating in the Mediterranean region and travelling across the northern parts of India. Air masses from higher latitudes are known to have higher specific activity than those of the equatorial regions, which may be the reason for higher cesium-137 levels in the samples from Gangtok and Srinagar.

Internal dose due to cesium-137 in milk — Milk is a major contributor of cesium-137 to the human body and the contributions due to other items of food generally consumed by the population of India contribute much less cesium-137 to the body (except a few leafy vegetables). For the purpose of evaluating the hazard due to the level of cesium-137 in milk, the contribution to body burden by milk is calculated assuming an average intake of half a litre of milk or milk products equivalent to half a litre of milk per person per day. It is assumed that cesium-137 is fully absorbed from the GI tract. The results are given in Table 3. The corresponding dose contribution is calculated on the basis that for a standard 70 kg man, 1 $\mu\mu\text{c}$ cesium-137 per g of potassium present in the body gives a dose of 0.02 m rad/year⁷.

TABLE 3 — DOSES AND BODY BURDENS FROM CESIUM-137 IN MILK AT BOMBAY AND ANAND

Year	Bombay		Anand	
	Body burden $\mu\mu\text{c}$	Dose m rad/year	Body burden $\mu\mu\text{c}$	Dose m rad/year
1961	524.0	54.75×10^{-3}	227.6	32.43×10^{-3}
1962	328.1	46.85×10^{-3}	191.4	27.37×10^{-3}
1963	590.0	84.27×10^{-3}	378.5	54.05×10^{-3}
1964	947.0	135.10×10^{-3}	346.0	49.45×10^{-3}
1965	431.0	61.50×10^{-3}	157.1	22.41×10^{-3}
1966	334.25	47.65×10^{-3}	155.9	22.23×10^{-3}
1967	147.1	21.00×10^{-3}	57.75	8.25×10^{-3}

The maximum permissible body burden of cesium-137, as recommended by ICRP, is 3.0 μc for an individual member of the population⁸. The estimated contribution due to milk produces a body burden very much smaller than the maximum permissible level. As the injection of cesium-137 in the atmosphere has reduced with the cessation of large-scale testing of nuclear weapons, the levels are likely to go down. The present dose rate and the body burden in India are, therefore, not likely to be of concern as a health hazard.

Summary

Naturally occurring potassium and man-made cesium-137 constitute the two important sources of gamma activity in milk and foodstuffs. Since milk

is the major source of uptake of cesium-137 by human beings and is the major diet of growing children, its measurement on a countrywide scale has been continued at the Bhabha Atomic Research Centre, Bombay, since 1958. The results of these measurements from 1962 onwards are presented. Some of the salient features discussed in detail are: (i) yearly variation of cesium-137, (ii) higher values for some of the stations, (iii) dependence on rainfall, latitude, etc., and (iv) the radiation dose and body burden from the observed levels of cesium-137 in milk.

Acknowledgement

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to thank the various milk supply agencies for their interest and prompt supplies.

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Quaternary Equilibria in Liquid Extraction

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THE distribution of a solute between two phases can be represented directly by the distribution coefficient—the ratio of its concentrations in the two phases. The failure of this quantity to be a true constant over the entire range of concentrations encountered in practice has given rise to many methods for representing the distribution equilibrium over a wide range of concentrations. The most commonly used among these, perhaps, is the triangular diagram method, in which the solute and the two solvents in which it is distributed are each allotted an apex of an equilateral triangle. This considerably simplified the problems of predicting and/or estimating the distribution of one component between two others and found wide use in liquid extraction, metallurgy and other fields. The interpretation of various representations in such a graph and the operations for design calculations required in practice have been gone into exhaustively.

The preferential extraction of a solute from its admixture with the original solvent by an extractant is governed by selectivity (β)—the ratio of the solute extracted by the applied extractant to the original solvent extracted by the same. In cases where the desired solute is not very different in nature from the solvent it was originally dissolved in, the selectivity is naturally low, and the extractant suffers from the standpoint of technological feasibility, because of the high extent of contamination involved.

A glaring instance is afforded by the recent trends in the practice of utilization of the byproducts of coal carbonization. Low temperature carbonization of coal yields large quantities of tar (7-8%

of the coal charged, by wt), rich in phenols, the technological utility of which is well known. The practice of extracting the coal-tar phenols with caustic soda suffers from many disadvantages, so that now there is a world-wide preference for the replacement of this method by physical methods, such as liquid extraction. In spite of this, however, the change-over in industrial practice has not yet been possible to any significant extent because of the low selectivity of almost all the solvents used so far.

It has been shown, however, that extraction in conjunction with a light hydrocarbon fraction, such as hexane, gives better results. This is obviously because of a preference of the light hydrocarbons towards the neutral components present in the tar oil from which the phenols are to be extracted. But for adoption of this method to industrial practice, one has to make design calculations for a system involving four components. Investigations with quaternary systems are so meagre and sketchy that no reliable generalization can be established.

The purpose of the present article is a critical review of the methods of representing quaternary equilibria so far introduced in literature, showing their limitations, which, it is hoped, shall pave the way for further investigations towards the general application of this important system.

Pioneering work in the field of quaternary equilibria was done about 25 years ago by Brancker *et al.*^{1,2} who studied the equilibrium of the acetone-acetic acid-water-chloroform system. The method of representation is the most logical and general to date. Four components can obviously give

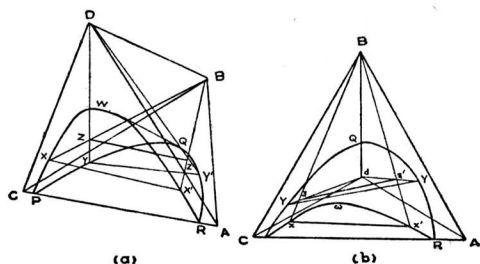


Fig. 1—(a) Space model of a distribution diagram and (b) its orthogonal projection [PQR, ternary distribution curve for system C-B-A; PWR, ternary distribution curve for system C-D-A; XX', YY', ternary tie-lines; XBX', YDY', planes passing through ternary tie-lines and the apex juxtaposite; and ZZ', quaternary tie-lines. In Fig. 1(b) all projected points are represented in small letters]

rise to four ternary systems and if each were to be represented by a triangle and the triangles to be placed symmetrically, then the entire model shall take the shape of a regular tetrahedron, each apex of which would represent one pure component. Since it is awkward to do mathematical computations on a solid figure, resort was taken to orthogonal projection on to one triangular face. The system studied, of course, is relatively simple, since only two ternary systems are heterogeneous. In each of these ternary systems again there is only one pair of partially miscible components, i.e. each of these is what is known as a type 1 system. Side views of the space model and its orthogonal projection are presented in Figs. 1(a) and 1(b) respectively.

In order to plot the orthogonally projected figure, it is necessary to add to the actual weight fraction of each component one-third of the weight fraction of the component represented by the projected apex. The properties of the projected diagram are equivalent to those of the ordinary triangular diagram, which permits the graphical addition and subtraction of the different components.

The main feature of this pioneering work, however, was to find a correlation between the ternary and quaternary equilibria, as represented by their tie-lines. According to these workers, a quaternary tie-line is located by the intersection of the planes passing through a tie-line in each ternary system and the apex juxtaposite to it. This is shown with a hypothetical distribution diagram in Fig. 1(a). In such a case, where the quaternary system is built of two type 1 ternary systems, it is possible, therefore, to obtain the entire quaternary equilibrium data from only the equilibrium data of the individual ternary systems^{3,4}.

It is yet to be ascertained, however, whether this condition holds good when a ternary system with two pairs of partially miscible components, i.e. a type 2 ternary system, is involved. Moreover, as shown by Smith and Braun⁵, the quaternary tie-line cannot be defined by equilibria in two of the ternary systems, when more than two ternaries are heterogeneous. The effect of the third ternary system, obviously, is to rotate the quaternary

tie-line out of the intersection of planes mentioned before.

Wiegand⁶ applied the concepts of analytical geometry to the solution of computation problems suggested by Hunter⁷. He chose to represent the quaternary system as a right tetrahedron rather than a regular one, so that the weight fraction of three of the components could be represented by the three axes common in analytical geometry, and the fourth could be defined by material balance. The equations for the different planes could then be developed and the prediction of equilibria at desired finite points made possible, once the determination of the different constants and their statistical average had been made⁶.

The method, however, is not very economical in labour, considering the complexity of the equations, and the complications which could arise from an inconsistency of the constants used in the equations. Moreover, the entire development of the equation is based on the rule of intersection of tie-line planes, which Wiegand believes, however, to be characteristic of a 'normal' type of distribution in liquid-liquid systems, analogous to Raoult's law for liquid-vapour equilibria. The logic behind this postulation, however, is not clear.

Stage calculations on the orthogonal projection become laborious if a number of stages are involved, for instance, in countercurrent distribution. To overcome this, Smith⁸ has suggested a logarithmic plot, which considerably simplifies the calculational procedure. However, this method also is limited in its application to only type 1 systems and is based on a generalization, the authenticity of which is not completely beyond doubt. According to this generalization, the distribution in a type 1 ternary system may be represented by an equation of the type:

$$\log (d_e/e_e) = g \log (d_f/f_f) + \log k$$

where d_e , d_f are the weight fractions of D , the solute, in the E -rich and F -rich layer respectively; e_e , weight fraction of E in the E -rich layer; f_f , weight fraction of F in the F -rich layer; and g , k are constants.

By plotting $\log (d_e/e_e)$ against $\log (d_f/f_f)$, therefore, the equilibrium in such a ternary system can be represented by a straight line. It has also been shown by Smith that both the ternary systems used by Hunter may be represented in this way. In order to represent both the ternary systems comprising the quaternary system in the same plot, a quantity is chosen as the sum of the weight fractions of two components which are not included in the same heterogeneous ternary. In this way, for one ternary, the above quantity actually represents only one component, while for the other ternary, the same quantity represents the other component.

Thus, in the quaternary system acetone-chloroform-acetic acid-water consisting of the two heterogeneous ternaries chloroform-acetone-water and chloroform-acetic acid-water, if a quantity (m) be chosen to represent the sum of the weight fractions of acetone and acetic acid, it shall represent the weight fraction of only one component in the case

of either ternary. Thus representing the weight fractions of water and chloroform by w and c respectively and defining $X = m/w$ and $Y = m/c$, a plot of $\log X$ against $\log Y$ shall yield the distribution line for both the systems on the same plot. In order to define the equilibrium completely, two more ratios are needed and the ratios $L = m/w$ and $R = h/a$ (where h and a are the weight fractions of acetic acid and acetone respectively) are selected for the purpose. Two complementary charts, obtained by plotting values of L and of R against $\log Y$ and the distribution lines obtained by plotting $\log X$ against $\log Y$ now make all calculations possible on rectangular coordinates.³

The construction of these charts, of course, is also laborious and somewhat complicated, but once they have been constructed, design calculations should not be difficult. The difficulty, however, is in the lack of generality of these charts, which are not applicable to type 2 systems and even not to some of type 1 systems, in the case of which the plot of $\log X$ versus $\log Y$ yields a curve rather than a straight line, warranting exhaustive data for interpolation to be possible. For example, the system butadiene-naphtha-isobutene-furfural, which involves a type 2 ternary system (naphtha-isobutene-furfural), does not yield the correlations mentioned by Smith. This system, again, has been studied and represented in a manner from which no generalization can be drawn^{5,8}.

An independent attempt at rectilinear plotting of quaternary equilibrium data has been made by Chang and Moulton⁹ based on the study of the system water-benzene-ethylisovalerate-ethanol. This also comprises two type 1 ternary systems but differs slightly from the systems studied by Hunter. In the case of the latter, the problem could be visualized as the distribution of two different solutes in a pair of solvents, whereas in this case, the problem is one of the distribution of a solute between two pairs of solvents with one solvent common between the pairs. From the technological viewpoint, of course, Hunter's choice of systems seems to be of greater significance.

The graphical method employed by Chang and Moulton⁹, however, seems to have a greater potential for general application. The method is based on the assumption that when there is an orderly change in the composition of the overall mixture, there should be a corresponding orderly change in every one of the four components in both the phases. If the change in the overall mixture is described by a 'reduced' quantity, the corresponding change in each of the four components in any one phase can be shown by four plane curves, which together shall define the composition of the phase. Four similar curves will, of course, be required to define the other phase, and these eight curves shall be correlated by the change in the overall composition of the mixture.

The basic assumption in this method of representation appears to be of more general application. Nevertheless, computation on these charts, which should be considered nomographic in nature, is also laborious, and theoretical generalization based on

these charts to any great extent cannot reasonably be expected.

The foregoing discussion, admittedly, has been rather sketchy. As a matter of fact, it could not have but been so. In spite of the importance of these systems in technological practice, little attention has been given to them. The scanty data and a general lack of continuum in the studies made add to the difficulties of making a generalization out of these. There is wide scope for intensive research in the field.

For the understanding of quaternary equilibria, it is imperative to make an exhaustive study of systems akin to those used by Hunter and to test the generality of the assumption of intersection of ternary tie-line planes. Even if this generality cannot be claimed, it should be useful to test Wiegand's contention of such being the 'ideal' case, since extrapolating from the analogy used by him — the treatment for deviations from ideality can be rendered by theoretical consideration, as has been done in the case of liquid-vapour equilibria deviating from Raoult's law.

Next, the effect of the third ternary in rotating the quaternary tie-line out of place is an important aspect, since in practice technological demand may necessitate such quaternary systems to be used where a third ternary may easily form. This is particularly true of cases where mixed solvents have to be used to modify the physical, chemical or toxic properties of the major solvent.

Once the generalizations in the case of quaternaries involving type 1 systems — by far the easiest to handle — have been made, their extrapolation to those involving type 2 systems should be fairly easy, particularly where the immiscibility or very slight miscibility between certain pairs of components — a logical limiting case of partial miscibility — simplifies the system considerably.

Intensive research on these lines may some day, perhaps in not too remote a future, yield a fair understanding of the quaternary equilibria.

Summary

The theories put forward to date to represent and predict equilibria in quaternary systems in liquid extraction are critically reviewed. It is concluded that the theories proposed so far are inadequate and there is need for further basic research in the field.

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REVIEWS

ULTRASONICS — THEORY AND APPLICATION by G. L. Goberman (The English Universities Press Ltd, London), 1968. Pp. xii+210. Price 45s.

The book is written for undergraduates interested in the study of ultrasonics as a basic course. The author has also kept in view the requirements of a fresh research worker who is interested in using simple ultrasonic techniques for his investigations. Hence the author has mostly confined himself to low power ultrasonics. There is, however, one chapter on high power ultrasonics.

The book is divided into eleven chapters. The first two chapters deal with basic theories of acoustic wave propagation and radiation in a medium. The next two chapters are on ultrasonic transducers. A good deal of attention is given to piezoelectric transducers, wherein the topic on equivalent circuits of piezoelectric transducers is written very well. Chapter 5 is devoted to high power ultrasonics where ultrasonically induced cavitation is treated in great detail and is discussed from the standpoint of the simple theory of Noltingk and Neppiras.

Chapters 6, 7 and 8 successively describe the theories of ultrasonic absorption, discussion of experimental results and experimental techniques for measuring ultrasonic absorption in fluids. The next chapter on shear wave propagation in viscoelastic medium is, indeed, very nicely written. Here all the salient facts of this newly developing subject are discussed in a cogent way.

Study of ultrasonic propagation in solids is fast developing and to keep pace with it is very difficult. The author has, therefore, only touched on the most important aspects of this subject in Chapter 10. The same can be said about the applications of ultrasonics in industry and elsewhere. The author has given in Chapter 11 some of the more important applications of ultrasonics. However, the bulk of space in this chapter is devoted to flaw detection in industry and medicine.

In Chapter 6, the author has made use of the theory developed by Andrae and Lamb to discuss the excess ultrasonic absorption in a fluid medium. The advantage of this theory is that it is equally applicable to thermal and structural relaxation processes. But the theory involves the use of a large number of internal degrees of freedom of the fluid molecules with consequent cumbersome mathematical expressions. The author should have avoided this theory, which is very difficult for a beginner. In the alternative, the author should have treated the same theory taking only one degree of freedom and made the chapter less heavy. Besides, the author has subsequently discussed the results involving only one degree of freedom. However, the discussion and evaluation of the dispersion and absorption curves towards the end of the chapter are excellent. Chapter 7, dealing with experimental results, which should prove very interesting, is, unfortunately, made very brief.

A few printing mistakes exist which, it is hoped, will be rectified in a subsequent edition. The book is well got up and the figures are very clean and instructive. The book can be used profitably as a preliminary text-book by students and research workers interested in ultrasonics.

SURYANARAYANA MANTHA

CHARACTERIZATION OF ORGANOMETALLIC COMPOUNDS (Part 1) by Minoru Tsutsui (Wiley-Interscience Publishers Inc., New York), 1969. Pp. vii+371. Price £ 15.00

The present book is the twenty-sixth in the series of monographs on 'Analytical Chemistry and Its Applications' being published under the general editorship of Professors P. J. Elving and I. M. Kolthoff. Almost all the publications in this series are considered standard texts and the present book also has maintained this reputation.

The most noticeable development in science during the last 2-3 decades is the gradual disappearance of the traditional dividing lines between various disciplines. In the field of chemistry, such a development is clearly noticed in the rapid strides made in the synthesis of organometallic compounds of newer and newer varieties during the last two decades. A remarkable breakthrough in this direction was the discovery and characterization of ferrocene in 1951, which opened a new era in the chemistry of transition metals. A prospective investigator in this fastly growing field has, thus, to face a formidable challenge; he must be fairly conversant with the recent developments in much broader areas of chemistry and must be prepared to borrow and use knowledge from all branches of chemistry (organic, inorganic and physical).

Although the techniques of analysis and characterization described in the present book are fairly well known, and a number of recent reviews and treatises are available on each individual technique, the present account appears to be the first effort at focusing the attention of the reader to the manner in which these techniques have been employed to elucidate the composition and structure of organometallic compounds. All the authors have taken care to present a brief yet lucid account of the fundamental principles of the technique before illustrating their applications.

The contributors to the present volume are mostly well-known authorities in their respective fields. The topics include chemical characterization (Otto Schwarzkopf and Francine Schwarzkopf), infrared spectroscopy (K. Nakamoto), mass spectrometry (R. W. Kiser), X-ray diffraction (N. C. Baenziger), microwave spectroscopy (R. Varma) and Mössbauer spectroscopy (R. H. Herber), following a brief and excellent introduction to organometallic chemistry by F. K. Cartledge and H. Gilman.

The introduction deals with the typical classification of organometallic compounds followed by the general methods useful in the synthesis of each

class of compounds. At the end of the chapter, the authors have provided a well-chosen list of most general and recent references, the usefulness of which has been increased considerably by arranging them groupwise. The chapter on 'Chemical characterization' lays particular emphasis on the methods of handling sensitive compounds as well as volatile liquids and gases.

Infrared spectroscopy is extremely useful not only in identifying the organometallic compounds but, in many cases, in determining their structures also. Nakamoto's article, as could be well expected on the basis of his vast experience in the field, is very informative and well illustrated. Emphasis has been particularly laid on far infrared, a region in which a large number of metal-carbon bands appear and information about which had not been reviewed exhaustively. Typical examples have been chosen both from sigma- as well as pi-bonded derivatives.

The next two chapters give exhaustive and well-chosen examples of the applications of mass spectroscopy and X-ray diffraction. Due to non-availability of commercial equipment and also because of certain initial reluctance arising from the fear of contamination of the instruments with the decomposition products, it is only during the last few years that the powerful tool of mass spectroscopy has been applied widely in the field of organometallic chemistry. Kiser has not only made outstanding contributions in the field, but the usual lucid style, in which he has dealt with the instrumentation techniques and the methods employed, makes the account really useful even for the beginner and non-expert in the field. The author has illustrated the account with examples of metal alkyls and aryls, transition metal carbonyls and cyclopentadienyls, arene and π -allyl complexes, and several mixed ligand-metal compounds. Looking at the chemical literature of the last five years, it becomes evident that X-ray diffraction has become the most important tool in finally defining the structure of organometallic compounds, particularly the electron deficient molecules and π -complexes. The chapter by Baenziger is thoroughly illustrated and a pleasure to read. Most of the information has been tabulated carefully.

Organometallic chemists have only recently started to apply microwave and Mössbauer spectroscopy to their compounds and the last two chapters not only summarize the known and relevant data in the field, but also indicate how much more could be expected by these two penetrating techniques.

In spite of being a collaborative effort, the chapters have been so well chosen and arranged that there is hardly any repetitions and the whole book gives an overall impression of coherence. The paper, printing and binding are excellent and there are hardly any printing mistakes [the reviewer could detect only the following very minor ones: (i) RB in the last line in the equation near the bottom of page 20 should be RBr; (ii) $(CH_3)_2SnX$ in the first line of para 3.2.8.2 on page 102 should be $(CH_3)_2SnX_2$; (iii) Pn in equation 4.30 on page 162 should be Pb].

The editor and the authors are to be congratulated for bringing out such a coherent account of the diverse techniques employed in the characterization of organometallic compounds. The book will certainly prove very useful and almost a boon to the graduate students and research workers in the expanding field of organometallic chemistry.

R. C. MEHROTRA

FIBRE REINFORCED MATERIALS by G. S. Holister & N. C. Thomas (Elsevier Publishing Co. Inc., New York), 1968. Pp. xiv+154

The demands of the nuclear age as well as the supersonic-cum-space age have served as the mother of invention of many new high strength structural materials. Many of the structural components, as in the case of high temperature reactors, turbines and aero-space vehicles, have to withstand high thermal stresses induced by very high temperatures to which these structures are subjected. Thus in the last 20 years, materials research has acquired a new dimension. The conventional approach of finding new alloys such as miraging steel, titanium, etc., as suitable high strength structural materials has met with great success. An alternate approach in the search for finding new high strength structural materials has led to the development in the last 15 years or so of the two-phase composite structure where conventional materials (either metals or plastics) are reinforced by high strength fibres. Research in the area of two-phase composite consisting of a matrix in which high strength fibres are embedded has been extensive as well as intensive, particularly during the last ten years. The book under review is a brief survey of the recent research work on certain problems pertaining to this area of fibre reinforced materials and has been written, to quote the authors, to "be of use to other workers in this rapidly expanding field which represents probably the most promising and exciting aspect of current materials research". To a large extent, the above-mentioned aim of the authors has been fulfilled in spite of the fact that the book is only of 150 pages.

The small size of the book obviously limits the coverage. The authors have taken a balanced point of view between theory and experiments and have shown as far as possible correlation (or lack of it) between theoretical predictions and actual experimental results. Certain problem areas where further work, both theoretical and experimental, is necessary are also mentioned.

The first chapter summarizes the standard existing techniques for the manufacture of the two-phase composite, i.e. for embedding properly the fibres in the matrix. As this chapter is a quick survey, one cannot hope to find full technological details of the fabrication techniques or the metallurgical processes involved.

The second chapter of the book deals with the various existing theories to determine the longitudinal and transverse stress distributions along the fibre-matrix interface. The composite structure is assumed to be subjected to tensile load. The theoretical results are compared with the available experimental results.

In the next chapter, the determination of the ultimate tensile and compressive strengths of the composite structure is taken up. One of the sections of this chapter discusses the effect of temperature on the ultimate strength of the composite. It is shown that while increase of temperature reduces the ultimate strength of the composite (reinforced metal or alloy), this reduction is much smaller than in the case of the metal or alloy without any reinforcement. Thus, the conclusion is drawn that the reinforced metal/alloy is better than the unreinforced metal/alloy for withstanding high temperatures.

The last three chapters are short ones, of less than 15 pages each. Chapter 4 gives a brief phenomenological description of the fatigue behaviour of the composite (reinforced plastic and reinforced metal) when subjected to repeated tension or reversed bending.

In a two-phase composite, the orientation of the fibre axis has an important role as far as the tensile strength and physical properties of the composite are concerned. The influence of fibre orientation on the tensile strength is discussed in Chapter 5, whereas in the next and the last chapter of the book, its influence on physical properties of the composite, such as modulus of elasticity and Poisson's ratio are discussed. It is necessary to state that this book, which is essentially a survey of recent research work done on certain selected problems, does not deal with many interesting problems such as the bending behaviour of composite structural elements — beams, plates and shells, and composite cylinders subjected to pressure, internal and/or external. But then, the small size of the book imposes severe limitation on coverage of the material. On the whole, this book is a very useful introduction for those who wish to start research work in the area of fibre reinforced materials.

K. A. V. PANDALAI

THE DIRECTORY OF SCIENTIFIC RESEARCH INSTITUTIONS IN INDIA, compiled by T. S. Rajagopalan & R. Satyanarayana with the assistance of K. Ramaswami & V. Sarangan (Indian National Scientific Documentation Centre, New Delhi), 1969. Pp. 1120. Price Rs 75.00, \$ 30.00, £ 10

There has been a tremendous upsurge in the research and development effort in the country since Independence and a large number of new research institutes have come up. Attempts at taking stock of the situation have been handicapped by lack of a centralized reference source of information regarding the research institutes scattered all over the country. This lacuna has been admirably filled by the *Directory of Scientific Institutions in India* compiled by the Indian National Scientific Documentation Centre.

The Directory brings together, for the first time, current information on 913 institutions engaged in fundamental and applied research and in develop-

mental work in the fields of science and technology, including psychology, demography, forensic medicine and geography. Academic institutions having facilities for postgraduate research work are also covered.

The institutions have been arranged according to major complexes, such as CSIR, ICAR, ICMR, Defence Research and Development Organization, Atomic Energy Commission, Central Government Institutions, State Government Institutions, Private Research Institutions, Academic Institutions, Co-operative Research Associations, Public Sector Industries and Private Sector Industries.

The Directory provides details on the budget, i.e. recurring grant for the year 1967-68, staff engaged in research, the divisions and field stations with the names of their heads, history giving a chronological account of the structure and growth of the institutions, functions, achievements, fields of research, special facilities, consultancy services offered, special collections maintained, facilities for postgraduate work, etc., and current periodical publications brought out by the institutions.

Three indexes, viz. Name index, Index of periodical publications and Subject index, have been provided. The name index provides entries for all the heads of the institutions and the divisional heads. The index of periodicals provides entries for periodical publications only and the annual reports and other reports have been excluded. The subject index provides information about fields of research/divisions, special equipment and other facilities, significant items of achievement and some important and special functions. The appendices include lists of institutions by subject, location and year of establishment.

The Directory covers nearly 90% of the institutes engaged in research and development work and will be of immense use to scientists, administrators, planners and international organizations.

PUBLICATIONS RECEIVED

ACTINOMYCIN — NATURE, FORMATION AND ACTIVITIES by Selman A. Waksman (Interscience Publishers—John Wiley & Sons Inc., New York), 1968. Pp. ix+231. Price \$ 8.95

TOPICS IN PHARMACEUTICAL SCIENCES: Vol. 1 — PENICILLINS AND CEPHALOSPORINS: EFFECTS OF HORMONAL STEROIDS ON CELLULAR PROCESSES (Interscience Publishers—John Wiley & Sons Inc., New York), 1968. Pp. ix+136. Price \$ 7.95

BIOLOGICAL OXIDATION by Thomas P. Singer (Interscience Publishers—John Wiley & Sons Inc., New York), 1968. Pp. ix+722. Price \$ 19.75

IONIZATION CONDUCTIVITY AND BREAKDOWN IN DIELECTRIC LIQUIDS by Ignacy Adamczewski (Taylor & Francis Ltd, International Scientific Publishers, London), 1969. Pp. xv+439. Price £ 8.10.0

Ion implantation — New technique for making semiconductor devices

A new technique, known as 'ion implantation', is being increasingly applied in the manufacture of semiconductor devices. The new process, which is more versatile and controllable than the earlier methods, injects into solids, accelerated charged particles, rather than doping them by the conventional diffusion methods which are not always suitable or convenient. Although ion doping was known as early as 1954, yet it was only in 1961 that the first use of this technique in commercial production was made by the Ion Physics Corporation of Burlington, Mass. Normally, the dopants are introduced by diffusion at high temperatures; the principal advantage of the ion implantation technique is that it is a relatively low temperature process in which the depth and uniformity of doping in the solid can be precisely controlled. Particles enter the solid as a directed beam with little or no sideways deflection. Thus, in contrast to diffusion, the doped areas can be closely defined. Since it is not a thermal equilibrium process, the choice of dopants is no longer limited by diffusion kinetics or even by solubility limits.

The ion implantation equipment (as the one at Harwell) uses energies only up to 180 keV. To achieve uniform doping over the entire surface of the semiconductor, the ion beam is swept across the specimen by electrostatic deflector plates. Alternatively, the specimens can be moved mechanically through a stationary beam. Dust must be scrupulously eliminated. After bombardment, the specimens must be annealed. The temperatures required are much less (650°C) than in the case of diffusion (1000°C). Certain specific devices have been found feasible for manufacture with advantage by the ion implantation method.

(i) Avalanche photo-diodes and nuclear radiation detectors: In these instruments it is important

to have a uniform junction that can withstand high reverse voltages. The enhanced diffusion which tends to occur along defects in the crystal leads to a spiky junction and localized breakdown. Ion implantation eliminates this.

(ii) Bipolar transistors: Ion implantation greatly reduces here the 'push-out' effect, by which the strain introduced during diffusion of one electrode causes an undesirable movement of a previously introduced junction.

(iii) Metal oxide-semiconductor transistors (MOSTs): The conventional diffusion method for making this kind of transistor causes uncontrolled spreading of the dopant under the oxide layer. This would lead to an overlap of the various electrodes. Such difficulties are eliminated by using the ion implantation method.

At present studies are concerned mainly with elemental semiconductors like Si or Ge. The compound semiconductors like GaAs, CdS in which diffusion is difficult, if not impossible, possess valuable properties like high electron mobilities or wide band gaps, making them ideal for high frequency transistors, oscillators or for electro-luminescent lamps and display panels. Successful extension of the ion implantation technique to these compound semiconductors may radically transform the fabrication of semiconductor devices with obvious advantages. Already, thermo-electric junctions have been successfully produced in GaS, SiC, CdTe, CdS and other compounds by the ion implantation method instead of by the conventional diffusion technique, which poses formidable difficulties in these compounds.

One more significant point about ion implantation is that it is useful not merely in semiconductors but also in modifying the properties of superconductors, magnetic films, phosphors and optical materials. Ion implantation can be visualized as a new tool for materials research and technology, with vast potentialities of industrial exploitation [*New Scientist.*, 41 (1969), 282].

Studies conducted at the Bell Telephone Laboratories have indicated the possibility of storing up to 1000 holograms in a single crystal of lithium niobate. Such an 'optical memory' from which the desired information could be retrieved or erased easily is based on a special characteristic of niobate crystals, viz. change in refractive index induced by laser radiations.

In ordinary holographic plates, part of the beam is absorbed by the transmittance variations of the plate. This absorption results in lowering of the efficiency of reproduction of the image (about 6%). On the other hand, in lithium niobate crystal, the holograms are recorded by changes in the refractive index of the crystal along the path taken by the interfering laser beams recording the hologram. Since there is no absorption, reconstruction efficiencies with lithium niobate crystal as the holographic material are as high as 42%.

To reconstruct a holographic image stored in a lithium niobate crystal, the reconstructing beam must be directed towards the crystal at a particular angle determined by the wavelength of the laser light and the angle of the original reference beam used in recording the hologram. If this condition is not satisfied, the laser beam will pass through the crystal unimpeded. An additional hologram can be stored in the crystal by directing the object and reference beams towards the crystal at a slightly different angle (even a fraction of a degree). Because of this property, it is possible to store many holograms in a single crystal. Holograms stored in the crystals can be erased by heating the crystals to 170°C [*Bell Lab. Rec.*, (Jan. 1969), 30].

Absolute rate of atomic reactions

Two new techniques of measuring the absolute rates of atomic and radical reactions have been developed at the US National Bureau of Standards.

In the first technique, vacuum UV flash photolysis has been

coupled with vacuum UV spectroscopy to obtain the first direct measurement of the absolute rates of carbon atom reactions. Carbon suboxide, C_3O_2 , on flash photolysis liberates carbon atoms in the 3P ground states and the 1S and 1D excited states, and carbon monoxide. The concentrations of the different species of the carbon atoms and hence the kinetics of their reactions can be followed by monitoring their vacuum UV absorptions; 3P carbon absorbs at 1650 Å and 1D at 1930.9 Å. Using this technique, the kinetics of carbon atom (both 3P and 1S) reactions with methane, hydrogen, oxygen, nitric oxide and carbon monoxide have been studied.

The reactions of atoms such as nitrogen, oxygen, bromine, sulphur, hydrogen and chlorine with different substrates have been followed by the resonance fluorescence method, wherein the fluorescence produced on absorption of resonance radiation is measured. A reaction vessel is filled with a potential atom source (e.g. CCl_4 for Cl) and the sample flashed with vacuum UV radiation, to produce a concentration of 10^{11} - 10^{12} atoms cc^{-1} . The atoms continuously absorb radiation from a resonance lamp filled with a gas mixture containing the element studied, and emit fluorescence. A fraction of this fluorescent radiation is detected with an electron magnetic multiplier placed at right angles to the plane of the mutually perpendicular flash and resonance lamp directions, and the individual photons displayed as oscilloscopic blips. When the atoms react and disappear, the signal decays proportionately. In a representative experiment, the reaction, $Cl(^2P_{3/2}) + H_2 \rightarrow HCl + H$, was studied; the rate $[(1.4 \pm (10\%) \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ sec}^{-1})]$ was proportional to hydrogen pressure and independent of the pressure of added helium.

An advantage of the fluorescence technique is that as the atoms are produced and monitored at the centre of the vessel, complications arising from wall reactions can be minimized. By suitably varying the light intensity, total gaseous pressure and the reactant pressure one can study atom-radical reactions also [*Chem. Engng News*, 47 (1) (1969), 37].

Rare earth metallocenes

An organometallic compound exhibiting interaction between the f -electron of a metal atom and the π -molecular orbital of cyclic organic compounds has been synthesized for the first time at the University of California, Berkeley [*J. Am. chem. Soc.*, 90 (1968), 7364].

Cyclooctatetraene was treated with dry potassium in dry oxygen-free tetrahydrofuran at $-30^\circ C$ and the resulting yellow solution of the cyclooctatetraenyl ion stirred overnight with a solution of uranium tetrachloride in tetrahydrofuran. On adding water to the reaction mixture, green crystals of bis-cyclooctatetraenyluranium precipitated in 80% yield. The new compound has been named uranocene in analogy with ferrocene (bis-cyclopentadienyliron), another sandwich type organometallic compound, wherein the d -electron of the iron atom interacts with the π -molecular orbital of the cyclopentadienyl ion. Incidentally, uranocene is the first reported sandwich compound of uranium.

Uranocene opens up a new area of rare earth chemistry. Along with its analogues, it may provide an efficient method of separating the actinides and lanthanides. It may also open up an area of new industrial catalysts [*Chem. Engng News*, 47 (1) (1969), 36].

Biochemistry of spore formation in fungi

J. E. Smith of Applied Microbiology Department, Strathclyde University, Glasgow, and J. C. Galbraith of Biology Department, Paisley College of Technology, Paisley, have in a recent article [*New Scientist*, 41 (1969), 334], dealing with the biochemical basis of morphological differentiation in microorganisms, such as fungi, attempted to clarify the paradoxical behaviour of various cell types in higher organisms, which despite similar genetic make-up differentiate into dissimilar morphological types. The study is an extension of Jacob and Monod's classical work on bacteria showing how differentiation can proceed without altering the primary genetic code.

Most of the microfungi manage to outlive adverse environmental

conditions like nutritional deficiency by producing asexually well-protected reproductive structures—spores—which can be transferred to favourable situations. The spore formation could, however, be completely inhibited if an appropriate energy source is available during early stages of morphogenesis, although at a later stage the cells are irrevocably committed to differentiation, and the presence of nutrient makes no difference. In the unicellular fungus *Blastocladiella emersonii* the development of a particular type of sporangium (spore container) depends on the presence or absence of bicarbonate in the incubatory medium. This not only clearly shows pluripotency of developing cells but also illustrates how relatively small environmental changes can cause profound alterations in the growth pattern. If bicarbonate is added before any point up to two-thirds of the time required for the germinated spore to produce a sporangium, the cell develops into a resistant thick-walled, pigmented sporangium, but in its absence, an ordinary colourless, thin-walled sporangium develops, showing thereby that there is a period of commitment in cellular metabolism after which the developmental pathway cannot be altered. This is due to marked change in the enzymatic activity of mitochondria. The presence of bicarbonate creates a lesion in the Krebs cycle of the developing cell blocking further metabolism of an intermediate, α -oxoglutarate, which is converted back to isocitrate to be further metabolized by isocitrate lyase—an enzyme not otherwise present—to yield glyoxylate leading through glycine to extensive RNA synthesis needed for differentiation. Similarly, in a filamentous fungus, *Aspergillus niger*, used industrially for citric acid production, spores are formed only after inclusion in the medium, of certain intermediates of Krebs cycle—particularly α -oxoglutarate, and here again exists a 'point-of-no-return'. It, therefore, implies that in these two fungi the bicarbonate ions and α -glutarate are respectively acting as inducers of differentiation.

The study lends support to an earlier suggestion that progressive development is based on sequential

induction, in which the product of one enzyme reaction induces the enzyme responsible for the next reaction, and that the responsiveness of the cell to an inducing stimulus depends on its stage of metabolism. Furthermore, differentiation occurs in an essentially endogenous, self-sufficient system isolated from the environment by permeability barriers, so that the developing cell being partly independent of external nutrients has a greater control on its metabolism; differentiation in such a 'closed system' appears by no means unique to microorganisms which in fact mimic differentiation in higher organisms.

Nonenzymatic preparation of N-(5'-phosphoribosyl) anthranilic acid in solution

An extremely simple, rapid and convenient method of preparing N-(5'-phosphoribosyl) anthranilic acid (PRA), an intermediate in tryptophan biosynthesis, has been reported from the Dept of Biology, Yale University, New Haven, Connecticut. The method makes use of the observation that after continuation of the reaction between anthranilic acid and D-ribose-5P for 4 min at room temperature, when the amount of PRA formed is maximum and that of 1-(O-carboxyphenylamino)-1-deoxyribulose phosphate (CDRP), another intermediate of tryptophan biosynthesis, is nil, isomerization of PRA to CDRP can be prevented through 100-fold dilution of the reaction mixture with cold 0.1M triethanolamine-HCl buffer (pH 8.6) at 0°C. The amount of PRA formed in this way is 30-40% of the theoretical maximum. The intermediate is distinct from CDRP in its not being converted to InGP (indole-3-glycerol 5-phosphate) by an enzyme which is able to utilize synthetic CDRP and in its inability to reduce tetrazolium dye under appropriate conditions. The spectral properties and stability of the intermediate are similar to those of a labile intermediate previously shown to be produced enzymatically from anthranilic acid and PP-ribose P.

In view of the lability of the intermediate, its further purification and hence structure determi-

nation has not been possible at this stage. It is still unknown which of its possible structure isomers is biologically active. Although the PRA prepared is unstable and is contaminated at least with unreacted anthranilic acid and ribose-P the ease of preparation makes it very feasible for use as substrate in enzymatic studies. The intermediate (PRA) is rapidly formed on mixing equal volumes of 1.0M anthranilic acid in 95% ethanol and 1.0M D-ribose-5-P at room temperature. After 4 min, the reaction mixture is diluted 100-fold with cold 0.1M triethanolamine-HCl buffer (pH 8.6) and kept at 0°C for not more than 4 hr before use. Appropriate further dilutions are made just prior to use. After dilution, no detectable CDRP is formed. The basis for the absence of any detectable isomerization of the PRA after dilution is not known so far; probably it is the result of either dilution of ethanol or a change in pH. Control mixtures free of PRA and CDRP are prepared by individually diluting the anthranilic acid and the ribose-5-P solutions into the same buffer to give the equivalent mixture as above [J. *biol. Chem.*, **243** (1968), 5605].

Method for the routine preservation of microorganisms

For carrying out experiments with microorganisms over a long period of time, it is essential to ensure that the source material remains unchanged. Earlier studies have shown that the viability of *Escherichia coli* B decreases with storage time. After nearly 2 years, 30% of the population remains viable. Methods used currently for the preservation of microorganisms have other limitations too. To overcome these difficulties, a simple method, based on a rapid freeze in liquid nitrogen followed by storage at -70°C, has been developed by Dr C. S. Cox of Microbiological Research Establishment, Porton Down, Wiltshire.

A culture is prepared with the inoculum from a freeze dried sample or other suitable source. Using a pipette which delivers 36 drops/ml, drops of culture are allowed

to fall into a Dewar flask containing liquid nitrogen. On freezing the drops sink to the bottom of the flask. When 300 or more frozen drops have formed, they are transferred to a container previously cooled at -70°C, and maintained at this temperature until required. The procedure is then to remove a single frozen drop with chilled sterile forceps and to put the drop into an empty sterile test tube. After rapid thawing the suspension formed can be used either to inoculate a nutrient agar plate by stroking with a sterile loop, or to inoculate a growth medium direct. In practice, the plate method is preferable because it enables the sterility to be checked and also a plate can be used for inoculation for 1-2 weeks.

Esch. coli K12HfrC and *Esch. coli* K12HfrH have been successfully stored for nearly a year by the above method. If a -70°C refrigerator is not available, an alternative is to use solid carbon dioxide or a liquid nitrogen storage container. Care should be taken with CO₂, however, because unless the pellet is well sealed, CO₂ dissolves into the frozen pellet and can cause a severe change in pH, especially on thawing [Nature, Lond., **220** (1968), 1139].

School of Research & Training in Earthquake Engineering, Roorkee

The annual report of the school for 1967-68 presents an account of the research activities and achievements in its main areas of work, viz. (1) Instrumentation, (2) Engineering seismology and seismo-tectonics, (3) Structural dynamics and model techniques, and (4) Soil dynamics and foundation design.

During the year, design and fabrication of three types of function generators capable of producing vibratory motion of varying amplitude and period was completed. In the first generator, the vibrations are imparted by the free fall of a simple pendulum, the rate of impact and height of fall being controlled automatically. In the second one, a spring loaded hammer is released at various speeds, while the third one is a cam driven function

generator. Other instruments developed include: (1) an automatic device to give free vibration with initial displacement; (2) an equipment for testing a soil sample under static sustained and dynamic pulsating loading; (3) a portable water tube tiltmeter for measuring the slow deformation of the earth's crust due to the gradual accumulation of elastic strain in active seismic regions (accuracy up to 10μ); (4) a silica tube extensometer for measuring the slow changes in the plane of the earth's crust due to the elastic strain build up in active seismic regions; and (5) an improved structural response recorder.

Geological mapping of 800 sq miles area around Delhi was completed. Following the Koyna earthquake, a detailed study of the geological and seismo-tectonic set-up of Maharashtra has been undertaken. The number of structural response recorder stations working in different parts of the country rose to 37 during the year.

Studies on the strength behaviour of reinforced brickwork in lean mortars have revealed that the addition of small percentages of reinforcement (steel) does not add much to the flexural strength, but increases their deflection capacity and energy absorbing capacity, which are extremely useful for their earthquake resistance. Notable among the projects under investigation in the field of structural dynamics and model techniques were: behaviour of structures during earthquakes; strengthening of brick buildings against earthquake forces; earthquake resistant design of brick buildings; behaviour of brickwalls with opening when subjected to vertical and lateral loads; behaviour of framed unreinforced brick shear walls and braced multi-storeyed frames; behaviour of reinforced concrete columns under cyclic loading; non-linear behaviour of multistoreyed frames; behaviour of bridge piers, arches and cylindrical shell roofs under dynamic loads; blast resistant design of structures; vibration analysis of dams; etc. Soil dynamics problems under investigation include: behaviour of piles under dynamic lateral loads in cohesionless soils;

dynamic earth pressure behind retaining walls; effect of size and shape of foundation on elastic coefficient in a layered soil mass; natural frequency of block foundations; liquefaction and settlement characteristics of sands; bearing capacity of footings subjected to moments; dynamic strength characteristics of soils; stability of rock slopes; shear strength characteristics of crushed rock aggregates; deformation modulus of rocks; etc.

Central Glass & Ceramic Research Institute, Calcutta

The annual report of the institute for 1967 presents an impressive record of achievements in its effort to make the country self-reliant in essential glass and ceramic raw materials, products and processes. Processes pertaining to (i) production of high alumina refractories from indigenous raw materials, (ii) production of autoclave plaster of Paris, (iii) metallization of nonconductors, and (iv) production of corrosion-resistant cast iron enamels were released for commercial utilization. Three patents were filed.

The production of optical glass was stepped up and the total production during the year was about 9 tonnes. A process was worked out for the casting and annealing of large glass slabs required for making atomic radiation shielding windows of about 1 sq m surface area used in hot cells of atomic reactors. Work on the development of infrared filters for headlamps was completed. Glasses suitable for sealing with tungsten and glass-to-metal compression seals were produced for use in solid state electronic devices. A process was developed for chemical pretreatment of heat-resistant steel parts of aircrafts before enamelling.

Lead-free enamels were developed for use in wire-wound resistors. A process was developed for the production of high alumina (75-90%) refractories for the steel industry, using technical alumina, bauxite or diaspore. The use of diaspore, which does not need calcination, reduces the cost of production of the refractories. Castable refractories satisfying ASTM specification were developed

using different types of fireclay and kyanite along with high alumina cement. Mortars satisfying ASTM specification were also developed using kyanite and sodium silicate.

Standard strain discs for use in the determination of the quality of annealing in glassware were made by careful heat treatment of flat glass pieces, so that each disc had a nominal light path retardation of 22.8 $m\mu$. The discs give performance comparable to that of imported discs.

A method was developed for making hard, dense plaster of Paris for use as a substitute for the imported cementing material employed in high tension switchgear industry and for the plaster used for making models and case models in the pottery industry. An opaque white glass enamel, maturing at 560° and having high acid resistance was developed.

A special glass composition was developed which when subjected to a special treatment reported earlier from the institute for improving its mechanical strength and chemical durability acquires an average value of the modulus of rupture of 50,000 lb/sq in. and resistance to thermal shock and water attack comparable to those of borosilicate glasses.

Decorative panels were developed by applying under pressure a very thin coating of a mica composition directly on plywood or other backing materials. The panels are characterized by their attractiveness, resistance to boiling water, organic acids, etc. and easy machinability. Studies were undertaken on the development of know-how for the fabrication of a variety of products, on behalf of different organizations. These include: (1) spark plug suppressor caps, (2) ceramic seals and spacers, (3) ceramic bulb-holders for aerodrome lights, (4) silica candle filters for fertilizer plants, (5) chlorination cells, (6) porous cups for tensiometers, and (6) arc-resistant laminates.

Improvements were effected in the processes developed earlier for the production of (1) low density insulating materials from waste mica; (2) corrosion-resistant vitreous enamels for cast iron; (3) electrically conducting glazes for reducing corona effect on high

tension insulators; (4) high tension insulator porcelain bodies; and (5) sanitaryware from low grade fireclay.

A number of projects were in hand concerning the evaluation, beneficiation and utilization of glass and ceramic raw materials. The possibility of correlating the concentration of mica with the petrographic characteristics of pegmatites and the associated rocks has been investigated. Analysis of the petrographic characteristics of samples studied so far has revealed that practically in every mine (i) commercial muscovite is associated with quartz and plagioclase (mostly oligoclase) and not with microcline; (ii) in the mica-rich zone, the plagioclase grains associated with mica are often partly altered to kaolinite or sericite; (iii) inclusions of minute muscovite flakes occur in plenty in the oligoclase associated with commercial muscovite; and (iv) pegmatite veins containing much tourmaline are poor in mica content.

DTA experiments conducted to understand the influence of added mullite on the nature of crystallization peak of kaolinite showed that there is no shift of the crystallization peaks on either side, but the intensities of the crystallization peaks diminish gradually with increasing addition of mullite to a definite weight of kaolinite. From studies on the vitrification behaviour of mixtures of boric oxide with different forms of silica (gel, raw and ordered cristobalite) it has been observed that mixtures of amorphous silica and boric oxide, besides being highly reactive, are metastably converted into glass below the equilibrium liquidus temperatures. At higher temperatures, quartz is probably transformed to a metastable amorphous phase and then to glass before any cristobalite can be formed.

A simple and accurate method has been developed for the estimation of silicon in steel and other metal alloys. A new method

developed for the estimation of fluorine involves the precipitation of fluorine as silico-fluoride, filtration and titration of the HF liberated after hydrolysis of the silico-fluoride.

Announcements

■ *The Seventh International Congress on Electron Microscopy* being organized by the French Society of Electron Microscopy, under the auspices of the International Federation of Electron Microscopy Societies, will be held in Grenoble (France) from 30 August to 5 September 1970.

The congress will deal with electron optics and related techniques, apparatus design, specimen preparation, and the multiple applications of electron microscopy in biology, medicine, biochemistry, crystallography, metallurgy, etc. Contributions of interest to both physicists and biologists will be favoured. Further information can be obtained from the General Secretary, Prof. Jean André, Bâtiment 444, Faculté des Sciences, 91-Orsay, France, or the local Chairman, M. Adrien Saulnier, VIIe Congrès International de Microscopie Electronique BP454, 38-Grenoble, France.

■ *The Twenty-first Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy* will be held in Cleveland, Ohio, USA, during 1-6 March 1970. About 300 papers on all aspects of analytical chemistry and spectroscopy will be presented. Symposia on the following subjects are being arranged: (1) SSP award symposium on absorption spectroscopy; (2) Coblenz Society symposium on laser Raman spectroscopy; (3) Analytical techniques on the horizon; (4) Polymer characterization; (5) Analytical chemistry—A fading discipline?; (6) Computers in the analytical laboratory; (7) High speed chromatography (gas and liquid); (8) Analyses of gases and vapours; (9) Recent developments in X-ray and optical spectroscopy;

(10) Analytical techniques in space; and (11) Molecular spectroscopy of minerals and related inorganics.

Papers are not restricted to the symposium topics and original papers on all phases of analytical chemistry and spectroscopy are invited. Authors who wish to present papers at the conference should submit three copies of a 150-word abstract and include the names and addresses of the authors, and the name of the laboratory in which the work was done. Abstracts should be sent to Edward L. Obermiller, Consolidation Coal Co., Research Division, Library, Pa 15129, USA.

In addition to the programme of technical papers, there will be an exhibition of the newest analytical instrumentation. More than 250 companies will display instruments, chemicals and equipment.

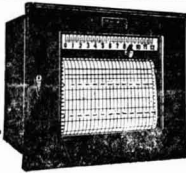
■ *An International Chemical Engineering Conference* is to be held in Melbourne and Sydney during 18-26 August 1970. The conference is being organized jointly by the Australian Academy of Science and the National Committee of the Institution of Chemical Engineers. Among the topics listed for discussion are froth flotation and mineral processing, computer control of chemical processes, dust and mist collection, biological fermentation, food engineering, biomedical functions, and the application of scientific decision-making to research and development, to design and evaluation, and to process operation.

Further information about the conference can be had from the Secretary, Australian Academy of Science, Gordon Street, Canberra City, ACT 2601, Australia.

■ *The Symposium on Chemicals and Oil from Coal* previously scheduled to be held at the Central Fuel Research Institute, Dhanbad, during February 1969, but subsequently postponed to the winter of 1969, will now be held during 6-8 December 1969.

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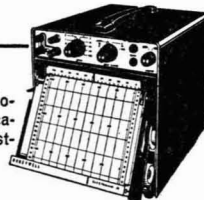
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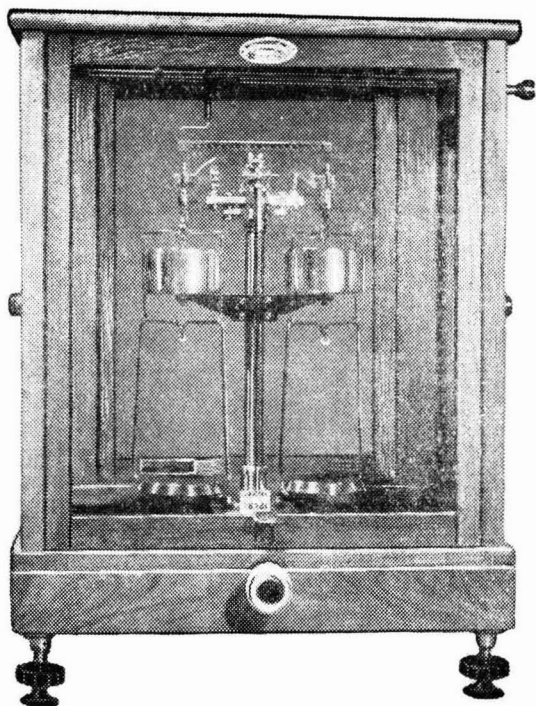
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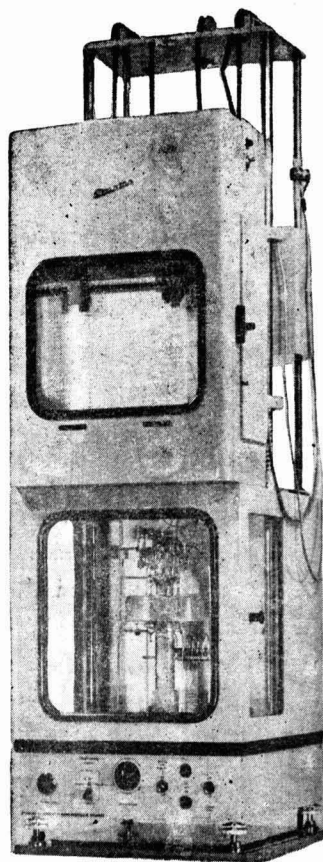
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