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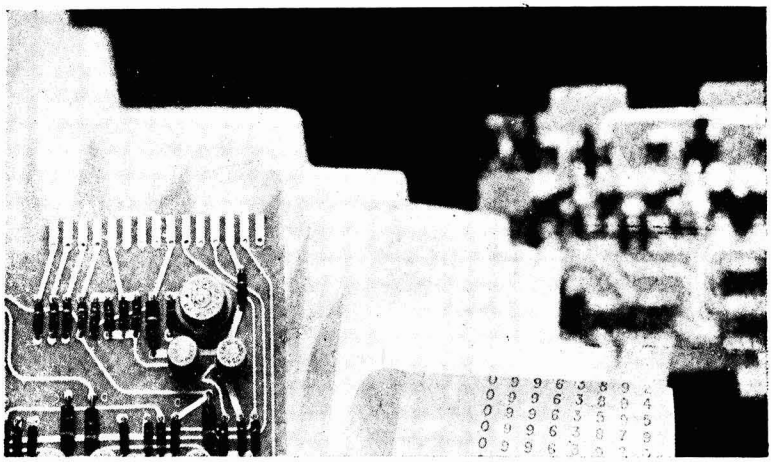

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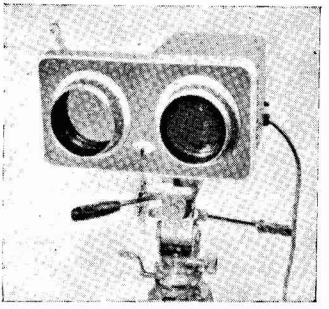

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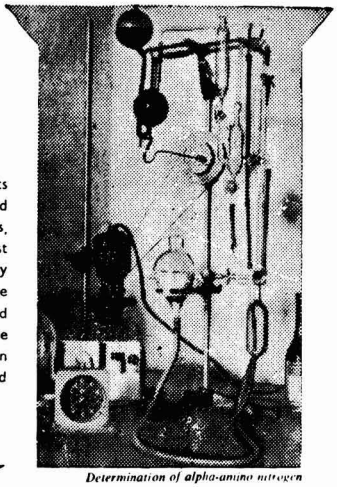

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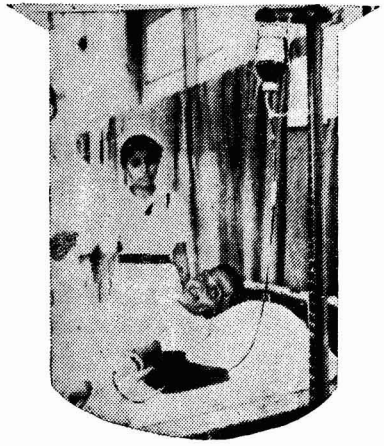

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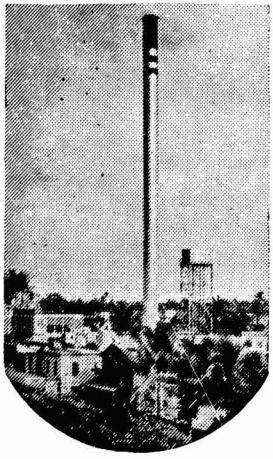

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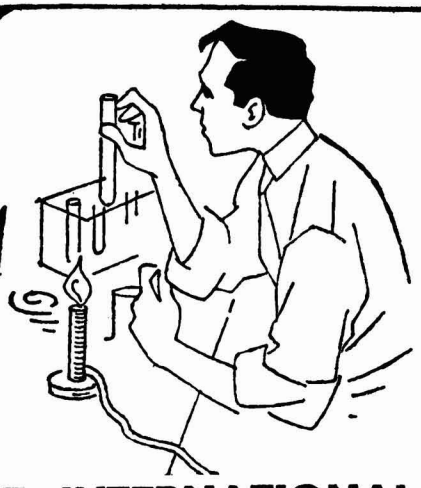

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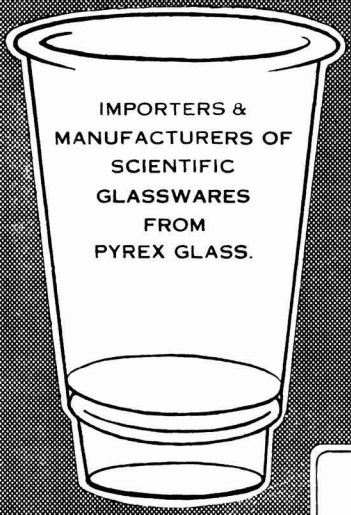

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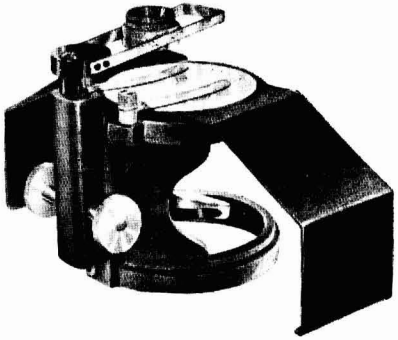

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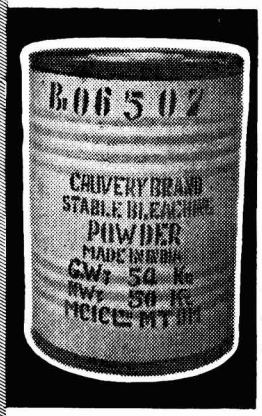

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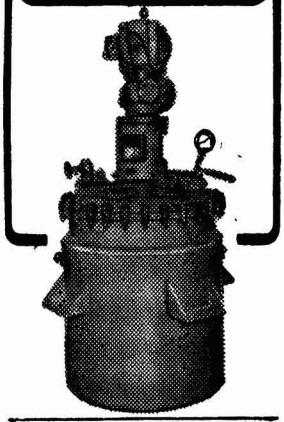

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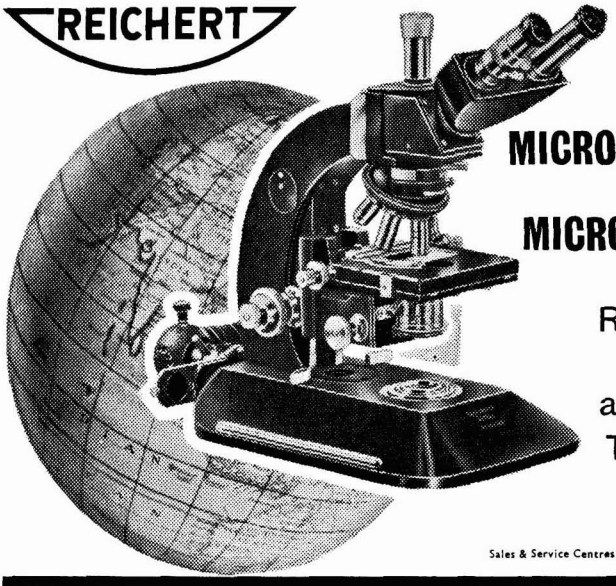

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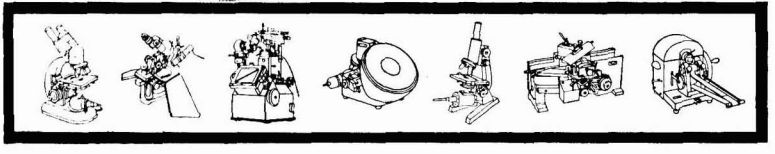

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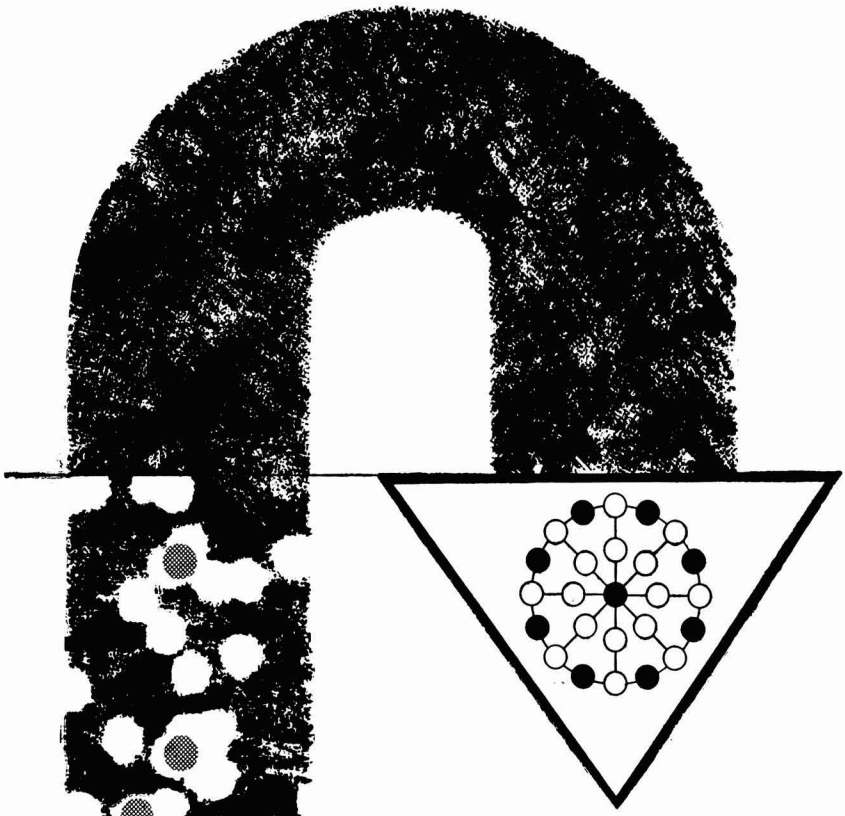

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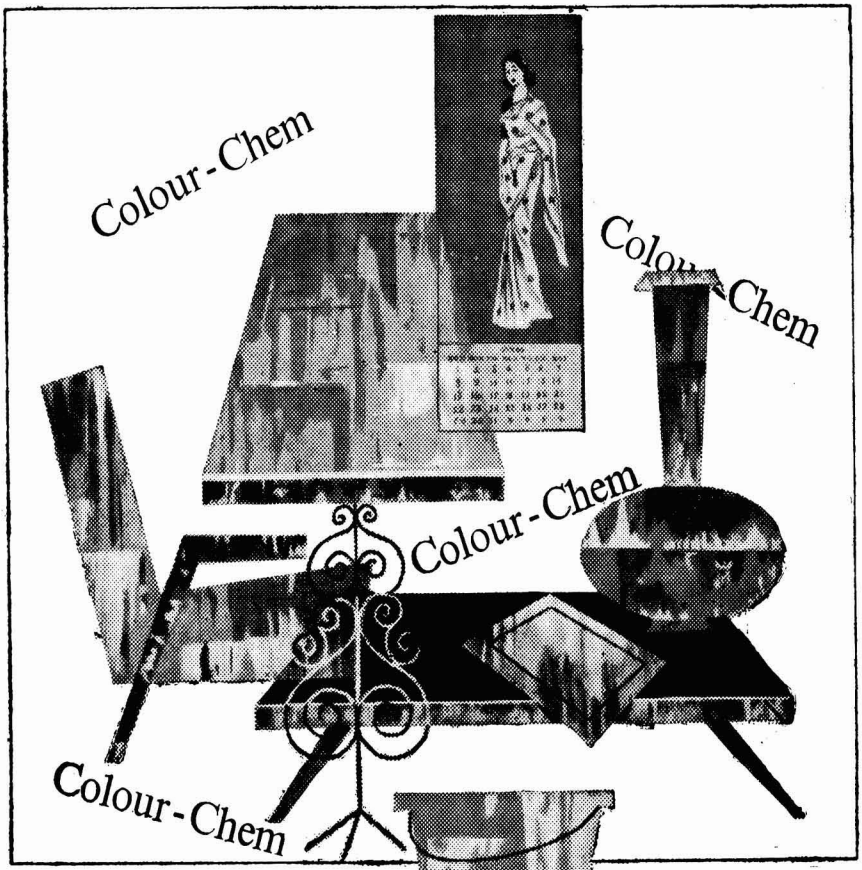

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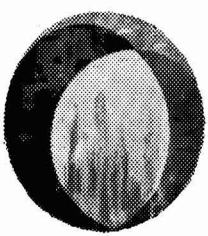

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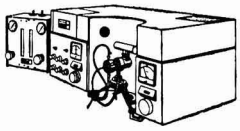

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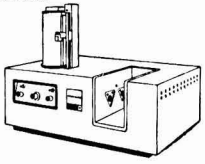

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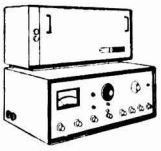

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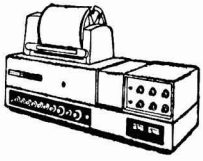

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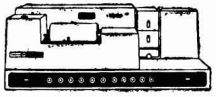

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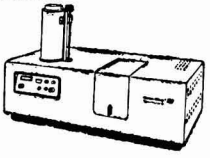

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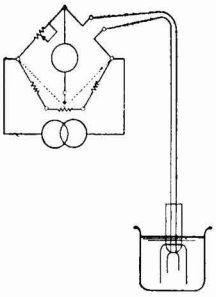

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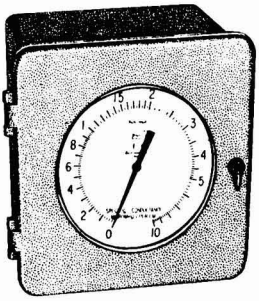

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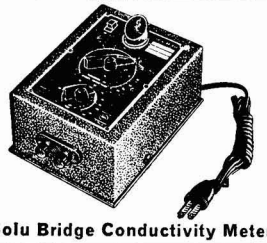

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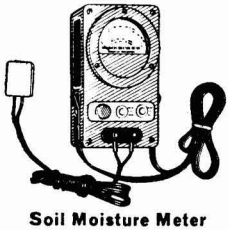

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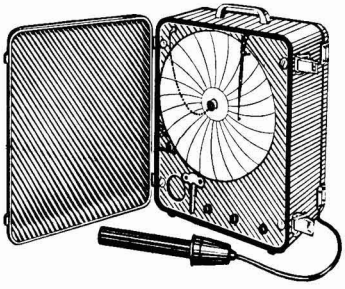

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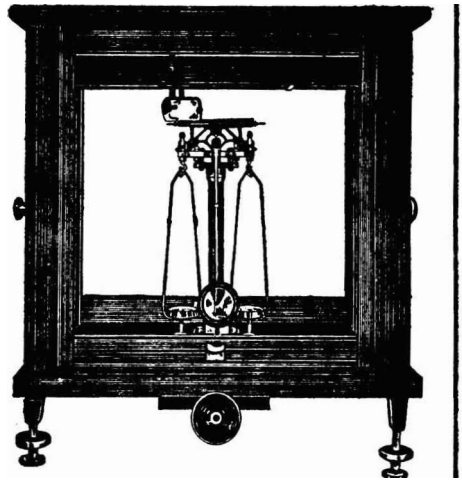

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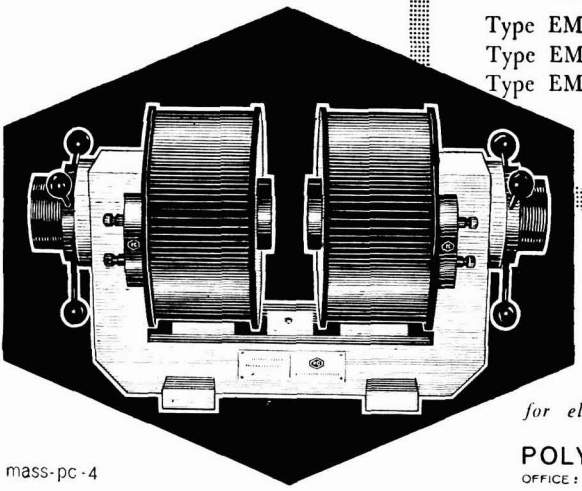

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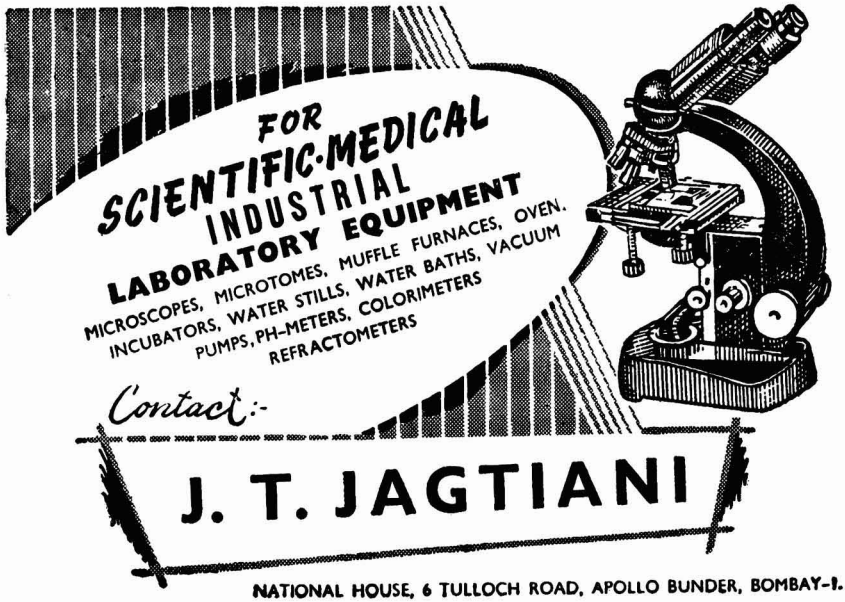

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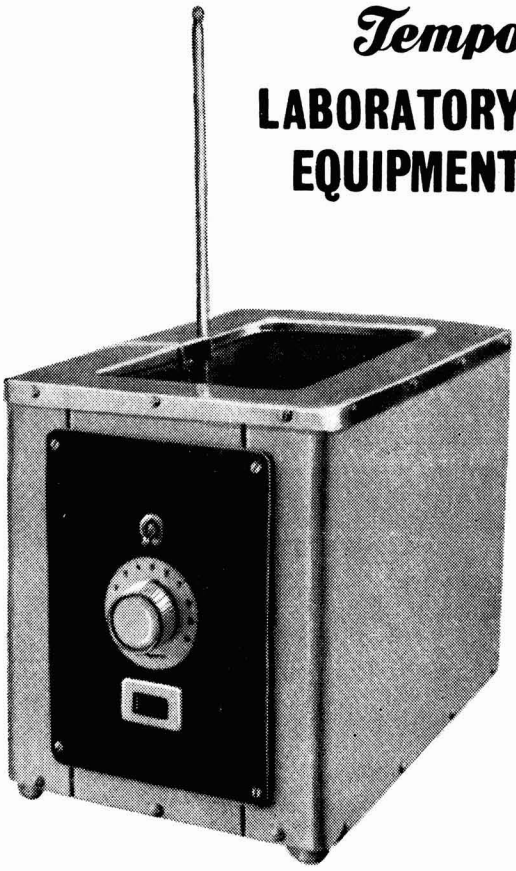

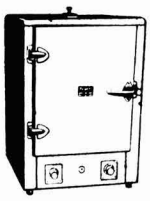

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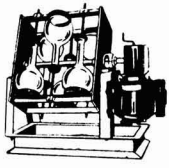

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Current Topics

Output & Dissemination of Indian Physics Literature

A DETAILED study of primary periodicals concerned with physics (including physical chemistry), recently carried out by the American Institute of Physics, New York, is based on an analysis of issues of *Physics Abstracts* published during 1961, and provides information on 20,287 articles published in 405 periodicals from 39 countries. The report* of this study contains interesting data on the output of physics literature and its dissemination by abstracting periodicals which are becoming increasingly important sources of reference. It also provides information in respect of the position India occupies in the world of physics.

According to the report, India ranks sixth by number of journals and ninth by number of abstracts published. There were 18 periodicals publishing papers in physics during 1961 and 352 abstracts of papers are included in the 1961 issues of *Physics Abstracts*. The majority of these abstracts are of papers published in *Indian Journal of Physics* (97), *Proceedings of the National Institute of Sciences: Section A* (78), *Journal of Scientific & Industrial Research: Section B — Physical Sciences* (56), and *Proceedings of the Indian Academy of Sciences: Section A* (55). The contribution of the remaining 14 periodicals is 66 articles.

A peculiar feature about the appearance of these abstracts in different issues of *Physics Abstracts* published during 1961 is that 220 articles out of 352 articles appearing in various periodicals have been abstracted in the November and December 1961 issues of *Physics Abstracts*; the only exception is the *Journal of Scientific & Industrial Research* from which the articles have been regularly abstracted and published in each issue of *Physics Abstracts*. This indicates that, for various reasons, the papers published in Indian periodicals are not abstracted promptly and expeditiously, and there is considerable lag between the publication of papers and their noticing by the abstracting periodicals. The reasons for the delay in the abstracting of Indian physics literature need to be examined.

Analysing the report, Rajagopalan and Sen† have pointed out that the number of papers in the field of physics published in Indian periodicals during 1961 was 502 and of these only 352 have been abstracted; the former figure has been arrived at taking into consideration a few more periodicals not covered by *Physics Abstracts*. The low percentage (70) of papers abstracted has been attributed to the fact that not all journals containing papers relating to physics have been covered. This omission may also be due to the fact that only a few Indian journals are exclusively devoted to physics.

Examining the position in 1964, Rajagopalan and Sen have reported that, in addition to 18 journals covered by *Physics Abstracts* in 1961, 23 other journals containing about 70 papers in the field of physics are not covered. According to the *Bibliography of Scientific Publications of South and South East Asia*, the total number of articles in physics published in Indian periodicals during 1964 was 648 (including 162 articles in physical chemistry). The publication of *Indian Science Abstracts* should enable abstracting services elsewhere to make their coverage of Indian physics literature exhaustive.

It is of interest to note that the *Indian Journal of Pure & Applied Physics*, which replaced the physics part of the *Journal of Scientific & Industrial Research: Section A — Physical Sciences* in 1963, published 141 papers in 1964 (as compared to 84 and 87 papers published in the *Proceedings of the Indian Academy of Sciences: Section A* and the *Indian Journal of Physics* respectively) and the abstracting of the papers published in *Physics Abstracts* was 100 per cent up to Volume 2, No. 9 of the journal. Also, the abstracts appeared regularly in each issue of the *Physics Abstracts* and the time taken for the appearance of the abstracts was 4-5 months.

Speedy abstracting — an important requirement in the dissemination of scientific information — could be facilitated in many ways, apart from the regularity in the publication of periodicals. Many periodicals do not carry abstracts of papers published. By providing satisfactory and acceptable abstracts, prepared according to the specifications laid down by the International Council of Scientific Unions' Abstracting Board, it is possible to cut down the time taken for the abstracts to appear in abstracting periodicals. Adequate care should also be taken to see that carefully edited and author approved abstracts are published with each article. Publication of abstracts in two or more languages other than English would also be helpful.

The speedy abstracting of papers published in the *Indian Journal of Pure & Applied Physics* is largely due to (i) preparation of satisfactory abstracts enabling abstracting periodicals to use them as such or with very slight modification, and (ii) the *Advance*

**The Journal Literature of Physics — A comprehensive study based on Physics Abstracts (Science Abstracts, Section A), 1961 Issues* by Stella Keenan & Pauline Atherton (American Institute of Physics, New York), 1964.

†T. S. Rajagopalan & B. K. Sen, *Ann. Lib. Sci. Doc.*, **11** (1964), 87.

Contents and Abstracts Service [see this journal, **19A** (1960), 144] initiated by the Publications & Information Directorate in 1960 covering all the research periodicals published by it. Under this service, about 100 abstracting periodicals in different countries are supplied, by air mail, contents pages and abstracts of papers published in a particular issue of a journal 6-8 weeks in advance of the publication of the journal. This service has ensured quick and world-wide dissemination of scientific information published in the periodicals brought out by the Publications & Information Directorate.

Sir Edward V. Appleton: 1892-1965

Sir Edward V. Appleton, pioneer in radio science and discoverer of the ionosphere, died on 21 April 1965 at the age of 72 leaving a vacuum unlikely to be filled in the near future. Born in 1892 in Bradford, England, he was a pupil of both Sir J. J. Thomson and Lord Rutherford at the University of Cambridge in which he took up research and teaching after World War I. Later he moved to the London University where he earned his D.Sc. degree, and became the Wheatstone Professor of Physics. In 1936, he returned to the Cambridge University to become the Jacksonian Professor of Natural Philosophy. During World War II he joined the British Government as Secretary of the Department of Science and Industrial Research, a post he occupied till 1947. He was made KCB in 1941 and GBE in 1946. He was awarded the Nobel Prize for Physics in 1947. In 1949, he joined the University of Edinburgh as its Principal and Vice-Chancellor and he remained there till his death.

The history of ionospheric research is inextricably linked with the name of Sir Edward. In 1927, he and Barnett obtained the first direct evidence for the existence of an ionized layer in the upper atmosphere (which he later called ' E-layer ') by comparing the intensities of fading of radio signals received simultaneously on a loop and a vertical aerial. Soon afterwards he presented his famous paper entitled ' The existence of more than one ionized layer in the upper atmosphere ', in which he produced evidence for a higher layer (which he called F-layer) and a lower layer (D-layer). Thus evolved the nomenclature of the ionospheric layers. In 1932, he presented the now famous magneto-ionic theory which quantitatively describes the conditions guiding the propagation of radio waves through the ionosphere. During World War II he and Beynon developed simple and easily applied expressions for transmission of radio waves at oblique incidence, and laid the ground work for the application of ionospheric data to radio communication problems.

In an epoch-making work in 1947, he showed that the electron density of the F-region has a symmetrical variation with *geomagnetic* latitude (and not with geographic latitude), with an unsuspected trough of low values near the magnetic equator, showing unmistakably the profound control of the earth's magnetic field in the upper regions of the ionosphere. The discovery of the equatorial trough, and the subsequent discovery of the swinging of the equatorial belt from a trough at noon to a crest at night, again by Appleton, showed the anomalous nature of the equatorial ionosphere and prompted Appleton to urge time and again the need for extensive ionospheric work at the equator, which was, and is, still inadequate. With Roy Piggotts, he initiated, while still at DSIR, work on ionospheric absorption, which continued for more than a solar cycle. Again in collaboration with Piggotts, he carried out one of the most extensive studies on the morphology of ionospheric storms.

At the Edinburgh University, which he joined in 1949, his interest in ionospheric work continued, although it now became predominantly theoretical. With Lyon, he showed that the apparently well-behaved E-region of the ionosphere has small but unmistakable anomalies, which could be separated. With a very simple modification of the generally used ' equilibrium ' equation, he showed how any height variation of the loss coefficient, vertical drift velocity or temperature can distort the E-region.

It is appropriate to record here that when Prof. S. K. Mitra started his Ionospheric Laboratory in Calcutta, at that time the only one in Asia, Sir Edward was one of its most ardent supporters. When, during World War II, the Ionospheric Station was closed down for security reasons, Appleton's own intervention was responsible for persuading the Allied Services Authorities to reopen the station. His interest in the increasingly vigorous ionospheric research schools in India remained unabated.

Sir Edward was awarded many honours, and was intimately associated with all major international radio organizations. He was the President of the International Scientific Radio Union (URSI) from 1934 to 1952 and was instrumental in shaping the course of the IGY ionospheric programme.

Sir Edward was a legendary figure in ionospheric research. The crucial part he played in the creation of modern radio science will not be easily forgotten.

A. P. MITRA

Evaluation of Research Projects & Personnel

AQUEIL AHMAD

Survey & Planning of Scientific Research Unit, Council of Scientific & Industrial Research, New Delhi

FOR successful implementation of research projects in the sense that outcomes correspond with the objectives with minimum wastage of time, money and manpower, it is necessary to have the projects evaluated periodically[1]. This evaluation should include both (1) programme evaluation, and (2) evaluation of personnel working in the project. The major purpose of programme evaluation is to check planning efficiency and progress of work, while evaluation of personnel determines whether each scientist attains maximum productivity. Programmes can be evaluated by checking outcomes and their utilization against reasons for selecting projects, project objectives and expenditure of time, money and manpower. Personnel evaluation, on the other hand, requires evaluation of an individual scientist's useful contribution to the project. Neither adequate quantitative measures of programme efficiency or individual contribution to research are, however, available; nor are there any statistical standards against which the two can always be evaluated. In most cases at present, the evaluation of programme efficiency and individual contribution both turn out to be qualitative assessments of the adequacy of programmes and personnel by experts in supervisory capacities[2]. The intention here, however, is neither to work out quantitative measures nor to evolve standards of programme efficiency or individual contribution, but to suggest some objective criteria through which programmes and personnel could be evaluated for: (1) efficiency in selection, execution and utilization of research[3], and (2) usefulness (on the basis of total contribution) of research workers in their projects.

Programme Evaluation

Programmes can be evaluated in terms of the following matchings:

Reasons for selecting the project: Justifications for those reasons — Reasons for which a project is selected are viewed here as different than the objectives of the project. Projects may be selected for various scientific or (as in most cases) extra-scientific reasons quite independent of project objectives. Such reasons may include prestige value of a project, its grant-getting potential, fillip to one's career through research, economic motives, etc. (extra-scientific); or, in a relatively few cases, researchers' interest in research for the sake of research itself, or a genuine need to gain certain types of knowledge without which an existing research would not progress further. After working on a project for some time, the selector should be able to judge whether its selection for a particular reason was justified. In this kind of evaluation, the original reasons for selection can be used as a standard against which the merits of selection can be judged. For example, an industrial concern with dwindling profits may sponsor a research with the intention of improving the quality of its goods or developing newer ones for market exploitation. After intensive research, a new product may be developed and put on the market but may not necessarily increase company sales; or, possibly, it might become evident, even before the product reaches the market, that someone else has already developed a better and/or cheaper one. Evaluation against initial reasons for selection can thus help the selector keep the broad purposes of research in view and drop out projects that have lost their utility.

Objectives: Outcomes and their utilization — Outcomes and their utilization should be evaluated in the light of the objectives that were set forth in the beginning of a project. A project may be selected with the objective, for instance, of developing the prototype of a machine to be used for a specific purpose. In a case of this sort, matching outcome against objectives is a fairly simple job that can be performed at every stage provided the stages are earmarked in the plan. Variations of objectives at every stage and their consequences on the project as a whole can also be studied. In many cases, however, the objectives may not be as specific as the development of a prototype. Some projects are carried out with rather vague objectives, such as the likely usefulness of results in a particular branch of industry, agriculture or medicine, and the researcher may not be sure for a long time whether the findings can actually be utilized in practice. But if the outcomes match the objectives to the extent that the researcher achieves success in carrying out the basic intentions of the research, for instance, testing of a hypothesis, his project would be evaluated as successfully completed. Here again the objectives themselves offer a rough standard against which outcomes can be evaluated.

Most often a research outcome is considered well utilized if it finds application in some field of activity, but such application may not always be possible. Besides practical application, other ways in which research outcomes can be utilized include their dissemination through publications, reports, lectures, etc., or their use in other researches in the same field or other fields of science. In evaluating the degree and type of utilization of research outcomes, information will be needed on such points as: (1) nature of outcomes, i.e. discovery of a method, process or relationship, (2) patents secured, (3) name of the user and the actual field or activity where utilized, (4) titles of publications, reports, lectures, etc., related to the research undertaken. This information when matched with plans and objectives will reveal the degree of correspondence between utilization as it was envisaged and actual utilization. It will be interesting to note that in some cases findings cannot be utilized for various

reasons, while in others, utilization is effected in a way that was neither planned nor expected.

Time targets: Outcomes — Time targets for completing parts or whole of a research programme are fixed in the planning stage to show that a certain amount of work is intended to be completed during a specific period. While evaluating research programmes it is necessary to check, firstly, if such time targets were fixed, and secondly, the extent to which these targets were maintained without substantially altering the original plan. In this connection, the following alternatives are possible: (1) all the stages of the project, like designing, pilot study, data collection, reporting, etc., were time scheduled and the schedule was maintained all through; (2) all or a few of the stages were time scheduled and the schedule was maintained for some of these stages; (3) all or a few of the stages were time scheduled but the schedule could not be maintained at all; (4) a time target was fixed for one stage only, such as pilot plant, and the target was maintained; (5) time target was fixed for one stage only but this target could not be maintained; and (6) no time targets were fixed for completion of the whole or any part of the project.

Non-fixation of time targets may be due to: uncertain nature of research, uncertainties regarding resources, or lack of planning, while inability to keep a set schedule will indicate poor planning, lack of organization, and/or material difficulties involved in carrying out the work. In any case (whether or not time targets were set or kept) the amount of work produced, including experiments, publications, patents, etc., and the time spent thereon must be noted. Time taken over and above the scheduled time targets and researchers' reason(s) for such delay must also be recorded. It has to be emphasized, however, that time targets for completing a certain amount of work are entirely arbitrary and hence they cannot serve as standards for judging if a project could accomplish as much as it should have during a specific time period. Time targets are fixed on the basis of experience through which a researcher learns, only roughly, how much work should be expected during a certain span of time. This ' how much ' may vary a great deal for different types of researches, but still it is useful to have projects time scheduled in order to have a yardstick by which progress over time can be measured.

Expenditure of money and manpower: Outcomes — Evaluation here will depend on two things: (1) estimation of what is achieved at what cost, and (2) estimation of the extent to which cost corresponds with the budget. For the first, cost on equipment, salaries, maintenance, etc., for a particular period should be matched with the quantity of outcomes (number of patents, papers, reports, etc.) during that period. For the second, the amount of money allotted and the amount spent, or expected to be spent, on various phases of research during a specified period of time will be noted down. Variations, if any, between money allotted and actual expenditure along with causes of these variations should also be recorded. Such variations may be due to expansion or narrowing of a project's scope, lack of perspective in planning or rise in cost of equipment and services. In any case, evaluation of research programmes against allocation of money and its expenditure will be useful in improving financial aspects of research planning.

Variations in expenditure of manpower, like those in money, may also depend on how well these resources are utilized. Information relating to the number of persons working in a project and the number of man hours devoted by each every day for project work will be collected. This information when reflected against the quantity of work (number of patents, papers produced, experiments performed, etc.) during a specific period of time is a sound criterion for matching outcomes against expenditure of manpower in a particular project. It should, however, be noted again that like time targets, allocation of money and manpower to a project depends on rough approximations based on experience; there are no set standards in research to decide how much of these should be spent on a particular type of project. Evaluation of research programmes in terms of allocation of money and manpower, and its expenditure resulting in certain outcomes will, therefore, simply indicate how well the financial aspects of a research programme were planned and managed, rather than serve as a guideline on resource requirements and allocation. Such guidelines or standards can perhaps be evolved, though roughly, through comparing allocation and expenditure of money and manpower in research projects of similar objectives and similar magnitude (if such a similarity can at all be determined).

Evaluation of Personnel

Proper utilization of available human resources in research is a matter of paramount importance. Proper utilization here means an individual scientist's maximum usefulness in the research work he is engaged in. In order to check how well a scientist is performing his role as a member of a research team, it is necessary that his efforts be periodically evaluated. As objective criteria for such evaluation are lacking, it is suggested that a serious attempt be made towards this end.

Most often an individual scientist's usefulness in research is quantitatively measured by the number of his publications. Though a genuine indicator of one's research productivity, an evaluation based solely on the number of publications may sometimes be quite misleading because: (1) in many types of researches quick publication of results is not possible, and (2) such an evaluation leaves quality of work unaccounted for. The distinction between quantity of work and its quality may hold true not only for publications but for all kinds of scientific effort and should always be kept in mind while evaluating the work of a scientist. Hard working people are usually more productive, but hard work may not always produce good results.

Productivity (quantity of work) in research can be evaluated in more definite terms than its quality. For instance, number of papers or reports written or published, lectures delivered, seminars attended,

patents taken out, experiments performed, etc.— all this activity performed by a scientist in connection with a particular project compared with his qualifications and training, duration of association with a project, and man hours of work regularly devoted to research — will provide a fairly valid index of his productivity in a particular capacity in a project*. To evaluate the quality of one's work, however, a thorough analysis of the content of the work is needed. Work of a good quality can be identified in a number of ways, although exact measurement of quality is a rather difficult matter. One indicator of quality is creativity or originality of the work concerned. A careful analysis of any scientific work and its comparison with works of similar nature can always reveal whether it purports to the discovery or invention of a method, process or product; whether the work generates a new theory, a new hypothesis, or corroborates an old one in a new way; and the like. Identifying a hitherto unnoticed problem and presenting it in a workable form also shows an element of creativity. A research supervisor may encounter countless instances of such creative thinking in reports and notes which never reach the publication stage. Besides these, creativity may also be indicated in several other situations, such as planning of research and its day-to-day execution. It is often quite difficult to detect, as well as evaluate, an individual's creative ability in these circumstances, specially when the individual is a member of a large team engaged in a similar type of activity. Keen judgement of supervisors and colleagues is the only instrument for recording, and their honest assessment the only way of evaluating creativity in such diffuse forms as mentioned here.

Psychologists have often tried to associate creativity with such traits of personality as initiative, dominance, self-sufficiency, enthusiasm, adventurousness, etc.; motivational drives to read and write more; biographical variables such as better grades in undergraduate and graduate work; and general factors like lack of preference for any particular religion, few or no commitments to community affairs and lack of interest in combining research with non-research activities such as teaching and administration[4]. Although the evaluation of the creative value of a scientific work would be quite independent of the fact that a scientist possesses these attributes or not, a knowledge about this may greatly enhance the value of personnel evaluation in research. For instance, inability to produce creative work by a scientist who, as we may know, possesses these attributes, might be related to a serious placement difficulty. To quote Gibson[5], " The secret of the efficient use of manpower either on laboratory scale or on nationwide basis lies in assigning to each mind a job suited to its attributes . . ." A creative mind requires creative work; but a thorough analysis of staff structures in research projects would reveal that scientists are sometimes misplaced in this respect. Misplacement may be due to wrong selection or hasty allocation of work without giving due consideration to matching human abilities to the technical requirements of a particular job. Misplacement of a scientist in most cases is probably not due to wrong selection but to hasty decisions regarding allocation of work among team members. In either case, a periodic evaluation of personnel in research teams may reveal the mistake and a little reshuffling would surely enhance the usefulness of each worker and, consequently, the efficiency of the team as a whole.

Evaluation of the quality of scientific work, being a delicate problem, should be based on careful considerations. Quality credit, for instance, is not to be reserved only for originality — complete originality in scientific work is rare and only a few geniuses in every age can be classified as truly original. In many instances a scientific work is valuable simply for its utility in an academic and/or practical activity. Trend studies of all types based on simple statistics are not works of creative value but they are very useful in predicting and planning the future.

The discussion so far on the qualitative evaluation of a scientific work has left the impression that such evaluation greatly depends upon the opinion of other competent persons in the field. In the case of unpublished work, such as departmental reports, monographs, research proposals, etc., the audience is limited to a few colleagues who may rate each others' work formally or informally; while a published work has an unlimited audience and the opinion of all those who have read it cannot be accounted for. A research supervisor in such cases can base his evaluation on a ' citation count ' of the work as well as on the opinion of his immediate colleagues, although the " citation count evaluation of the research impact of a paper can be measured years after its publication . . . "†.

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2. QUINN, J. B., *Proceedings of the 11th national conference on the administration of research* (Pennsylvania State University Press, Pennsylvania), 1958, 66.
3. *Administration of research and development grants, Study No.* 1, Report of the Select Committee on Government Research (US Government Printing Office, Washington DC), 1964.
4. CHAMBERS, J. A., *Science*, **145** (1964), 1203.
5. GIBSON, cited in *An introduction to research procedures in social sciences* by M. H. Gopal (Asia Publishing House, Bombay), 1964, 18.

*Technical papers and patents are quite often used as measures of productivity in research [cf. Van Zelst, R. H. & Kerr, W. A., *J. abnorm. Soc. Psychol.*, **46** (1951), 470; and Shockley, W., *Proc. Inst. Radio Engrs*, (1957), 279]. In my opinion, however, a periodic evaluation of an individual's productivity in group research should include, besides technical papers, patents, etc., the whole gamut of academic activities performed in connection with a particular research; because in many types of researches quick results in the form of papers and patents are not possible.

†An interesting ' citation count ' technique is presented by W. M. Miles [cited in *Operations research in research and development* by B. V. Dean (John Wiley & Sons, New York), 1963, 97]. Through citation count one can only determine the amount of reaction of the scientific community to a particular piece of literature, not whether the reaction was generally positive or negative or equally both (unless of course each reference is thoroughly read and analysed).

Chemistry of Natural Products*

INDIA possesses rich and extensive forest resources with varied types of vegetation, viz. dry deciduous, moist deciduous, moist evergreen, subtropical, temperate and Alpine. A survey of Indian flora which may be classified as medicinal, poisonous and aromatic plants has shown that India possesses nearly 20,000 botanical species, of which only a few hundreds (nearly 600 species) have been examined. India's vegetable *Materia medica* offers, therefore, a vast field of study and all that has been done to date can be considered to have touched only the fringes of this vast and complex problem. But the work done so far in this field has brought out the merits of various Indian herbs which have secured an important place in world medicine.

In recent years the increasing strains and stresses of modern life, faster and more dangerous means of locomotion, stiffer competition for professional opportunities and the uncertainties of the community in a socially unsettled world have raised the number of psychosomatic and mental disorders and their attendant physiological consequences to unexpected levels. Sedatives and hypnotics are standard aids in overcoming excited mental states, particularly the tranquillizers can control such mental derangements including schizophrenia. So I felt interested in the chemical examination of the medicinal herbs in order to search for drugs which might have therapeutic efficacies in psychoneurotic and other behavioural disorders, as also in epilepsy which may also be a neuropsychiatric problem. Plants reputed to cure such mental ill health as mentioned in Ayurveda were selected for chemical investigation and also other plants of the same botanical families or their biogenetically related families were examined chemically. In this connection one hundred plant species of sixteen botanical families — Apocynaceae, Buxaceae, Loganiaceae, Rutaceae, Euphorbiaceae, Piperaceae, Compositae, Leguminosae, Meliaceae, Menispermaceae, Acanthaceae, Dilleniaceae, Magnoliaceae, Ebenaceae, Umbelliferae and Marsileaceae — were examined. More than thirty new alkaloids, ten coumarins besides several triterpenoids, furanoid bitter principles and carotenes, the precursor of vitamins, have been isolated. The work on some of these plants has been extremely interesting. Mention may be made about *Rauwolfia, Alstonia, Rhazya, Kopsia* and *Chonemorpha* (Apocynaceae); *Securinega* and *Trewia* (Euphorbiaceae); *Citrus, Luvanga, Zanthoxylum* and *Aegle marmelos* (Rutaceae); *Angelica* and *Prangos* (Umbelliferae); *Inula* (Compositae); *Calycotome* and *Cassia* (Leguminosae); *Tinospora* (Menispermaceae); *Swietenia* (Meliaceae); and *Marsilea* (Marsileaceae).

Research in the field of Indian medicinal plants in our laboratory has been pursued in the following directions. (1) Alkaloids belonging to the series: (a) indole alkaloids (i) monomeric and (ii) dimeric; (b) indoloquinazoline alkaloids; (c) quinoline alkaloids; (d) isoquinoline alkaloids; (e) pyridine alkaloids; (f) diterpene alkaloids; and (g) steroid alkaloids. (2) Polyphenolic compounds: (a) coumarins and (b) dianthraquinones. (3) Furanoid bitter principles. (4) Macrocyclic ketone. Besides, attention has also been given towards studying the mechanism of the following organic reactions: (a) mechanism of hydramine fission; (b) studies on the Claisen rearrangement; and (c) stereochemical correlations in nitrogen heterocycles. Last but not the least is the work in the field of analytical chemistry in which successful application of periodic acid as a suitable reagent for the detection and quantitative estimation of isolated double bond deserves mention. Periodic acid has also been found to be a selective reagent for the stereospecific synthesis of *trans* glycols.

ALKALOIDS

Indole Alkaloids

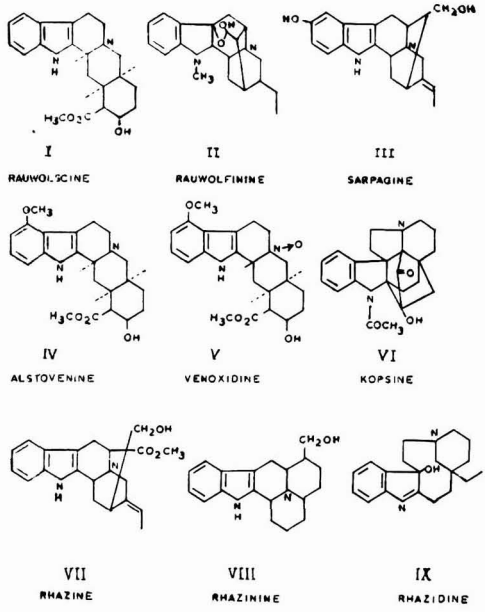
More than thirty indole alkaloids have been isolated from various species of the genus (i) *Rauwolfia*, (ii) *Alstonia*, (iii) *Rhazya*, (iv) *Vinca* and (v) *Kopsia*, all belonging to the family Apocynaceae and their chemistry and biological activities have been studied.

Nine *Rauwolfia* species were thoroughly screened and as a result reserpine and a number of new indole alkaloids were isolated. All these bases were shown to possess hypotensive and mild sedative action. Of the *Alstonia* alkaloids, echitamine possesses antimalarial and antidysenteric properties and the dimeric indole alkaloid, villalstonine, makes the irregular heart pulsation regular. Chemotherapeutic potentialities of kopsine (from *Kopsia arborea*) and rhazine and its congeners (from *Rhazya stricta*) are being revealed. The alkaloids which were isolated for the first time and whose constitution was eventually established were rauwolscine (I), rauwolfinine (II), sarpagine (III) from *Rauwolfia* species, alstovenine (IV), venoxidine (V) from *Alstonia venenata*, kopsine (VI) from *Kopsia* and rhazine (VII), rhazinine (VIII) and rhazidine (IX) from *Rhazya* species. Besides, various other indole alkaloids, viz. echitamine, echitamidine, macralstonine, macralstonidine, villalstonine, venenatine, nerifoline, etc., were isolated and their chemistry was elaborated.

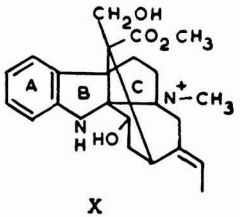
Classical as well as modern chemical approaches (application of physical tools, e.g. ultraviolet, infrared, nuclear magnetic resonance spectroscopy and mass spectrometry) were made in establishing the structure and stereochemistry of various natural products studied.

Echitamine (X) was shown to possess the pyrrolidino-indoline (ABC) moiety which was confirmed by X-ray study. Incidentally, it needs mention that the presence of such a moiety in the family

*A dissertation by Dr (Mrs) Asima Chatterjee, Khaira Professor of Chemistry, Calcutta University, Calcutta, on the occasion of the presentation of the Shanti Swarup Bhatnagar Memorial Award in Chemistry for the year 1961 at the National Physical Laboratory, New Delhi, 14 January 1965.



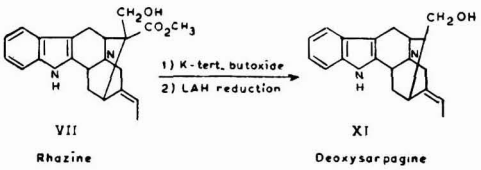
Apocynaceae was observed for the first time by us. Echitamidine, a congener alkaloid of echitamine, was assigned an akuammicine type of structure on the basis of physical and chemical evidence and from biogenetic considerations.



With the physical and chemical data at hand the dimeric indole alkaloids, macralstonidine and villalstonine, are supposed to contain β-carboline nucleus of the sarpagine and pleiocarpamine types as the units in their molecules.

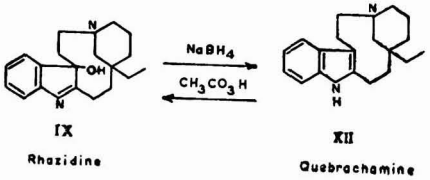
The structure elucidation of the *Rhazya* alkaloids and of the alkaloids, kopsine, alstovenine and venoxidine, has been possible very recently and a short account of their chemistry is discussed below.

Rhazine — The alkaloid rhazine, $C_{21}H_{24}N_2O_3$, was shown to be a tetrahydro-β-carboline derivative from ultraviolet and infrared spectral measurements as also from degradative reactions. Functional group analyses coupled with NMR spectral measurements permitted recognition of (i) four aromatic protons (2·5 τ), (ii) an indole-NH (2·0 τ), (iii) an ethylidene function (methyl doublet at 8-8·2 τ and methine quartet centred round 4·0 τ), (iv) a primary hydroxyl group and (v) a carbomethoxyl function. Based on these data and from biogenetic consideration a 16-carbomethoxy-10-deoxy sarpagine structure (VII) for rhazine was proposed which was eventually settled by its conversion to deoxysarpagine (XI).



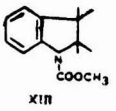
Rhazidine — From chemical and various spectral measurements, the alkaloid rhazidine, $C_{19}H_{26}N_2O$, was shown to have a 3-hydroxyindolenine structure. The strong peaks at m/e M-17 and at M-29 in the mass spectrum of rhazidine suggested the presence of a tertiary hydroxyl and a C-ethyl function in its molecule, the presence of the latter was also discernible in its NMR spectrum.

Isolation of 3,5-diethylpyridine from the Zn-dust distillation product of rhazidine suggested an aspidospermine like skeletal pattern which was subsequently confirmed by conversion of rhazidine to quebrachamine (XII) by sodium borohydride reduction. On the basis of these results rhazidine is represented by the structure (IX). The β-hydroxyindolenine structure (IX) for rhazidine has been finally confirmed by its reduction with lithium aluminium deuteride to monodeutero quebrachamine and also by the conversion of quebrachamine (XII) on oxidation with peracetic acid to rhazidine.

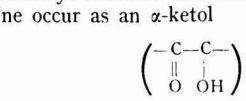


Rhazinine — The structure elucidation of the alkaloid rhazinine (VIII), a novel pattern of indole alkaloid representing a linearly fused indolo-lupine system, has been possible by the combination of classical degradative methods and utilization of modern physical tools.

Kopsine — The alkaloid kopsine, $C_{22}H_{24}N_2O_4$, from ultraviolet, infrared and NMR spectral measurements was shown to have an N-carbomethoxy dihydroindole chromophore (XIII), a five-membered ring



ketone and a tertiary hydroxyl group. Relevant oxidation and reduction experiments revealed that the carbonyl function and the hydroxyl group in kopsine occur as an α-ketol



system. Isolation of 3,5-diethylpyridine as one of the products on Zn-dust distillation of kopsine suggested an aspidospermine type of skeleton for kopsine which was eventually settled from mass spectral analysis of the base. A detailed study of the mass spectral fragments of kopsine and a number of its derivatives permitted a heptacyclic structure (VI) assignment for this compound. The mechanistic pathway for the genesis of some important fragments out of the molecule of kopsine is given in Chart 1.

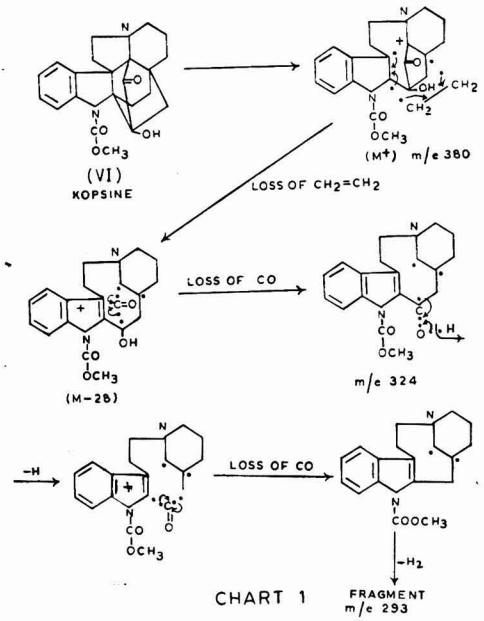
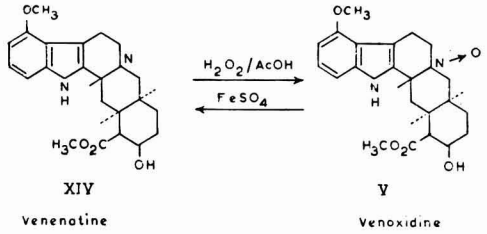


CHART 1

Alstovenine — Alstovenine, $C_{22}H_{28}N_2O_4$, isolated from *Alstonia venenata* R. Br., was recognized as a 5-methoxytetrahydro-β-carboline derivative from various spectral measurements. Isolation of yobyrine and 5-methoxy yobyrine by selenium dehydrogenation of the base settled a 9-methoxy yohimbane skeleton for alstovenine. From various degradative and stereochemical reactions, alstovenine has been shown to have the formulation (IV).

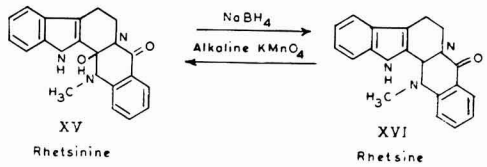
Venoxidine — Venoxidine, $C_{22}H_{28}N_2O_5$, is a water-soluble alkaloid of the stem bark of *Alstonia venenata* R. Br. On catalytic hydrogenation as also on reduction with Zn/AcOH or $FeSO_4$, venoxidine furnished a tertiary base, $C_{22}H_{28}N_2O_4$, which was identified as the C-3 epimer of alstovenine, i.e. venenatine (XIV). Based on this result, venoxidine was assigned the structure (V) which was confirmed by conversion of venenatine (XIV) to venoxidine by H_2O_2/AcOH oxidation.



Indoloquinazoline Alkaloids

Rhetsinine, rhetsine (= evodiamine) and rhetine (= rutaecarpine), isolated from *Zanthoxylum rhetsa* DC and *Zanthoxylum oxyphyllum* (Rutaceae), were shown to be indoloquinazoline derivatives by degradative studies. By a number of degradative reactions and finally by synthesis the structure of rhetsinine has been settled as (XV).

A correlation between rhetsine (XVI) and rhetsinine (XV) was also revealed by (i) treating rhetsinine with $NaBH_4$ to get rhetsine and (ii) by treating rhetsine with alkaline permanganate to get rhetsinine.



Quinoline Alkaloids

Graveolinine — The quinoline alkaloid, graveolinine (XVII), $C_{17}H_{13}NO_3$, isolated from *Ruta graveolens* Linn. (Rutaceae), was synthesized according to the sequence of reactions shown in Chart 2.

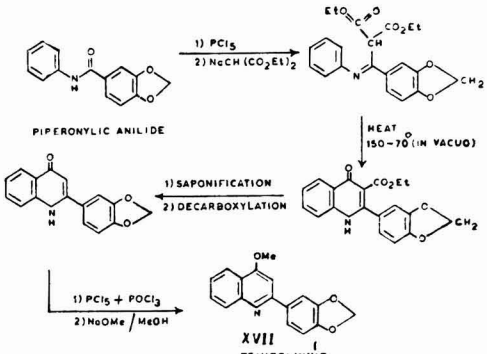


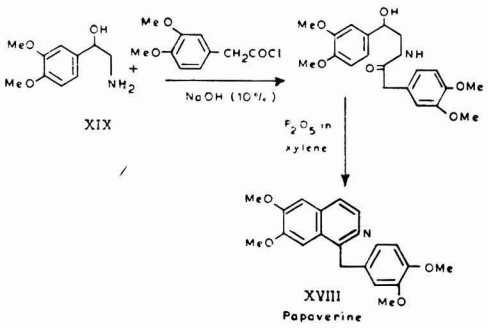
CHART 2

Isoquinoline Alkaloids

As a suitable starting material for the synthesis of isoquinoline alkaloids, different β-phenylethanol-

amines were synthesized by a simplified and novel procedure which involves an initial condensation of an aromatic aldehyde with HCN and subsequent $LiAlH_4$ reduction of the corresponding hydroxynitrile to β-phenylethanolamine. Incidentally, these β-phenylethanolamines are very important from chemotherapeutic viewpoint and have found use as pressor drugs.

Papaverine — Synthesis of papaverine (XVIII) was achieved in two steps, starting with β-hydroxy-β-(3,4-dimethoxyphenyl)ethylamine (XIX). Attention was also given towards the synthesis of naturally occurring alkaloids under ' physiological conditions '. The synthesis of calycotomine (1-hydroxy-methyl-6,7-di-methoxyterahydroisoquinoline) from DOPA (dihydroxyphenylalanine) may be mentioned in this connection.



Pyridine Alkaloids

Piperlongumine, nudiflorine and allosecurinine are the three pyridine alkaloids that have recently been isolated in this laboratory and their structures completely established.

Piperlongumine — Piperlongumine, $C_{17}H_{19}NO_5$, is an alkaloid of *Piper longum* Linn. Functional group analyses and spectral measurements indicated the presence of three aromatic methoxyl, two olefinic double bonds and a conjugated amide linkage in its molecule. Alkali hydrolysis of piperlongumine furnished 3,4,5-trimethoxycinnamic acid and piperlongumic acid, $C_{17}H_{21}NO_6$. Piperlongumic acid on catalytic reduction furnished a tetrahydroderivative which from various chemical reactions and spectral measurements was recognized as a condensation product of δ-aminovaleric acid and 3,4,5-trimethoxyphenyl propionic acid. Isolation of α-piperidone by alkali hydrolysis of tetrahydropiperlongumine and succinaldehydic acid by ozonolysis of piperlongumic acid settled the structure of piperlongumine as (XX). The correctness of this structure was verified by an unambiguous synthesis of tetrahydropiperlongumic acid (Chart 3).

Nudiflorine — Nudiflorine, $C_7H_6N_2O$, was isolated from *Trewia nudiflora* Linn. The presence of a 2-pyridone nucleus and a nitrile function in its molecule was indicated from ultraviolet and infrared spectral measurements. NMR spectrum of nudiflorine indicated the presence of an N-methyl and 1,2,4-trisubstituted aromatic splitting pattern.

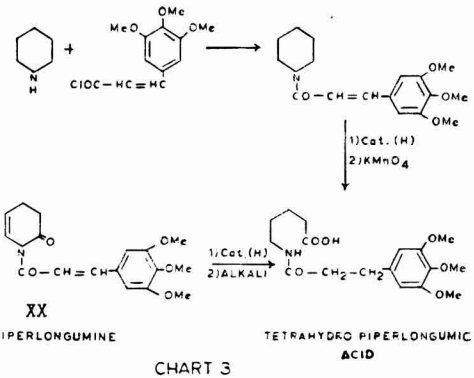


CHART 3

Structural feature of nudiflorine was revealed by acid hydrolysis of the compound which furnished 1-methyl-2-pyridone-5-carboxylic acid. Based on these results nudiflorine was assigned the structure (XXI) which has been finally confirmed by its synthesis (Chart 4).

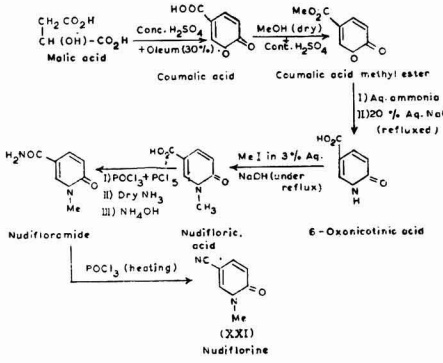
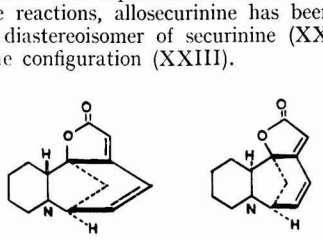


CHART 4

Allosecurinine—Allosecurinine, $C_{13}H_{15}NO_2$, an alkaloid isolated from the roots of *Securinega suffruticosa* (Pall) Rehd, has been found to possess strychninoid activity. From a careful analysis of the various spectral data of allosecurinine and of its derivatives coupled with the experimental results of the degradative reactions, allosecurinine has been shown to be a diastereoisomer of securinine (XXII) and it has the configuration (XXIII).



XXII Securinine — XXIII Allosecurinine

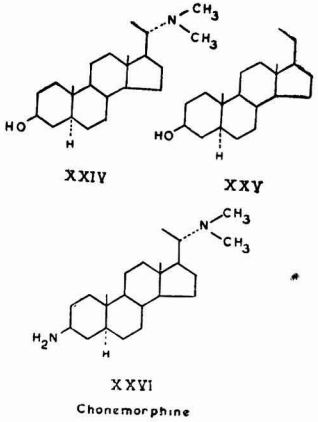
Diterpene Alkaloids

From *Inula royleana* DC (Compositae) two diterpene alkaloids, royline (≡ lycoctonine) and

inuline (≡ anthranoyllycoctonine), have been isolated. The isolation of these diterpene alkaloids from a plant of the family Compositae has an important bearing as it establishes its biogenetic correlationship with the family Ranunculaceae, a potent source of such diterpene alkaloids.

Steroid Alkaloids

Chonemorphine — Chonemorphine, $C_{23}H_{42}N_2$, is an alkaloid of *Chonemorpha macrophylla* G. Don (Apocynaceae). Isolation of Diel's hydrocarbon by selenium dehydrogenation of the base indicated its steroidal nature. Deamination of chonemorphine to 3β-hydroxy-20α-dimethylamino-5α-pregnane (XXIV) and its subsequent Hofmann degradation to 3β-hydroxy-5α-pregnane (XXV) revealed that chonemorphine is 3β-amino-20α-dimethylamino-5α-pregnane (XXVI). The structure proof was also available from its unambiguous synthesis.



Chonemorphine stands as a promising hormonal drug and a suitable starting material for the synthesis of progesterone and dihydrocortisone.

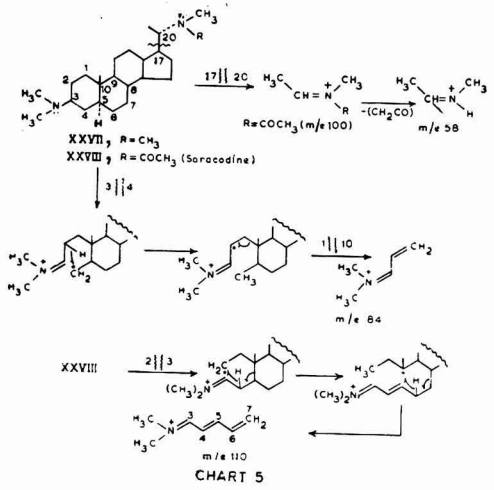


CHART 5

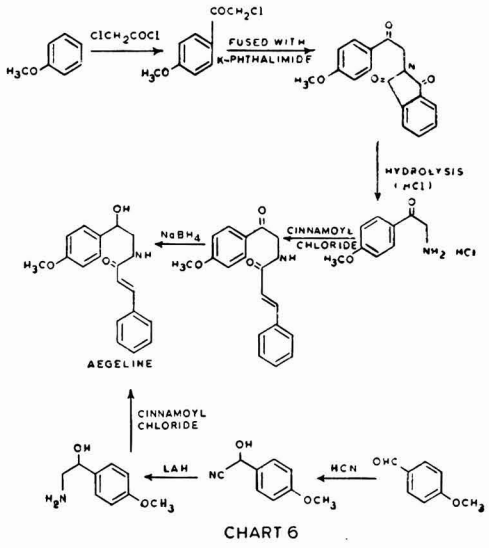


CHART 6

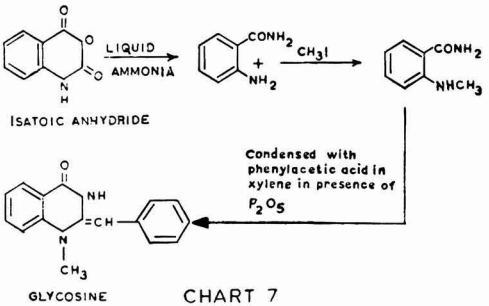


CHART 7

Saracodine —Saracodine, $C_{26}H_{46}N_2O$, is an alkaloid of *Sarcococca pruniformis*. From chemical studies, NMR and mass spectral measurements and lastly by conversion of saracodine to dimethyl chonemorphine (XXVII), the structure and stereochemistry of the alkaloid have been conclusively settled as (XXVIII). The mass spectral fragmentation pattern of saracodine is shown in Chart 5.

Besides the work on these different alkaloids, studies on the stereochemistry and synthesis of aegeline, an alkaloid amide, structure elucidation and synthesis of quinazoline alkaloids, glycosine and aegelenine (Charts 6 and 7), and isolation and characterization of liriodenine, an aporphine alkaloid, also deserve mention.

The structure of glycosine has been confirmed from NMR studies, deuterium exchange of —NH and from ultraviolet spectra in acid solution.

POLYPHENOLIC COMPOUNDS

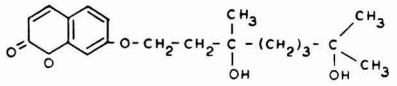
Coumarins

Of a number of coumarins, isolated from different plant sources in recent years, the following deserve

mention: (i) marmin, (ii) bergamottin, (iii) xanthotoxin, (iv) marmesin, (v) oxypeucedanin, (vi) prangolarin, and (vii) archangelin. It has been shown that xanthotoxin and imperatorin (also isolated in our laboratory), occurring in many Umbelliferae and Rutaceae plants, can restore melanin in melanin-depleted skin and thus find a very useful application in the treatment of leucoderma. Bel (*Aegle marmelos*) and Kakali (*Luvanga scandens*) fruits are rich sources of imperatorin and xanthotoxin.

Marmin — Marmin, $C_{19}H_{26}O_5$, m.p. 124·5°, $[\alpha]_D^{30°}$ +26·85° (ethyl alcohol), isolated from *Aegle marmelos*, has been proved to be an α,β-unsaturated δ-lactone from its spectral measurements and chemical evidence.

On acid hydrolysis, marmin yields umbelliferone, $C_9H_6O_3$, and a neutral compound, marminol, $C_{10}H_{19}(OH)_3$, which has been identified as 1,5-dihydroxy-2,6-dihydrogeraniol from oxidation reactions and consequently the structure of marmin has been postulated as 1,5-dihydroxy-2,6-dihydrogeranyl ether of umbelliferone (XXIX).

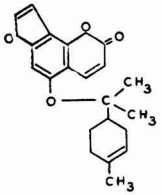


XXIX
Marmin

Bergamottin — Bergamottin, $C_{21}H_{22}O_4$, m.p. 60°, isolated from bergamot oil, is formulated as bergaptol geranyl ether. Its structure has been confirmed by an unambiguous synthesis of bergamottin by us from bergaptol and geranyl chloride.

Prangolarin — Prangolarin, $C_{16}H_{14}O_5$, m.p. 104-5°, $[\alpha]_D^{30°}$ +20° (chloroform), has been isolated from *Prangos pabularia* Lindl. Prangolarin on acid cleavage loses a C_5 unit ($-C_5H_9O$) and yields bergaptol and on chromic acid oxidation it produces acetone. These degradative experiments together with the fact that prangolarin is dextrorotatory prove that it is the optical antipode of oxypeucedanin.

Archangelin — Archangelin, $C_{21}H_{22}O_4$, m.p. 132°, and a number of lactones have been isolated from *Angelica archangelica* Linn. Various spectral measurements suggest the benzofuran structure for archangelin. The benzofuran structure has been confirmed from its degradation to isobergaptol. The mass fragmentation pattern, NMR data coupled with the fact that archangelin produces acetone on chromic acid oxidation and isobergaptol on acid cleavage suggest structure (XXX) for archangelin.



XXX
Archangelin

BITTER PRINCIPLES

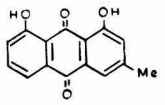
Research in the field of bitter principles and substituted β-furanolactones has culminated in the elucidation of the structure of tinosporine (≡ columbin) from *Tinospora cordifolia* and swietenine from *Swietenia macrophylla*. The functional groups and the structure of swietenine proposed along with their X-ray studies suggested their biogenetic origin from tetracyclic triterpene of euphol type.

Cassianin and Siameanin

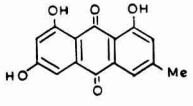
From the trunk bark of *Cassia siamea* Lam. three new pigments, cassianin, siameanin and siameadin, have been isolated.

Elemental analysis of cassianin and a number of its derivatives in combination with its mass spectrometric molecular weight determination settled the molecular formula of cassianin as $C_{30}H_{18}O_9$. Characteristic colour reactions and ultraviolet and visible spectral analysis revealed that cassianin is a polyhydroxy anthraquinone. Of the nine oxygen atoms present in its molecule, five were recognized as phenolic hydroxyls from acetylation experiment, the remaining four occurring as quinone carbonyls as evidenced from its reductive acetylation to a nonaacyl derivative.

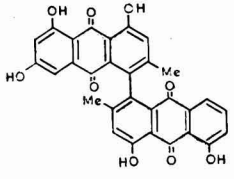
These data coupled with the fact that cassianin on Zn-dust distillation gives β-methylanthracene as the only degradation product indicated that cassianin is a dianthraquinone derivative in which both the anthraquinone moieties bear a β-methyl group. This is further supported by other degradation reactions. Structural feature of cassianin was completely revealed by its reductive cleavage with alkaline sodium dithionite (under mild conditions) to chrysophanol (XXXI) and emodin (XXXII). Based on this observation and from biogenetic consideration the structure of cassianin has been elaborated as (XXXIII). NMR spectral data of cassianin and its derivatives are consistent with this formulation. Siameanin, $C_{30}H_{18}O_8$, likewise was shown to have the structure (XXXIV) as it could be cleaved into two molecules of chrysophanol by treatment with alkaline sodium dithionite.



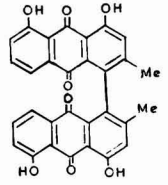
XXXI
Chrysophanol



XXXII
Emodin



XXXIII
Cassianin



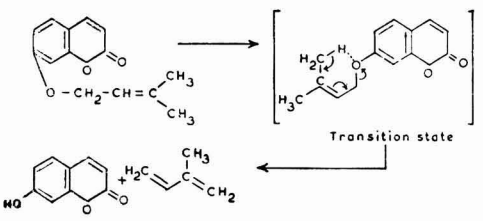
XXXIV
Siameanin

Mechanism of Hydramine Fission

Hydramine fission of β-hydroxyphenylethylamine and some of its substitution products in the presence of hydrochloric acid was studied and it was shown that the nature of the substituents has a profound influence in steering the course of hydramine fission of these compounds. It was observed that electron releasing groups substituted in *para* and *ortho* positions to the ethanolamine moiety favour the formation of aromatic aldehydes, whereas their *meta* orientation leads to the formation of aromatic ketones which are also produced when the *para* or *ortho* substituent is an electron attracting group. An interesting observation made in this connection is that unsubstituted β-phenylethanolamine upon hydramine fission produces β-phenylnaphthalene. A mechanism for these reactions was postulated.

Claisen Rearrangement

Claisen migration of γ,γ-dimethyl allyl ethers of 5-hydroxy-, 6-hydroxy-, 7-hydroxy- and 8-hydroxycoumarins was studied and it was observed that these γ,γ-dimethyl allyloxycoumarins do not undergo any molecular rearrangement, but instead suffer degradation to phenolic coumarins and isoprene under the experimental conditions at which Claisen rearrangement is performed. As a plausible explanation for this abnormal reaction the following mechanism has been put forward:



Stereochemical Reactions in Nitrogen Heterocycles

Various stereochemical reactions in rauwolscine-yohimbine system were performed of which stereospecific reduction of rauwolscone and yohimbone and conversion of yohimbine to β-yohimbine deserve mention.

It was observed that rauwolscone or yohimbone on reduction with $NaBH_4$ or $LiAlH_4$ affords only one epimeric alcohol, viz. alloyohimbol or epiyohimbol. Reduction of yohimbone with aluminium isopropoxide, however, produces both yohimbol and epiyohimbol. Similar reduction of rauwolscone gives only one product, i.e. alloyohimbol. A mechanism for these reactions has been discussed.

Conversion of yohimbine to β-yohimbine has been achieved by a three-step process which involves modified Oppenauer oxidation of yohimbine to yohimbinone and its subsequent reduction with $NaBH_4$ to β-yohimbic acid which on methylation gives β-yohimbine.

Indian Cryptogams

Although we have been studying the chemistry of higher plants, our attention has also been drawn to the Indian Cryptogams or flowerless plants, specially the Indian ferns, a field which remains practically unexplored. The work already done with the Cryptogams, particularly with *Penicillium* species, has led to the discovery of many important antibiotics and others have shown promise of utilization in medicine and in industry. In recent years several ferns belonging to *Marsilea* species have been examined by Chatterjee and coworkers. *M. minuta* is found to contain a sedative, anti-epileptic and anticonvulsant drug, marsilin, which is a macrocyclic ketone. Marsilin is effective in curing behavioural and psychological disorders and is shown to be a promising rehabilitation drug.

The chemists are now extremely interested in studying the biogenetic pathway to the natural products from plant materials. This investigation has been possible since the development of isotopic tracer technique. By feeding the plants with isotopic labelled compounds many experiments on biosynthesis of natural products have been performed. It appears that carbon dioxide inhaled from the atmosphere by the plants is first converted into acetate. Then the orderly arrangement of the acetate units gives rise to isoprenoids and terpenoids, some of which constitute many important essential oils. The polyacetate system also produces various coumarins and bitter principles, whereas these acetates with ammonia upon appropriate biosynthetic sequence give rise to alkaloids of innumerable structures and configurations.

However, the ultimate aim of studying plant chemistry is not only to divulge the secrets of nature and to explore her treasures, the drugs and chemicals of economic importance that can be used for human welfare but also to find out the process of biological evolution.

Some Aspects of Biochemical Pharmacology of Myocardial Infarction*

ACCORDING to Kagan *et al.*[1], an individual most prone to develop coronary heart disease (CHD) may be described as a mesomorphic, obese, middle-aged male, with high serum cholesterol, high blood pressure, low vital capacity and an abnormal electrocardiogram. He eats too much of too rich foods, smokes cigarettes to excess, and is physically inactive both in occupation and in recreation. He is ambitious, aggressive and subject to frequent deadlines and other emotional stresses. The closer an individual comes to fitting this pattern, the greater should be the efforts of his physician to alter, where practicable, these characteristics of the patient and of his environment.

Researches carried out in recent years have created the basis for an approach to effective primary prevention by demonstrating that the abnormalities leading to CHD proneness are ameanble to correction or control. It is clear now that hypercholesterolaemia can be reduced in most persons by dietary means. Hypertension can also be brought under control in most cases by dietotherapy (weight reduction of the obese and salt restriction) plus the modern drugs, if needed. This is particularly true when elevated blood pressure is detected early, when still moderate in degree and uncomplicated by renal or other vital organ damage. Chronic overeating (with consequent obesity) and heavy cigarette smoking are habit patterns, often deeply ingrained, but nevertheless treatable and correctable, albeit not easily. Diabetes can be controlled by diet and (if needed) by drugs. Other risk factors — hypothyroidism, renal damage, physical inactivity — are also amenable to effective intervention. Data are available indicating that cessation of heavy smoking, reduction of high blood pressure, control of diabetes and correction of obesity are all associated with significant decrease in the risk of developing CHD in middle age. There is reason, therefore, for cautious optimism concerning the possibility of prevention based on correction of abnormalities making for CHD proneness.

Role of Diet

There is increasing body of evidence disputing the solitary role of dietary lipids in the pathogenesis of CHD. Yudkin[2] has shown the association of a diminished glucose tolerance and atherosclerosis, and has impressed the concept of high levels of dietary sucrose in patients with occlusive atherosclerotic disease. This is against the earlier views expressed by a large number of workers showing the importance of blood lipids, egg yolk, cholesterol, etc., in the development of the disease. Block[3] and Lynen[4] re-emphasize the role of cholesterol. A survey of literature reveals that diet through its polysaturated fat content has been the most fascinating yet incompletely understood factor in the proneness to coronary artery disease. Yudkin[2] concluded that the relationship between coronary artery disease and fat intake was more apparent rather than real. A similar viewpoint has been extended recently by Shah[5] that there are other important factors such as physical inactivity, mental stress and psychosocial factor, apart from diet, playing a role in the etiology of CHD. This led Arora and coworkers to make an attempt in understanding the role of dietary fats in the genesis of CHD. The value of Bengal gram *C. arietinum* in preventing the development of atherosclerosis has been investigated. Pigs, aged 4 years, were fed on vanaspati and cholesterol for 15 months and the results were compared with those for pigs on normal diet and pigs on vanaspati plus cholesterol plus 60 per cent Bengal gram diet. The results showed that cholesterol level is raised in experimental animals as compared to control animals. EKG record showed evidence of acute myocardial infarction in the experimental animals, but, in contrast, the histological studies revealed no areas of myocardial infarction except for slight fatty degeneration at the apex of the ventricles and also showed no clear evidence of atheromatous patches. The thoracic aorta showed areas of sudanophilia which was more marked than in the case of abdominal aorta. Absence of atheromatous patches in the animals is suggestive of the role of Bengal gram which was the main dietary constituent in these animals. As $4\frac{1}{2}$ years is the average age at which spontaneous atherosclerosis develops in English pigs, these effects were rather surprising. Further work on the role of diet is in progress.

Role of Emotions

Another important factor in the genesis of CHD is the emotional factor[6]. A significant role has been ascribed to emotions in the precipitation of half the cases of acute myocardial infarction. The validity of this concept has not been established, although a great body of supportive evidence is currently accumulating. The fact that there has been an increasing tendency for the disease to occur in younger age groups, at least among men, further supports this concept. Emotional reactions or mental stress elevate blood fats (non-esterified fatty acids, lipid mobilizing factor, cholesterol, lipoprotein) also; they increase the daytime production of epinephrine and norepinephrine; they increase the systolic tension time index, i.e. myocardial oxygen consumption, independent of body oxygen consumption and possibly enhance blood clotting.

This view is gaining ground not only in USA and Europe but also in Russia. A review of the Russian research on the role of visceral reflexes in coronary insufficiency by Simson[7] emphasizes their reports on prolonged experimental neuroses on monkeys produced by conflicting conditioned reflexes, some of which developed electrocardiographic

*A dissertation by Dr R. B. Arora, Professor of Pharmacology, All-India Institute of Medical Sciences, New Delhi, on the occasion of the presentation of the Shanti Swarup Bhatnagar Memorial Award in Medical Sciences for the year 1961 at the National Physical Laboratory, New Delhi, 14 January 1965.

changes that were identical with those of human left ventricular ischaemia.

Osler[8] described the typical coronary patient as a 'keen and ambitious man', the indicator of whose engines is always set at 'full speed ahead'. Dunbar[9] and Arlow[10] both concluded that a clear-cut personality pattern can be detected in many individuals with coronary artery disease, characterized among other things, by habitual overdrive and compulsive drive to success by means of hard work and self-discipline. Physiological studies of coronary patients have confirmed this in part. In this regard, it is also of interest that among 100 patients with myocardial infarction suffered prior to age 40, most were either holding down two jobs or working more than 60 hr a week at one job just prior to their attack[11]. Russeks emphasized their personality as being aggressive and ambitious with an intense physical and emotional drive, a description much like that which was noted five decades earlier by Osler. Even as late as December 1964, the well-known US heart specialist, Howard B. Sprague of Harvard University, says in an article titled 'Tensions on the heart' that comparatively high incidence of mortality from CHD among professional and managerial groups is due to emotional tension.

If chronic exposure to the forms of socio-economic stress unique to industrialized societies is a major pathogenetic factor in the increasing incidence of clinical coronary disease, several possible mechanisms might be operative alone or synergistically. First, the effects of emotional stress on cardiovascular haemodynamics are profound and there is much evidence that organic vascular damage may result from such effects. This will be perhaps in part due to endocrine engendered angiotoxic effects where the resulting vascular damage may predispose or perhaps in part precede subsequent lipid deposition, of greatest severity in the presence of elevated plasma lipid content. A second possible mechanism implicates the direct effects of emotional stress upon the plasma lipids. A third possible mechanism by which the exposure to emotional stress might affect the coronary arteries is by precipitating thrombotic occlusion.

In any case, if atherogenesis is variously a function of time, haemodynamic factors, intimal damage and disturbed lipid metabolism, and if myocardial infarction is in many instances a function of thrombotic occlusion superimposed on an atherosclerotic substrate, then it already has been demonstrated by various data that each of these factors is adversely influenced by exogenously administered adrenal hormones. On the other hand, it is well known that emotional stress stimulates adrenal secretion, or, in other words, that cerebral cortical perturbation stimulates a self-engendered administration of endogenously derived adrenal hormones (Fig. 1).

Further, in the presence of emotional tension there may be the associated factors of diminished exercise and augmented dietary intake as well as altered blood flow. These relationships (Fig. 2) on the basis of which it is suggested that occupational stress in susceptible individuals, as increasingly found in middle and upper class males of

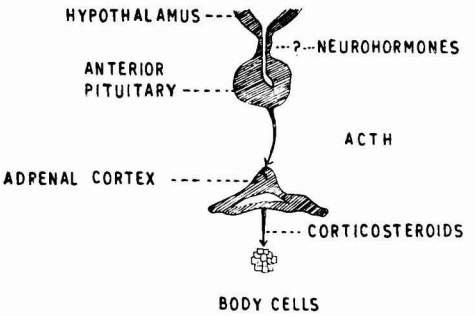


Fig. 1 — Hypothalmico-hypophysial adrenal relationship

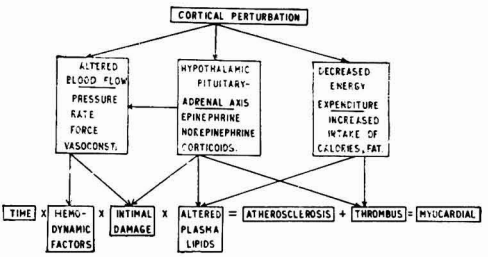


Fig. 2 — Possible pathways by which cortical perturbation may participate in mechanism of myocardial infarction

industrialized societies, more or less continuously stimulates such cortical perturbation[12].

In considering the hypothalamic-pituitary-adrenal cortical system as a functional unit, there are several points of approach which might provide useful information regarding the response of this system to stress. Although there appears little doubt that an essential chemical mediator is released by specialized cells in the hypothalamus, the identity of this chemical substance remains undisclosed. There is no evidence to date that the nature or quantity of this substance can be determined from analysis of readily available body fluids. However, it is probable that investigations into the nature and action of 'neurohumoral' substances may one day provide an important link in the chain of events which initiate man's hormonal response to stress.

In spite of recent researches, CHD still accounts for more than half of all the deaths in Britain, the United States, Australia, New Zealand and many other countries. According to a survey covering 22 selected countries, conducted by WHO in 1964, the percentage of deaths through heart disease amounted to 48 per cent of all deaths, with women having a lower proportion than men. Efforts to find a solution have been obstructed for want of an animal species in which the disease similar to human could be duplicated, so that data from animal experiments can be projected to man and drugs beneficial in animal atherosclerotic coronary disease would be equally effective in man.

With this background I would like to put forward my thesis for solving this riddle. My concept implies that the endocrine system, specially neurohormones, related amino acids and the enzymes concerned in their metabolism, play an important role in atherogenesis and clinical CHD.

Author's Thesis

My thesis is that emotional stress plays a major role in clinical coronary disease[13]. Body mechanisms or substances released within or administered exogenously to mammals, which prevent the release and/or increase the bound form of catecholamine and related hormones, are likely to produce a salutary effect in preventing and/or ameliorating myocardial infarction and clinical coronary artery disease.

In a searching analysis of the problem of coronary artery disease, Florey, in his Jephcott lecture[14] in 1960, declared that it might not be going too far to believe that the same factors were operating to produce the lesion in the pigs as the one present in man, and that it may now be possible to elaborate observations made on man in a more comprehensive manner on animals. A reappraisal of the feasibility of using the domestic pig was, therefore, deemed pertinent.

Among the numerous advantages of the pig are the uniformly regular coronary artery pattern and the basic genetic similarity among pigs of the same strain and a blood lipoprotein pattern similar to the human. The pig is adaptable to extreme changes of diet, and, while the vagus plays no part in the innervation of the coronary arteries in the dog, it has a pronounced effect in the pig. Further, angina pectoris, coronary failure and acute myocardial infarction in man have been shown to be closely related to coronary narrowing and occlusion in the pig.

Experimental Study

The present study[15-18] was, therefore, set out to examine the possibility of developing the animal for use in a study of homogeneous experimental myocardial infarction by a two-stage coronary artery ligation. To our knowledge this is the first time that successful experimental induction of a delayed development of ectopic ventricular arrhythmia and homogeneous experimental myocardial infarction in the pig has been reported.

When the ligature was tied 26 ± 3 mm. from the left auricular margin, the change in rhythm developed after 2·0-2·5 hr reached a maximum after 6 hr and persisted up to 23 hr. The post-ligation heart rate rarely exceeded 150-172 per min., about 15 per cent more than the control rate. It was thus a low frequency tachycardia, and even this continued only for a brief period (av. 4 hr). The lack of marked post-occlusion tachycardia in the pig could be due to the considerable vagal control, similar to what occurs in surviving human coronary thrombosis. The changes in the S-T segment and T waves in the electrocardiograms usually continued up to 9-48 hr after ligation.

Free wall and bundle branch blocks were frequently met with, showing bigeminal, trigeminal and other rhythms. The ectopic ventricular low frequency tachycardia in the domestic pig was quicker in onset, short-lived in duration and subsided earlier. Although the porcine experimental arrhythmia is similar to its human counterpart in the sequence of infarction followed by arrhythmia, it is multifocal and possesses a rate close to the sinus rate. Further, it is self-limiting and does not often prove fatal if the occlusion is left unreleased. No gross changes were seen in the heart for periods of ligation under 15 min. Maximum changes occurred when the occlusion lasted for 20 min. or more. The infarcted area was definitely demarcated from the normal on the tenth day. In the five and six weeks' infarcts the scar was remarkably thinned out and fibrosed. Usually by about the sixth week, the infarct was replaced by granulation tissue and finally by a collagenous scar.

After this was accomplished, Indian domestic pigs served as the experimental animals and the criterion for judging the beneficial effect of drugs was improvement in histopathology of homogeneous myocardial infarction produced by two-stage coronary artery ligation according to the technique mentioned above.

Neurohormonal and enzymatic changes during myocardial infarction in the pig heart, brain and adrenals were investigated to get an insight into the mechanisms of this process. These included estimations of catecholamine, 5-hydroxytryptamine, histamine, acetylcholine and enzymes like monoamine oxidase. Indirect evidence about the role of other enzymes, hormones or amino acids involved was obtained by seeing if administration of monoamine oxidase inhibitors, diamine oxidase inhibitors, catechol-O-methyl transferase inhibitors, decarboxylase inhibitor, reserpine, taurine, bradykinin, aldosterone, thrombolytic agents and Rastinon produce any beneficial or harmful effect in experimental myocardial infarction.

Clinical Studies

The next part of the study[19] was biochemical estimations of neurohormones and corticoids in patients of myocardial infarction to correlate with the experimental data, specially those which are connected with emotional stress.

Arora and coworkers have demonstrated that the concentration of catecholamines, 5-hydroxytryptamine and acetylcholine markedly decreases from the area of myocardial infarction in pigs over a period of few days. Russel *et al.*[20] also reported that the infarcted areas produced by coronary artery ligation in dogs lose about 75 per cent of their normal catecholamine within 24 hr. These studies are also compatible with the observation of Gazes and associates[21], where they indicated that plasma catecholamines are increased after acute myocardial infarction and also in angina pectoris after exercise. Moreover, Arora *et al.* have shown that urinary 5-hydroxyindoleacetic acid and catecholamine were increased in patients of acute myocardial infarction.

Arora and coworkers have found that the normal urinary excretion of 5-HIAA in healthy male adult ranged between 1·87 and 8·75 mg. (mean 3·82 mg.) per 24 hr urine and catecholamines ranged between 42·34 and 72·50 μg. (mean 58·03 μg.) per 24 hr

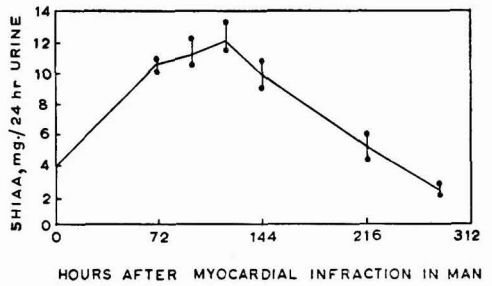


Fig. 3 — 5-Hydroxyindoleacetic acid content in 24 hr urine of the patients of myocardial infarction

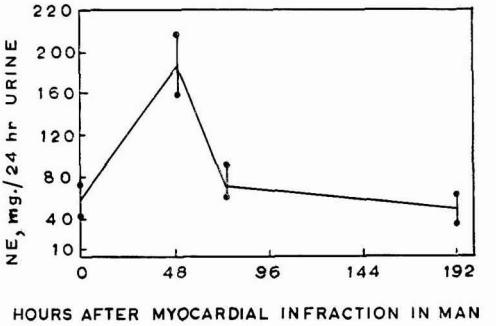


Fig. 4 — Norepinephrine content in 24 hr urine of the patients of myocardial infarction

urine. These investigations demonstrate a definite increase in the urinary excretion of catecholamine and 5-HIAA in all the patients at different intervals following infarction (Fig. 3). The maximum increase of catecholamines in urine occurs between 36 and 48 hr after infarction and returns to normal levels within 72 hr. However, it is after 72 hr of infarction that the urinary 5-HIAA level begins to register a rise reaching the peak levels in 120 hr and returns to normal after 264 hr.

The determination of urinary 5-HIAA and catecholamine levels in patients of myocardial infarction, which is technically easier to estimate with the least disturbance of the patient, represents an additional advance in the laboratory diagnosis of the condition. Thus demostration of elevated catecholamine levels during the first few days after acute myocardial infarction and followed by subsequent increase in urine of 5-HIAA in the proper clinical setting should serve as a highly valuable indicator of acute myocardial infarction.

During myocardial infarction, which is an emergency mechanism, there will be release of catecholamines as an alarm reaction[22]. This is a natural phenomenon which always takes place under conditions of stress and strain. As a consequence, there will be depletion of catecholamines; probably at first labile adrenaline and later even bound catecholamines also may be called for action. It appears from these studies that the sources of these increased levels of plasma catecholamines are probably the myocardial infarcted regions, which are deprived of their normal constituents of bound stores of norepinephrine and epinephrine (Fig. 4).

In recent years great deal of attention has been paid to kinins and their role in biological activity. It has been suggested that bradykinin is responsible for reactive hyperaemia. It has been claimed that in a weakly alkaline region between *p*H 7·5 and 8·5 the equilibrium lies with the inactive compound and a shift towards the acid side pushes the equilibrium to the side of the free component. But if such small *p*H changes would really affect the mechanism of kinin formation, then several problems can be explained. For example, acid metabolites such as lactic acid and carbon dioxide can bring about in the muscle during ischaemia, activation of kinins. It might be suggested that during myocardial ischaemia the acid products of catabolism are responsible for the activation of kinin from the enzyme and consequently increase in blood flow as well as for reactions like pain. Silva[23] suggested a relationship, still unexplained, between release of bradykinin and local discharge of catecholamines, as responsible for and expected consequence of its action. A vicious cycle might be then established, the increase in capillary permeability would cause more bradykinin to cross the capillary walls and more bradykinin would be released (Fig. 5). A suffusion of bradykinin locally discharged would dilate the blood vessels and help keep balance of the calibre of the smaller vessels. The above data give some support to the stress hypothesis as an important etiological factor of myocardial infarction and a more than casual relationship of catecholamine and related hormones with the clinical coronary artery disease.

Epinephrine, in addition to its action on carbohydrate metabolism, produces marked effects on fat metabolism. Cahill *et al.*[24] observed that both epinephrine and norepinephrine increase the incorporation of labelled glucose into glyceride, glycerol and increase the release of non-esterified fatty acids into the medium without affecting free fatty acid synthesis from glucose. These authors conclude that the primary action of catecholamine is to stimulate lipolysis.

ACTH, like epinephrine, increases lipid mobilization from isolated fat bodies. ACTH fails to

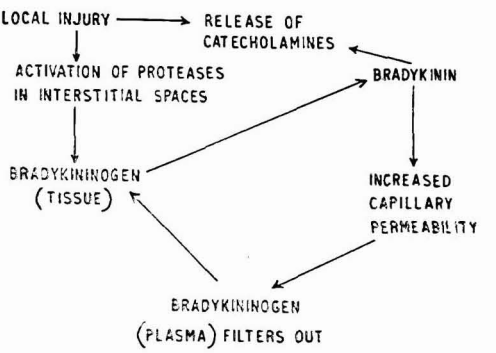


Fig. 5 — Vicious cycle at the site of injury

stimulate fat mobilization in adipose tissue of reserpinized animals, although it will do so in the presence of small amounts of epinephrine. This suggests that the action of ACTH on fat metabolism is dependent on the presence of epinephrine (or norepinephrine).

Role of Cortical Hormones

It has long been recognized that emotional stress significantly accelerates blood coagulation and there is reason to believe that this may be mediated not only by an augmented secretion of epinephrine but also possibly by adrenal corticoids. This latter view is supported by our findings of increased urinary aldosterone in patients of myocardial infarction[25,26]. The value was 2-3 times higher in acute uncomplicated myocardial infarction when compared with normal men. Sodium restricted diet and controlled posture in normal individuals caused a rise in the urinary excretion of aldosterone, but this rise was much less than that observed in myocardial infarction (Fig. 6).

Urinary aldosterone excretion in normal healthy dogs fed the same diet and later on in the same dogs with experimental myocardial infarction revealed similar increase in the absence of liver damage. Animal experiments also preclude the possible influence of drugs used in myocardial infarction on the level of urinary excretion of aldosterone. Therefore, extrapolation of the findings from animal experiments permits us to hypothesize that increased urinary excretion of aldosterone is due to myocardial infarction and not due to the associated factors, viz. diet, drugs and posture. Rise of sodium concentration in the infarcted myocardium appears to have a causal relationship with the aldosterone induced arrhythmia in myocardial infarction.

Role of Biogenic Amines

Arguing in the same way, Arora and coworkers have shown[27] the beneficial effect of a drug which raises biogenic amines, viz. nialamide, a hydrazide MAO inhibitor, in histopathology of experimental myocardial infarction in the pig.

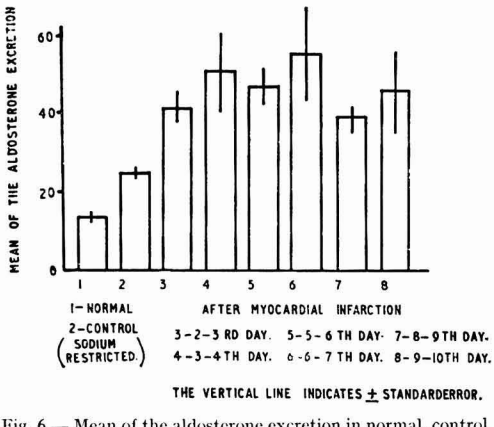


Fig. 6 — Mean of the aldosterone excretion in normal, control and myocardial infarction patients

In our studies, the hearts of the pigs after coronary ligation were examined at varying intervals from 6 to 30 hr. The earliest detectable pathological alteration was seen 6 hr after ligation. The heart muscle showed increased acid staining of the cytoplasm, vacuolation and changes in the nuclei. The myocardium showed hyaline changes and striations were lost. There was evidence of pyknosis, karyorrhexis and karyolysis. Nuclei had disappeared specially from the cells in the necrosed areas. Later on, the progressive myocardial infarction was present, e.g. leucocytic infiltration and fragmentation of muscle fibres and bundles. The nature of histological changes in the nialamide pretreated coronary ligated pigs is shown in Fig. 7.

In the case of other drugs, except glutamic acid-IH, there was no evidence of improvement in myocardial infarction. The criteria for judging myocardial infarction were oedema, presence of thrombosis, and the nature and degree of necrosis of myocardium (acid staining, fatty and hyaline degeneration of muscles, fragmentation of muscle bundles and leucocytic infiltration). Beneficial effects seen in arrhythmia, as shown in the electrocardiogram, and some reduction in the area of infarction after coronary artery occlusion in the four-day nialamide pretreated pigs indirectly indicated the presence of interarterial coronary anastomoses. However, absence of a definite transfer of injected material from the right to the left coronary artery in our experiments absolves functionally significant interarterial coronary anastomoses as a possible mechanism for the salutary effect of MAO inhibitors. The MAO inhibitors are known to cause coronary dilation in the isolated heart and in the intact animals. In our series of four-day nialamide pretreated pigs followed by two-stage coronary ligation, islands of cardiac tissue capillaries, showing some evidence of dilation, were observed histologically, but this finding may not be of great significance in the absence of direct evidence of coronary arterial collateral circulation in roentgenograms of the pig heart.

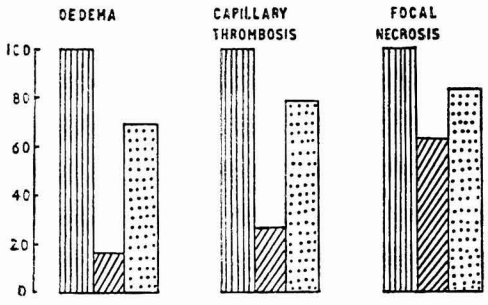


Fig. 7 — Quantitative assessment of the effect of nialamide and glutamic-IH [Pretreatment on myocardial infarction in the pig: Quantitative assessment of various characters were observed from oedema, thrombus in the coronary artery and histopathological study of the infarcted area 30 hr after coronary ligation. Vertical-lined columns represent those in the control untreated; oblique-lined, nialamide treated; and dotted, glutamic acid-IH treated animals]

In the light of the above, it may be inferred that amine oxidase inhibition plays an important role in exerting beneficial effects in myocardial infarction. But this does not appear to be the decisive or exclusive factor since glutamic acid-IH, which is a MAO inhibitor nearly equivalent in potency to nialamide, is only partly effective in myocardial infarction. It implies the existence of a pharmacologic effect of nialamide in addition to MAO inhibition which contributes to the ultimate response elicited.

However, inhibition of cardiac MAO to the extent of 98-100 per cent appears essential for demonstrable beneficial effect in histopathology of myocardial infarction in the pig by an MAO inhibitor like nialamide. This inhibition is associated with 95, 25 and 133 per cent increase in left ventricular (cardiac) norepinephrine, serotonin and adrenaline respectively. This preferential increase in myocardial catecholamine by a drug which produces a salutary effect is likely to implicate catecholamines for the beneficial effect seen in experimental myocardial infarction in the pig.

Studies have been made (unpublished work) on other types of enzyme inhibitors in experimental myocardial infarction. A catechol-O-methyl transferase inhibitor (viz. pyrogallol), decarboxylase inhibitor (viz. α-methyldopa) and diamine oxidase inhibitor (viz. isonicotinic acid hydrazide) were tried in equimolar dosages to nialamide, but they did not show any statistically significant salutary effect.

In ectopic ventricular arrhythmia complicating myocardial infarction, the observations[19] were of a similar nature. However, when the dosages of some of these enzyme inhibitors were increased, say 3-7 times, the molar equivalent of nialamide, the arrhythmia was reverted showing the complexity of the problem.

The effect of nialamide on ectopic ventricular arrhythmia appears to be markedly due to MAO inhibition and also to its capacity to raise the levels of catecholamines, although direct pharmacological effects of these drugs, in view of the similarity of the fit of the hydrazide to epinephrine which are explained later, cannot be entirely ruled out. The experiments in which the animals were pretreated with reserpine before the production of ectopic ventricular arrhythmia, resulting from coronary ligation, for testing the anti-arrhythmic effect of nialamide, support the view that the accumulation and/or replenishment of catecholamines in the heart plays an important role in the beneficial effect. The action appears to be rather specific, since nialamide does not revert either ouabain induced ventricular arrhythmia, aconitine induced auricular arrhythmia or injury stimulated auricular flutter in dogs. Further, the salutary anti-arrhythmic effect of these drugs seems to be unrelated to diamine oxidase inhibition or increase in blood levels of 5-hydroxytryptamine, dopamine or tyramine. The hydrazide moiety appears to be a requisite *sine qua non* for anti-arrhythmic activity as MO-911 (Pargyline) demonstrated no beneficial effect unless it was administered in seven times the molar equivalent dose of nialamide.

Catechol-O-methyl transferase inhibition produced by these drugs appears to play some role since pyrogallol and iproniazid were equipotent anti-arrhythmics; however, they were both much inferior to nialamide which implicates a particular chemical moiety and/or possible anchoring groups present in this drug as important additional potentiating factors explaining its superiority. Finally, the significance of accumulation of taurine, ethylamine-2-sulphonic acid during MAO inhibition has been discussed as a possible mechanism of beneficial effect by MAO inhibitors.

As mentioned earlier, the anti-arrhythmic action of MAO inhibitors in arrhythmia resulting from myocardial infarction in dogs can be annulled by pretreatment with reserpine. Further, reserpine pretreatment increases the mortality in dogs and pigs when it precedes experimental myocardial infarction stress more than a casual relationship between catecholamine depletion and development of myocardial infarction. It is now established by extensive biochemical and pharmacological studies that iproniazid and other similar MAO inhibitors markedly change the metabolism of biological amines, catecholamines, serotonin and other amines. As regards the role of MAO inhibitors, several possibilities exist for explaining their mechanism of action. These are explained below.

Catecholamine Hypothesis

It has been shown by Pletscher and Pellmont[28] that there is a marked rise in norepinephrine content of the mammalian heart after iproniazid administration and that coronary flow is increased. In sharp contrast, Bing found that iproniazid administered to dogs produces no alteration in coronary flow, myocardial usage of oxygen or coronary sinus oxygen, and catecholamines have an adverse effect in angina pectoris. That catecholamines have an adverse effect in atherogenesis and myocardial infarction has been supported by others.

This need not be an insuperable objection if the hydrazines inhibit the release of stored epinephrine or the formation of a harmful metabolic derivative of the hormone. The absence of a deleterious effect despite the increased content of total norepinephrine in the heart needs no explanation. Brodie and Shore[29] report that both 5-HT and catecholamines are bound to proteins and thus are protected against the action of monoamine oxidase system. The catecholamines are stored in the granules of the cell and are liberated only during stimulation or under the influence of reserpine. Similar conclusions were also reached by Blaschko and coworkers[30].

The action of acetylcholine and 5-hydroxytryptamine can be ascribed to the liberation of catecholamine by them. At about the same time that O-methylation was being established as the major means for the metabolic inactivation of circulating catecholamine, other evidence accumulated indicating that MAO does play an important role in the metabolism of endogenous catecholamines. From these results it may be concluded that the readily released portion of stored norepinephrine is enzymatically destroyed by O-methylation, either in the tissues or after having reached the circulation

whereas the more firmly bound norepinephrine was deaminated. In any case, the primary effect of MAO inhibitors in increasing catecholamines in tissues and in preventing depletion of amines by reserpine is blockade of MAO. This is further supported by the fact that reserpine releases the firmly bound portion of norepinephrine.

On the basis of statistically significant data[31] obtained from human and animal experiments, Arora and coworkers have concluded that a decrease of cardiac catecholamine and serotonin to unmeasurable levels within a few days after experimental myocardial infarction in the dog and pig, and an increase in the urinary excretion of these amines in these laboratory animals and in patients of myocardial infarction, point to heart as the probable source of depletion, specially in the absence of any significant change in brain, adrenal and intestine amine levels. It is suggested that MAO inhibitors which preferentially raise cardiac monoamines may be advocated as a logical approach to therapy in patients of myocardial infarction, and increased urinary excretion of 5-hydroxyindoleacetic acid and catecholamine when they occur together with the proper clinical setting be viewed with caution as forerunners of ischaemic heart disease.

Adrenergic fit — In further support of the catecholamines and related amine and stress thesis, I may put forward the theory of adrenergic fit to account for the beneficial effect of drugs which raise these amines in myocardial infarction. Iproniazid competes with norepinephrine at the receptor site on smooth muscle in the blood vessels. Also, it has been shown that iproniazid changes norepinephrine sensitivity of the rabbit blood vessels pretreated with MAO inhibitors. Further tests on contractility of the aorta, in response to norepinephrine *in vitro*, compared with controls showed that the contraction of the aorta was markedly depressed in iproniazid treated animals. This could be an adrenolytic effect. Zeller[32] has postulated an adrenergic blocking effect for iproniazid because of the similarity of the fit of the hydrazide to norepinephrine. Similar fit for nialamide has been postulated by Arora and coworkers (Fig. 8), which envisages that the effects of release of catecholamines during conditions of stress and other conditions are likely to be blocked. In this connection the infrared spectrum of nialamide has been studied for some indications of the possible anchoring groups present in it.

Anchoring group — It is now widely recognized that the biological activities of a drug are considerably influenced by its physico-chemical features. Such features may include the nature of the electrical characters of different portions of the drug molecule, hydrogen bonding, if present, and the overall molecular shape. The contribution of these forces to the biological activity becomes particularly significant in the case of drugs which exert their action by competing with a normal physiological component for an enzyme or receptor surface. A most useful method of gathering information regarding such subtle physico-chemical features of a molecule is to study its spectral characteristics. Ultraviolet and infrared spectral data of drug molecules offer

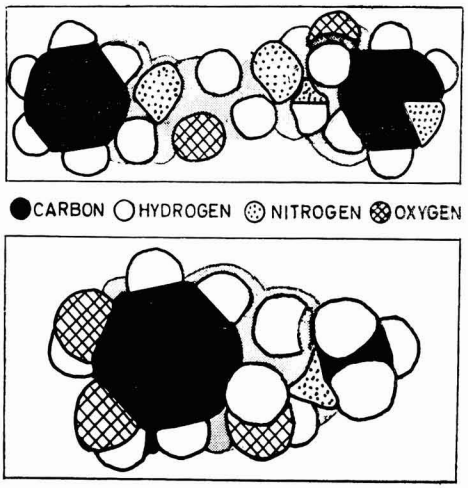


Fig. 8 — Adrenergic fit of nialamide

valuable information which has very often a direct bearing on the biological activities. From this point of view the infrared spectrum of nialamide shows two NH frequencies at 3325 and 3250 cm.$^{-1}$. The presence of two NH frequencies show that there are two types of NH groups in the molecule, one of them being associated (i.e. hydrogen bonded) either intramolecularly or intermolecularly. The peak with the lower frequency is to be attributed to the associated NH frequency and it is more intense than the other NH frequency. This shows that two NH groups (which must include at least one of the amide functions) are involved in some type of hydrogen bonding. The higher NH frequency may be due to the third NH which at any rate is less bonded than the other two. From this it may be inferred that nialamide probably forms hydrogen bonds with some suitable surface through some of its NH groups. In this connection it may be noted that iproniazid is also known to form hydrogen bonds and metal chelates (as a consequence of the former). However, this cannot be taken as conclusive evidence for any intramolecular hydrogen bonding.

Platelet clumping, ATP and catecholamine — Much evidence is available concerning the atherogenic effectiveness of epinephrine and norepinephrine both in animals and man; this effectiveness is enhanced by thyroid hormone, and, in general, emotional sympathetic over-stimulation may be assumed to be damaging the arterial walls.

In the adrenal medulla catecholamines are localized in cells, designated as chromaffin cells (their property of being brown or black in the presence of chromate and possessing the sedimentation characteristics of mitochondria; Blaschko *et al.*[33]). The ATP content of adrenal medulla tissue (approx. 5 mg./g. tissue) is higher than that of any tissue yet studied and also ATP content per g. protein of adrenal chromaffin granules is several times

greater than any intracellular particles examined. This fact coupled with the fairly constant ratio of ATP to catecholamine in the granules and the unusual stability of ATP has led to the conclusion that ATP may be functioning as the anion in a salt complex with the amines. At *p*H 7·4, a 4:1 complex of catecholamines and ATP exists in a variety of animals[34]. These suggest that ADP and AMP may also play some role in this complexing. After stimulation of the adrenal gland, there is a depletion of ATP without a proportionate increase in ADP and AMP *in vivo*[35]. The spontaneous loss of catecholamines from adrenal granules is associated with a proportional loss of ATP *in vitro*.

Virtually, nothing is known about the mechanism of catecholamine release from tissues other than the adrenal medulla. The intimate storage mechanism in granules of catecholamines, ATP and perhaps other molecules remains a matter for conjecture. The role of ATP in the release of amines from storage sites and the metabolic fate of ATP have thus far escaped solution, as has the mechanism of release induced either by nerve stimulation or by chemical agents.

When agglutination occurs, the platelets become closely applied to one another, but the actual physical nature of their adhesion is unknown. Platelets are known to contain considerable amounts of adenosine triphosphate, and it has recently been shown that adenosine diphosphate causes their agglutination. These observations have been carried one stage further and it has been shown that adenosine monophosphate inhibits the agglutinating action of adenosine diphosphate. Investigations of the properties of the platelets by biochemical means correlated with observations in the electron microscope are proceeding apace, and it is conceivable that within a few years we shall have a clear picture of what happens during the interesting process of thrombus formation.

However, any attempt to devise an effective blocking agent is profoundly handicapped by the fact that it is not known what unique features of the platelet surface are responsible for its 'stickiness' to surface or in what way ADP or the other possible aggregating compounds alter the platelet surface to permit platelet-to-platelet adhesion. Furthermore, the mechanisms thought to be involved in platelet aggregation are concerned in many other vital processes. Evidently, it will be difficult to find a therapeutically active inhibitor, but efforts in this direction appear worth while. The present scheme envisages work in this direction.

Possibly, with an effective drug, the damaged endothelial surface could be rendered benign and non-attractive to the platelets; in this connection nialamide has been shown[36] in some special circumstances to be effective. Arora and coworkers have confirmed these claims and have shown that a dose of 5 mg./kg. of nialamide in rabbits increases the platelet clumping time from 50 to 84 sec. Further, daily administration of nialamide did not cause much further rise in the platelet clumping time. After the withdrawal of the drug, platelet clumping time reached normal limits in 4 days. The drug was devoid of any toxicity. Support for this viewpoint was given by Maschouf *et al.*[37] who have shown that the inhibitory action of nialamide on thrombosis is presumably achieved by diminishing the platelets' contribution to this process.

Concluding Remarks

World statistics reveal that CHD is the civilized man's greatest cardiovascular problem. This disease claims the life during the most productive period of a person's life — period during which his talents are mature and can be of use to his family, his profession and his nation. During the past few years, intensive analysis of the etiological factors has increased our knowledge regarding the pathogenesis and possible prevention of atherosclerotic coronary heart diease.

I have already emphasized the importance of primary prevention and it is clear that a check can be maintained over some of the claimed but not proved causative and predisposing factors like diabetes, hypercholestrolaemia, hypertension, excessive smoking and overindulgence in very rich food. I have also shown the relative importance of the enzyme monoamine oxidase vis-à-vis catechol-O-methyl transferase, dopa decarboxylase and diamine oxidase, and the drugs which inhibit them with respect to their salutary effect or otherwise in experimental coronary infarction and the role of aldosterone in the biochemical pharmacology of myocardial infarction. Though the modification of all these factors appears to play a significant role in the etiology of myocardial (coronary) infarction, the major causative or predisposing factor appears to be neurohormonal, with special reference to depletion and/or destruction of cardiac catecholamines and related neurohormones released due to mental stress, which in turn produce changes not only in the coagulation factors leading to atherogenesis but also to consequent coronary insufficiency and so-called clinical heart attack.

In further support of the neurohormonal concept I quote the unpublished observations of my group made over a number of years on the role of environmental factors and coronary disease in laboratory animals at the All-India Institute of Medical Sciences and earlier at Sawai Man Singh Medical College, Jaipur, with special reference to the pig which show that emotional stress has an important role in the development of myocardial infarction. This is corroborated by a study by workers from abroad on other species of animals. For example, the frequency of coronary disease in mammals at the Philadelphia Zoological Garden has increased about tenfold within recent years. The greater part of this increase came between 1955 and 1962, during which period deaths from myocardial infarction also became relatively common, whereas none had occurred earlier. Furthermore, this animal collection has been fed on highly constant diets of high quality since 1935. Accordingly, an increase in the frequency of coronary disease in these animals may not be attributed to food intake nor to the type of diet. Here, then, are examples of coronary disease that have developed without reference to diet or to sex in a wide range of species[38].

One may object, of course, that observations on the effects of intraspecific interactions of caged animals in a zoo or laboratory cannot be applied directly to 'free-ranging creatures', or even to urban man. Careful study of natural populations, however, shows that the concept of the 'free-ranging creature' is a myth. Potential and actual home range depends upon individual aggressiveness and population density. The development of coronary arteriosclerosis in animals of wild populations apparently is a response to relatively intense intraspecific interactions.

Here a question may be asked with respect to aldosterone (in the absence of published data on other forms of stress) which Arora *et al.* have shown to be increased in patients of myocardial infarction. In this connection, Jensen *et al.*[39] have shown that the urinary excretion of certain 2,3,5-triphenyltetrazolium chloride reducing substances which are soluble in ether increases in healthy persons during emotional stress. This fraction (TZR) probably contains hydrocortisone, corticosterone, aldosterone and aldosterone derivatives as well as other substances. Thus, it is obvious that TZR is a sensitive new corticoid fraction which is activated by rather subtle psychosomatic changes.

On the basis of the experimental and biochemical investigations carried out by Arora *et al.*, the factors which play a part in CHD can be connected with the unitary concept of emotional stress and the resultant neurohormonal imbalances as the dominant factor in the etiology of CHD. This presumption is based on our results showing that body mechanisms or substances present within or extraneously given, which prevent or block the release or depletion of catecholamines and related amines, are very likely to produce beneficial effect in CHD. To say it in a simplified way, heart needs a certain optimum level of catecholamines and related amines for its normal functioning as we need certain optimum amount of resources for our maintenance. Heart cannot afford to lose such great amount of catecholamines and other amines as actually happens during CHD as shown by experiments and it suffers if it is robbed of them. But if we can prevent the usurper from doing so we feel sound and safe. For example, when MAO inhibitor prevents MAO from destroying the vital amines of the heart, i.e. catecholamines, etc., it prevents the catastrophe. But this is not as simple as that of vaccines preventing polio or antibiotics preventing infectious diseases whose root causes are known. On the contrary, nothing is known as to the cause of CHD.

Acknowledgement

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Symposium on the Chemistry of Natural Products

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ALMOST successful international symposium to discuss the Chemistry of Natural Products was held in Kyoto, Japan, from 12 to 18 April 1964, under the auspices of the International Union of Pure & Applied Chemistry and was organized by the Science Council of Japan. The meeting was the first of its kind in Japan and was truly international as it was attended by some 1250 delegates of whom 242 were from different parts of the world. An impressive feature of the conference was the efficiency with which the conference was organized and the delegates had every reason to be grateful to the Organizing Committee and particularly to Dr K. Tsuda, General Secretary, and to the Moderator, Prof. K. Nakanishi, and their colleagues for their untiring efforts for the successful conference arrangements.

The welcome addresses by Prof. M. Kotake, Chairman, Organizing Committee, and Prof. S. Tomonaga, President, Science Council of Japan, were followed by a lecture by Prof. S. Sugasawa on the ' History of Japanese natural products research '. The contributions of the three great pioneers of Japan in the field, Dr W. N. Nagai (1845-1929), Dr U. Suzuki (1874-1943) and Dr R. Majima (1874-1962), and of their schools formed the main topics of his talk. Prof. Sugasawa elaborated the work of Prof. Y. Asahina, the famous natural product chemist, who has still been pursuing researches in this field, although he is more than 80 years old.

Special Lectures

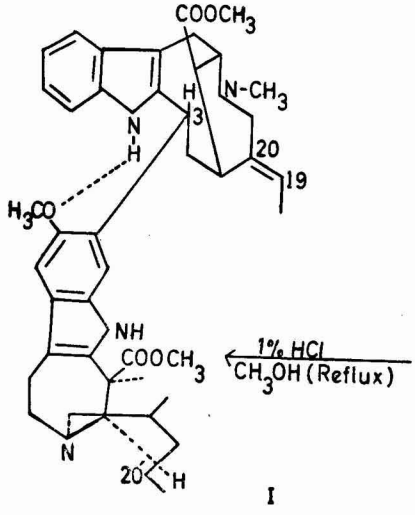
Ten special lectures were arranged during the symposium. A brief summary of the lectures is given below.

Chemistry of dimeric indole alkaloids — Prof. G. H. Büchi discussed in detail the fascinating chemistry of this group of complex molecules with special reference to voacamine (I) the structure elucidation of which was done in his laboratory and also by mass spectrometric studies carried out by Prof. K. Biemann at the Massachusetts Institute of Technology, USA.

Biogenesis of plant products — In the next lecture Prof. D. H. R. Barton dealt mainly with the mechanism of biogenesis of morphine and aporphine alkaloids and considered the coupling of phenolate radicals as an important step in the formation of these alkaloids. It was proposed that reticuline (II) and salutardine (III) are the two important intermediates in the genesis of morphine bases. Reticuline on phenolic oxidation gives the dienone; salutardine, which on reduction and dehydration furnishes thebaine, the precursor of codeine and morphine. The validity of this scheme has eventually been verified with the help of radioactive tracers.

The fairly recent discovery of dienol-benzene rearrangement and isolation of new class of proaporphine bases, viz. crotonosine, pronuciferine, etc., indicate that phenolic coupling is also operative in the genesis of aporphine bases.

Structure of tetrodotoxin — Prof. R. B. Woodward gave a talk on the structure of tetrodotoxin. Tetrodotoxin is the poisonous principle of certain variety of puffer fish (swell fish) (*Spheroides porphyres* and *S. rubripes*). The structure (IV) was assigned to this complex molecule, $C_{11}H_{17}O_8N_3$, by degrading the toxin into simple identifiable products (guanidine and a number of quinazoline derivatives are a few of many such important



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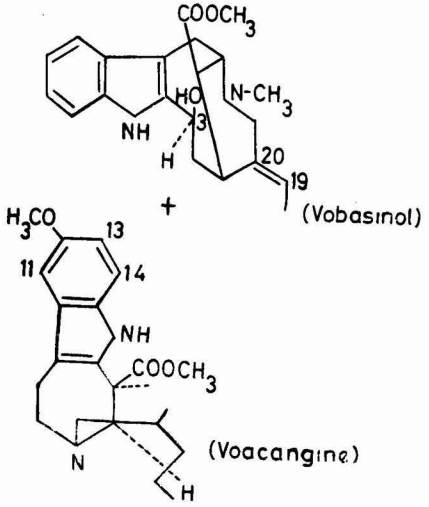

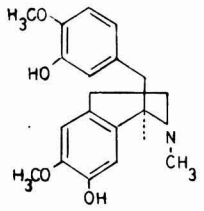

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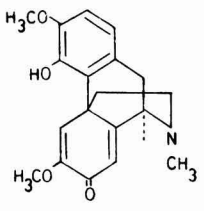

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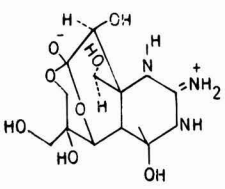

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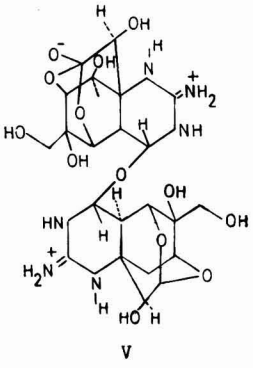


V

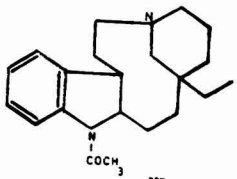
degradation products) and from a detailed NMR spectral study of these simple molecules, their chemical transformation products and deuterium labelled derivatives and also from X-ray crystallographic analyses of the derivatives of tetrodotoxin. The alternative suggestion by Dr K. Tsuda and his collaborators that the toxin possesses the structure (V), in which two units of (IV) are joined together by an ethereal oxygen, was given a serious consideration by Prof. Woodward who, however, established the improbability of such a formulation by single crystal X-ray diffraction studies of the free base.

Intramolecular rearrangements in peptide system — Prof. M. M. Shemyakin gave a talk on intramolecular rearrangements in peptide system and on the hydroxy and aminoacyl incorporation on a large number of linear and cyclic peptides, acylated by various hydroxy and amino acid residues. In the majority of cases the hydroxy and aminoacyl incorporation reaction takes place spontaneously and only in isolated cases requires heat or the presence of bases. The structures of the resultant linear and cyclic depsipeptides, cyclopeptides and peptides were proved with the aid of their infrared, ultraviolet, NMR and mass spectra and in certain cases by counter synthesis.

High resolution mass spectrometry — Prof. K. Biemann spoke on high resolution mass spectrometry. Mass spectrometric technique, in spite of its glorious success in the solution of structural problems, is not yet a self-sufficient device. Its chief limitation lies in the relatively poor resolving power of the conventional single-focusing mass spectrometer, as such an instrument fails to differentiate between the fragments which have the same nominal mass but which differ by fractional mass units (e.g. CO versus C_2H_4; CH_2 versus N). Further, in the conventional technique only the intense peaks are taken into consideration and the potentialities residing in the minor peaks are almost altogether ignored. Prof. Biemann realized these limitations of the conventional procedure and has developed a new technique, high resolution mass spectrometric technique, which has not only added a new dimension to mass spectrometric information but promises to be a self-sufficient device in structural investigation. The simplicity, advantage and precision of this newly developed technique were illustrated by his work on the structure of alkaloids of various structural patterns and other natural products.

Specification of stereospecificity of oxido-reductases by diamond lattice sections— Prof. V. Prelog, Switzerland, spoke on the specification of stereospecificity of some oxido-reductases by diamond lattice sections. It has been observed that multipurpose enzymes such as oxido-reductases often react with a high degree of stereospecificity. In order to ascertain what features in a given molecule are responsible for these stereospecific reactions, a large number of optically active substrates were used and the initial reaction rates of enzymic reduction or oxidation were determined under comparable conditions with a standard series of cyclohexane and decalin derivatives of known absolute configurations. The carbon skeletons of these substrates which are built from the chair form of cyclohexane are regarded as portions of a diamond lattice. By superposing the skeletons of all the substrates which react at a measurable rate with the enzyme in question, and also by using the same position for the reacting carbon oxygen atoms, a diamond lattice section characteristic of the enzyme is obtained. This section, according to Prof. Prelog, defines the space, in the transition state of the reacting complex, not occupied by the enzyme, coenzyme, inhibitors or tightly bound solvent. A consideration of the characteristic diamond lattice section, and the skeletons of the substrates which do not react, then furnishes important information concerning the steric hindrance in the complex. Experimental evidence supports this contention and, therefore, the characteristic diamond lattice section can, with reservation, be used to predict whether a particular substrate will react with the enzyme in question. Prof. Prelog suggests that the enzyme protein plays a decisive role in determining the stereospecificity.

Progress in the synthesis of polycyclic natural products—Prof. G. Stork described new approaches of some generality to the construction of polycyclic natural products, viz. steroids and alkaloids. The main topic of interest was the total synthesis of aspidospermine (VI). The first part of this investigation



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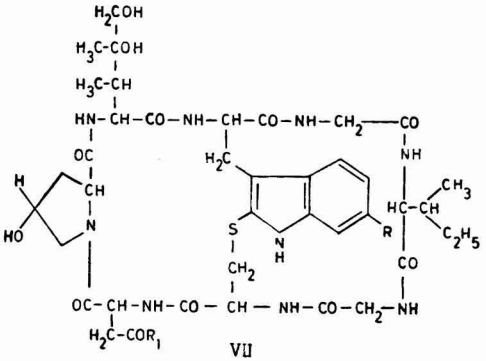

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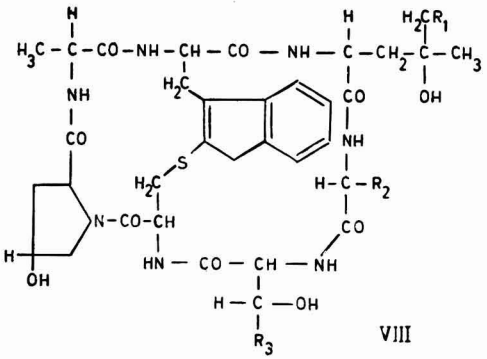


VIII

αC-Amanitin: R=OH, R_1=NH_2
β-Amanitin: R=OH, R_1=OH
Amanin: R=H, R_1=OH IX

Phalloidin: R_1=OH, R_2=CH_3, R_3=CH_3
Phalloin: R_1=H, R_2=CH_3, R_3=CH_3
Phallacidin: R_1=OH, R_2=$CH(CH_3)_2$, R_3=COOH

consists in the synthesis of a tricyclic pyrrocoline system on which the Fischer indole synthesis proceeded with the desired result. The starting materials for the first component en route to aspidospermine were enamine of butyraldehyde and ethyl acrylate. The resulting cyclobutane derivatives readily opened upon treatment with acid to the expected glutaric half aldehyde. The enamine alkylation reaction was then repeated again with the product that eventually led to the formation of the hydroquinolone. The addition of a third ring to the last bicyclic system was achieved by following the process of annelation as applied in the steroid field. The Fischer indole synthesis on this product followed by reduction (LAH) and acetylation gave the desired DL-aspidospermine.

Peptides of Amanita phalloides—Prof. Th. Wieland spoke on the peptides of *Amanita phalloides*. *A. phalloides* is one of the poisonous species of tuber leaf mushrooms. Structure elucidation of amanitines (VII), phalloidines (VIII) and amanin (IX), toxic cyclopeptide indole derivatives, isolated from these mushrooms, formed the subject matter of his lecture.

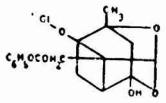
Isotope labelling and mass spectrometry of natural products — Prof. C. Djerassi gave a talk on isotope labelling and mass spectrometry of natural products. Application of mass spectrometry to structural problems in organic chemistry requires a reasonable prediction about the principal fragmentation paths of a given molecule upon electron bombardment. From the nature of the survived charged fragments and the neutral moieties that are lost, the mechanism of molecular fragmentation in a mass spectrometer can be elucidated. While there are many occasions where the nature of the generated fragments can easily be understood by simple consideration of the masses of the charged fragments, there are others, specially when heteroatoms are present, where this is not feasible because of the occurrence of isobaric fragments (e.g. CH_2 versus N). There are, however, three ways to overcome this difficulty: (i) substituent labelling, (ii) isotope labelling, and (iii) high resolution mass spectrometry. Of these, substitution of one of the constituent atoms by its isotope (usually D, ^{13}C, ^{15}N or ^{18}O) offers the most meaningful information. In view of the availability of many convenient methods and reagents for deuterium transfer in an organic molecule it is quite likely that deuterium labelling will soon become a *sine qua non* of modern mass spectrometric research.

Recent advances in X-ray analysis of natural products — Prof. J. M. Robertson spoke on recent advances in X-ray analysis of natural products. Of all the various physical methods that are now so important in helping to elucidate chemical structures, X-ray analysis is undoubtedly the most far-reaching and complete, but it is still perhaps the most difficult to apply. Prof. Robertson pointed out the difficulties in X-ray analysis of complex organic molecules and mentioned the progress that has so far been made in this line. He also mentioned that the most powerful and indeed the only approach that has proved successful in solving complex natural product structures by X-ray analysis has been the heavy atom or occasionally the isomorphous substitution method. The first application of both these methods to the direct solution of structures of organic molecules was in the case of Linstead's phthalocyanin compounds which contained about 40 carbon or nitrogen atoms in crystallographically unknown positions. He gave an account of his work on various natural products where structure elucidation has been possible exclusively by X-ray analysis.

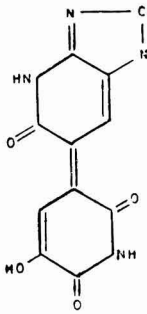
Other Papers and Discussions

Apart from these main lectures, about 240 papers were presented at the symposium, which were grouped into eleven sections. Some of these are summarized below.

Prof. S. Shibata elegantly elucidated the structure and stereochemistry of paeoniflorin (X), a monoterpene glucoside and a major constituent of Chinese paeony root. Dr H. Minato showed that linderane, a new crystalline component occurring in the root of *Lindera strychnifolia* Vill., is the first example



X



XI

of a ten-membered sesquiterpenic lactone having a furan ring. Dr V. Herout elaborated the structure of a hydrocarbon from *Petasites officinalis* Moesch and other species which is the first representative containing an eremophilane skeleton isolated from natural sources. Mme J. Polonsky discussed the structure of samaderine C and samaderol and their relationship with several bitter lactones from other Simarubaceae which is important from biogenetic viewpoint. The work of Dr T. Kubota on enmein and that of Dr S. Natori on new triterpenoids of rearranged hopane series from ferns deserves mention.

On biologically active substances and heterocycles several papers were read. Of these the most stimulating ones were the structure elucidation and synthesis of cytohaemin (porphyrin derivative), the prosthetic group of cytochromea and 'Atmungsferment' using material from horse heart muscle by Prof. F. Lynen and that of a bacterial pigment (XI) (*Corynebacterium insidiosum*) derived from $\beta\beta'$-dipyridyl by Prof. R. Kuhn.

Dr A. W. Johnson gave a talk on viomycin, an antibiotic isolated from *Streptomyces puniceous* and *S. fradiae*, and Dr S. Tatsuoka on chromomycin A_3.

The symposium showed great interest in the chemistry of alkaloids including their biosynthesis. Prof. T. A. Geissman discussed the structure of an aporphine alkaloid, guatterine, isolated from Anonaceae species, *Guatteria psilopus*, having the unusual 11-position for hydroxyl. Structural elaboration of several new aporphine and benzylisoquinoline alkaloids from 5 *Fagara* species of Rutaceae by Prof. V. Deulofeu revealed interesting data. The contribution of Dr M. Gorman was towards the structure determination of perivine (vobasine structure) and vindolicine (belonging to aspidospermine series). Dr K. Bláha discussed the structures of vincaminorine (ind-N-methyl-3-α-carbomethoxy quebrachamine) and its epimer, vincaminoreine, occurring in *Vinca minor* L. Total discussion on stereospecific synthesis of securinine by Dr Y. Tamura, on diterpene alkaloids, viz. DL-atisine, by Dr W. Nagata, and garryine by Dr S. Masamune revealed many novel reactions. Dr N. Matsume elaborated the structure of a diterpene alkaloid, kobusine and its correlation with pseudokobusine. Rauwolfia species, work on which appeared to be completely exhausted, was still found to produce a new yohimbinoid type alkaloid, deserpideine, the constitution of which was elaborated by Dr M. Shamma. Dr R. Goutarel spoke on steroid alkaloids of *Funtumia* and on alkalo ds baleabuxine and cyclobaleabuxine from *Buxus belearica* Willd., and Dr G. Habermehl on the study of the constitution by X-ray analysis of cycloneosamandiones and other minor alkaloids isolated from the venom of the two subspecies of *Salamandra maculosa*.

The contributions on the biogenesis and biosynthesis of alkaloids, their precursors and the probable mode of combination of these units were also significant. For example, the biosynthesis of anabasine and its analogues was discussed by Dr K. Mothes by different botanical families, viz. Leguminosae, Solanaceae and Chenopodiaceae, and the genesis of morphine bases, the tobacco alkaloids and iboga alkaloids were dealt with lucidly by Dr A. R. Battersby, Dr T. C. Tso and Dr E. Leete respectively, and biosynthesis of mimosine by Dr I. D. Spenser. These and similar other contributions in this field that were discussed in the symposium may, in near future, divulge many intricacies of nature.

Several lectures and papers dealing with the synthesis, structure determination and stereochemistry of steroids and steroidal reactions were presented. The papers on the microbiological transformation of steroidal alkaloids by Dr S. M. Kupchan, investigation of the hydride transfer reaction involved in the isomerization of steroidal sapogenins at C-25 by Dr F. Sondheimer and the structure of digacetigenin by Prof. C. W. Shoppee, and transformation of steroid toad poisons by microorganisms by Dr CH. Tamm showed how fast this branch of organic chemistry is progressing.

Significant contributions made in the field of amino acids and peptides and in the nucleosides include those of Dr A. Kjaer — occurrence of D-phenyl glycine in higher plants; Dr J. Kovacs — polypeptides related to anthrax polypeptide; Dr J. C. Sheehan — the chemistry of telomycin; and Dr J. J. Fox — the structure of gougerotin (a broad spectrum antibiotic where the sarcosyl group is attached in peptide linkage to the seryl moiety). Dr T. L. V. Ulbricht, Dr Y. Mizuno and Dr Y. Ishido spoke on the synthesis of various nucleosides.

In the carbohydrate section several interesting papers were presented. Synthesis of aminocyclitols by Dr N. Kurihara, the development of a new route to higher sugars and the structure elucidation of oligosides (new type of plant glycosides) by Dr N. K. Kochetkov, characterization and synthesis of neohesperidose from *Citrus* by Dr R. M. Horowitz, and structure elucidation and configurational studies of polysaccharides from *Polyporus giganteus* by Dr B. Lindberg deserve mention.

In sessional meetings on phenolics Dr G. Bendz reported the occurrence of anthocyanin pigments in moss. Dr J. D. Bu'Lock discussed the origin and function of some fungus pigments one of which is hispidin and Dr W. D. Ollis reported the

existence of the derivatives of 3-aryl-4-hydroxycoumarin and coumarinochromone as natural products (in the heartwood of *Derris robusta*). Dr K. Fukui spoke on the total synthesis of nepseudin isolated from *Neorautanenia pseudopachyrrhaiza*. Dr J. Craig discussed the isolation and stereochemistry of the antibacterial principle from *Haematoxylon braziletto*. Dr T. J. Mabry gave a talk on phenolics occurring in the genus *Baptisia* (Leguminosae). The observations made in this connection have an important significance in chemotaxonomy.

Another thread which was running through the symposium was the application of physico-chemical methods in structure determination of complex natural products where classical approach and ultraviolet, infrared and NMR spectra could not give the final answer. Dr K. Tsuda, Dr N. Yasuoka and Dr T. Goto deserve commendation for the successful application of X-ray method in the structure elaboration of a complex natural product, tetrodotoxin (the poisonous principle of the ovaries of swell fish) and its derivative, bromoanhydrotetrodoic lactone hydrobromide. Dr M. Natsume spoke how the X-ray study of acetyl-bromoacetyldihydroenmein helped to elucidate its constitution and stereochemistry. Dr K. Schreiber gave an elegant survey of molecular mass spectrographic investigations of a number of sterols, steroidal sapogenins and alkaloids, triterpenoids, some of their derivatives and other plant products. He discussed how molecular weight distributions in complex mixtures of closely related compounds have been accurately determined by application of negative ion molecular mass spectrography. Dr M. Fetizon described a novel method for the characterization of ring A in steroids and related compounds by mass spectrometry. Dr T. Urbanski spoke on the tautomerism of urea and presented evidence for its imidol form which reacts with diazomethane.

Indian Contributions

Several delegates from India who attended the symposium gave interesting talks on plant products. Prof. T. R. Seshadri discussed the constitution of pedicellic acid, a colourless crystalline compound obtained from *Didymocarpus pedicellata*, and this acid has been proved to be an $\alpha\alpha'$-disubstituted succinic acid derivative. The author [Prof. (Mrs) A. Chatterjee] spoke on piperlongumine (a biogenetic variant of piperine), an alkaloid isolated from *Piper longum* Linn. She conclusively established its structure as 3,4,5-trimethoxy cinnamamide of 2-$\Delta^{4,5}$-piperidone from an unambiguous synthesis. Dr S. C. Bhattacharya gave a talk on a terpenoid compound agarospirol isolated from the oil of infected variety of *Aquillaria aggallocha* Roxb., the structure of which was elegantly established from infrared and NMR spectral analysis and from various degradation reactions. Dr P. C. Dutta discussed synthetic studies on marrubin and in this connection spoke on stereospecific synthesis of the intermediate products. Dr N. V. Subba Rao spoke on scandenin, chandenin and nallanin, three phenolic components of the roots of *Derris scandens*. By a study of the absorption characteristics and degradation reactions, scandenin was shown to be 2,3-disubstituted chromanone containing in 2-position, *p*-hydroxyphenyl group and in 3-position, a tropolone ring or a dimedone system.

Conclusion

The proceedings of the main symposium were brought to a close by Prof. H. G. H. Erdtman who summarized the scientific achievements of the symposium and then Prof. S. Takei, Chairman, Kyoto Committee, gave a warm farewell speech on behalf of the Organizing Committee. All the delegates enjoyed the symposium which was made lively by various scientific exhibitions and excursions.

Symposium on Problems of Indian Arid Zone

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A SYMPOSIUM held in 1952 on the Rajputana desert concluded that appropriate steps should be taken to arrest the march of the desert, which was expanding at the rate of one mile per year into the fertile Indo-Gangetic plains. Meagre water resources, intense heat and wind erosion in the arid zone impose severe constraint on plant, animal and human life. Man himself has partly been responsible for the present state of affairs, because he has indiscriminately misused the natural resources of the area. He has now realized the seriousness of the problems of this vast desert area, comprising 80,000 sq. miles, and is taking advantage of the advances in science and technology for proper husbanding of the available resources.

The main object of the Symposium on Problems of Indian Arid Zone, organized jointly by the Ministry of Education and the UNESCO South Asia Science Cooperation Office, New Delhi, at the Central Arid Zone Research Institute (CAZRI), Jodhpur, from 23 November to 2 December 1964, was to take stock of the progress made in understanding the problems of the region and to plan future programmes of work. Dr P. C. Raheja was the Director of the symposium. About 100 participants and observers from India and a dozen scientists from overseas took part and presented 90 papers. The symposium, which emphasized the need for exploiting the mineral resources, solar energy and wind power, industrial crops, etc., in these regions, covered the following main subjects: Analysis of basic resources; Utilization of resources; Ecology of human factors; Pests and their control; Mineral resources and their exploitation; Archaeology; Geography of the desert and extension of boundaries; Macro- and microclimatology; and Solar energy, wind power and fuel energy. No paper was contributed on archaeology.

Analysis of Basic Resources

Hydrology and geohydrology — J. C. Chaudhari, T. N. Bhargava and A. D. Purohit (Defence Laboratory, Jodhpur) presented a paper on the physico-chemical characteristics of ground water resources of Barmer and Jaisalmer districts of Rajasthan. Waters of the Barmer district are generally more brackish, with higher total dissolved solids and carbonate content than the waters in the Jaisalmer district. Discussing the palaeogeography, sedimentological framework and ground water potentiality of the arid zone of Western India, P. G. Adhyalkar (Exploratory Tube-wells Organization, New Delhi) observed that the Lathis and the Bhuj sandstones are potential ground water reservoirs of great promise. P. C. Chatterji (CAZRI, Jodhpur) described hydro-geological investigations conducted recently in the region of Malani suite of igneous rocks of western Rajasthan. These investigations have revealed that the primary rocks of the central Luni basin are not good hosts for ground water accumulation. Though this region has fairly good ground water resources, their exploitation for irrigation is limited due to the great depth at which the water is located, high cost of power, undulating relief of the terrain, high cost of digging wells, failure of tube-wells and slow recharge rate of aquifers. Efficient use of water resources in Indian arid zone was considered by V. V. Dhruvanarayan, Shri Niwas and K. N. K. Murthy (CAZRI, Jodhpur) who suggested that the unexploited high yielding water zones found near foothills with deep water table could be utilized by installing economically cheap pumping sets. S. Pels (Water Conservation & Irrigation Commission of New South Wales, Australia) discussing the work being done on the hydro-geology of the riverine plain of SE Australia indicated that the younger alluvium has good aquifers. Work on similar lines could be undertaken for the successful exploitation of the ground water resources of Rajasthan, particularly the younger alluvium along the Luni and Jawai rivers.

Ecological and botanical surveys — T. A. Rao and K. R. Aggarwal (Botanical Survey of India, Calcutta) presented the results of ecological studies on Saurashtra coast and neighbouring islands. The Egyptian method of classification has been modified to suit Indian conditions, but certain areas are not easy to define on a botanical basis. Based on the types of situation and edaphic features, the vegetation of Beyt island has been classified into three main categories, viz. (i) strand, (ii) salt pans, slacks and mud formation, and (iii) inland mixed scrub forest. The same workers described in another paper the ecology of several plant communities of Saurashtra coast. These have been grouped into three ecosystems, viz. strand, salt marsh and semi-arid coastal plain. B. N. Mulay and M. C. Joshi (Birla College, Pilani) reported the results of several years of field observations in the Rajasthan desert on such problems as the influence of environment comprising physiographic, climatic, edaphic and biotic features on vegetation. The various ecosystems of Rajasthan desert and the choice of species for afforestation of the area were discussed. Dalbir Singh (Rajasthan University, Jaipur) gave an account of some useful cucurbits for cultivation in the arid zone in Rajasthan, which thrive on loose, sandy and well-drained soils, and show remarkable adaptation to survive in hostile environments. The possibility of extracting oil from the seeds of the cucurbits for industrial and edible purposes was indicated.

The ecology of the grasslands of Nokha and Roda villages in Bikaner district was discussed by K. A. Shankarnarayan (Indian Grassland & Fodder Research Institute, Jhansi). The main grassland community comprises *Dactyloctenium scindicum* and

Lasiurus sindicus type and falls under the category of 'poor' range land. However, the presence of 75 per cent of perennial grasses indicates that the area has adequate potentiality to become a good range land provided proper management practices are followed. *Indigofera enneaphylla, I. cordifolia* and *Tephrosia purpurea* are the only legumes encountered. Describing the geography of grasses, R. O. Whyte (FAO Grassland Adviser, Jhansi) indicated that *The grass cover of India* (published by the Indian Council of Agricultural Research) reveals the position of Indian arid and semi-arid grass communities in relation to other foreign communities to the west, north-east and south-east. The survey reveals a number of aspects of the geography of grasses which merit further research. Y. Satyanarayan (CAZRI, Jodhpur), discussing the habitat and plant communities of the Indian desert, observed that vegetation alone is not an adequate criterion for recognizing and demarcating plant communities. Grouping them on the basis of land forms and further subdividing them on the basis of structure and floristic composition were considered more rational. Medicinal plants suitable for cultivation in Indian arid zone were discussed by I. C. Chopra and B. K. Abrol (Regional Research Laboratory, Jammu). It was suggested that the following medicinal plants could be grown in the arid and semi-arid areas of the country without much effort: *Agave* sp., *Aloes, Datura stramonium, D. innoxia, Glyemrrhiza glabra, Balanites aegyptiaca, Cassia acutifolia, C. angustifolia, Commiphora mukul* and *Euphorbia resinifera.*

Geomorphology — Discussing the geomorphology and evolution of the Rajasthan desert, K. P. Rode (Rajasthan University, Udaipur) observed that the sheet movements are responsible for the making of arid landscapes. Geomorphic features around the semi-arid region of Bellary in Mysore State were described by R. Vaidyanathan (Andhra University, Waltair). A study of the mode of occurrence, altitude, shape and size of the various geomorphic features in this area and their interrelationship has led, for the first time, to the recognition of domes, whalebacks, flat domes, inselbergs, koppies, pediments and pediment passes. The utility of this study in pedology and civil engineering was emphasized. B. Ghose (CAZRI, Jodhpur) considered geomorphological aspects of the formation of salt basins in the lower Luni basin. According to him, salt basins and saline depressions are the relics of prior drainage channels. New salt basins are also being formed presently on the lower reaches of smaller tributaries by the same geomorphic processes. Orientation, distribution and origin of sand dunes in central Luni basin were discussed by S. Pandey, S. Singh and B. Ghose (CAZRI, Jodhpur). Based on field studies, the pattern and origin of sand dunes in the region have been worked out. The influence of variable factors, such as sand depth, proximity to the source of loose dry sand, vegetal growth, fluctuation of ground water level and geomorphological conditions, namely drainage lines and exposure of bed rocks on the above-mentioned pattern, was discussed, together with the origin of dune sand and its characteristics and dune cycle. K. Narayanan of the Oil & Natural Gas Commission (ONGC, Dehra Dun) discussed the stratigraphy of the Rajasthan shelf and the occurrence of a small marine *Permian* in the north-western part of Jodhpur district. He observed that there are indications that the underlying rocks in Jaisalmer and the neighbouring districts are oil bearing. Further survey in collaboration with other basic resources surveys are necessary to get more information about arid and semi-arid tracts.

Soil fertility and classification — Classification and fertility of soils of desert and semi-desert regions in India were dealt with by S. P. Raychaudhuri (Planning Commission, New Delhi). Based on the rainfall pattern, the soils of south and south-eastern Punjab and of western Rajasthan may be classified into: (i) pedocal sierozems of alluvial origin, (ii) pedocal brown soil of alluvial origin, (iii) grey-brown soil, and (iv) desert soil. The characteristics of these soils and their occurrence were described.

W. V. B. Sundara Rao (Indian Agricultural Research Institute, New Delhi) discussed some aspects of soil microbiology in relation to soil fertility, with special reference to arid zones. Moisture becomes a limiting factor in the arid zone and it is necessary to examine to what extent the microflora in these soils differ from those in other soils. The role of microorganisms in combating plant pathogens and building up of soil structure by synthesizing growth promoting substances in arid zone soils needs investigation. Studies have been carried out with respect to rhizobium inoculation of the leguminous seeds of food and fodder crops, the bacterization of seed with azotobacteria and phosphobacterin and with locally isolated microbes which solubilize phosphorus. However, the suitability of the various processes of enriching nitrogen in the soil under different conditions, and the role of manuring and crop rotation for proper microbial balance in soil need further investigation in arid zone areas. I. M. Qureshi and J. S. P. Yadev (Forest Research Institute, Dehra Dun) discussed forest soils of the arid regions of India. Analysis of 10 typical soil profiles from different localities has shown that most soils are poor in organic matter and nitrogen, are alkaline in reaction and contain fairly high concentration of both total and exchangeable calcium, while other soils are highly saline and calcareous. The availability of potassium, phosphorus and manganese is low. Genesis, morphology and management of arid zone soils were considered by C. T. Abichandani (CAZRI, Jodhpur). He made a number of useful suggestions for proper soil management, viz. adopting anti-wind erosion measures to protect soil blowing, soil moisture conservation, rehabilitation of sand dunes and sandy plains by afforestation; planting of shelter belts, grass strip planting, controlled grazing, wind strip cropping, stubble mulching, introduction of crop rotations including leguminous plants, and use of manures and fertilizers. To correct solenetz effect created by saline water irrigation, periodic dressing of soil with gypsum was suggested. Preparatory tillage and periodic soil working to increase water intake, bunding to hold

water and adoption of dry farming practices to help moisture conservation were emphasized.

Soil-plant-water relations — J. J. Chinoy (Gujarat University, Ahmedabad) discussed the effects of atmospheric and soil droughts on the growth and yield of crop plants. Agrobiological tests on different varieties of wheat, oat, barley and linseed have shown that the major factor determining grain yield, 1000-kernel weight and grain number was the temperature during ripening both under irrigation and 'dry' conditions. With late flowering varieties, grains ripen at a higher temperature as compared to early flowering ones, and the yields are poor. Some aspects of the management of soil-water-plant-climatic complex in the arid zone and characterization of soils were considered by B. Rama Moorthy (Indian Agricultural Research Institute, New Delhi). R. C. Mondal (CAZRI, Jodhpur) dealt with the quality of ground waters in Siwana, Jalore and Saila Development Blocks in the central Luni river basin. Most of the waters in Siwana area have sodium absorption ratios (SAR) in the range 0-10 and when used for irrigation the waters are not likely to produce alkalinity in the soil. A. N. Lahiri (CAZRI, Jodhpur) discussed the results of studies on moisture relationships of some xeric plant species of western Rajasthan. The high rate of transpiration in certain dune plants in summer under low soil moisture conditions has been found to be due to their deep root system, while the relatively lower rate during winter is related to low temperature conditions in the morning and decreased solar intensity in the evening. Evaluation of hydrological conditions of grasslands was discussed by N. D. Rege and S. C. Gupta (Ministry of Food & Agriculture, New Delhi). It was shown that 13·7 per cent of the total geographical area, serving primarily as grazing lands for the large number of livestock, has been badly overgrazed. In order to improve and to manage the grasslands properly, a survey to assess their condition is necessary taking into account factors such as the amount of ground cover with living and dead vegetation, kind and vigour of vegetation, degree of grazing, soil characteristics with respect to growth of grasses, amount and nature of soil erosion, characteristics and amount of run-off and duration and rate of stream flow.

Utilization of Resources

N. D. Rege (Ministry of Food & Agriculture, New Delhi) discussed the problems concerning the management of arable lands in arid and semi-arid regions and suggested that the methods found most suitable for the cultivation of lands in arid regions could safely be used in semi-arid areas. Problems of crop raising in arid regions of Bellary tract were discussed by S. Chattopadhyay (Soil Conservation Research Centre, Bellary). In this area, which is largely made up of black cotton soil, response to fertilizers has been low due to lack of placement of fertilizer rather than to inadequate moisture, but two rainfalls are necessary for the fertilizer to give the response. Boron and manganese have shown some toxicity. D. K. Misra (CAZRI, Jodhpur) described agronomic investigations in western Rajasthan conducted on stubble mulching, weed control, seed placement, mixed cropping, crop sequence and response to macro- and micronutrients on the millet crop. The special farming practice of stubble mulching has been found to provide protection to the cultivated land from soil blowing, increasing the yield of *Pennisetum typhoides*, both in grain and stalk. To get uniform germination and crop stand, 7·5 cm. soil depth of seed placement is optimum. The optimum seed rate ratio of cereal to legume appeared to be 50: 50. Application of micronutrients of iron and manganese showed significant increase in crop yield.

Irrigation — T. Kurian, E. R. R. Iyengar, M. R. Narayana and D. S. Datar (Central Salt & Marine Chemicals Research Institute, Bhavnagar) discussed the effect of sea water composition on the growth of tobacco and the possibilities of utilizing sea water and other highly saline waters for the cultivation of certain varieties of food crops and plants of industrial value on dune sands of inland and coastal regions. Sea water depresses the growth of tobacco plant, but addition of potassium salts improves the crop growth. The effect of sea water on some soil characteristics was discussed by M. R. Narayana, V. C. Mehta and D. S. Datar (Central Salt & Marine Chemicals Research Institute, Bhavnagar). The effect of high salinity and SAR value of these waters is minimized in sandy soils due to low clay content. High permeability of these sandy soils makes them suitable for irrigation and there is no salt accumulation in the root zone. D. R. Bhumbha, K. K. Mahajan, J. S. Kanwar and Bhajan Singh (Punjab Agricultural University, Hissar) have tried the use of water with different sodium levels and salinities on the growth of crops and the properties of soil by conducting experiments in small plots having loamy sand soil. No significant increase has been observed in wheat yield with change in SAR, but maize yield falls with water, whose SAR is 16. N. C. Rawal and dos M. Pais Cuddou (Central Water & Power Commission, New Delhi) described pre-irrigation soil survey as adopted by the Central Water & Power Commission and discussed the results of some projects conducted during the last three years.

Afforestation — R. N. Kaul and B. N. Ganguli (CAZRI, Jodhpur) presented the results of silvicultural studies on four indigenous tree species, namely *Prosopis spicigera, Albizzia lebbeck, Accia senegal* and *Tecomella undulata*, adaptability trials, afforestation of the principal land types and top feed species. Among the species tried from Israel, Australia, USA, USSR and Southern Rhodesia, *Acacia tortillis* (Israel) and *Eucalyptus camaldulensis* (Australia) have shown high degree of adaptability to the environment and have excelled the principal indigenous tree species in respect of growth and survival. On shifting sand dunes the species successfully afforested are *Prosopis juliflora, P. spicigera, Calligonum polygonoides, Zizyphus jujuba, Dalbergia sissoo, Ailanthus excelsa, Albizzia lebbeck, Azadirachta indica, Erianthus munja* and *Lasiurus hirsutus*. In shallow soils overlying hard calcareous pan, deep soil working and planting balled seedlings of *Azadirachta indica*

and *Albizzia lebbeck* have proved successful. In semi-rocky areas, where soil accumulates in local depressions, ridge-cum-trench type of soil working with seedling of *Acacia senegal* and *Prosopis juliflora* were successful, while in less soil accumulated conditions planting of pre-sprouted stumps of *Prosopis juliflora* and transplants of *Azadirachta indica* gave better results. Protection against all biotic interferences until the area gets reasonably covered with natural vegetation appears to be the most practical approach. Studies on palatability and chemical composition of 16 tree and shrub species occurring naturally in the grazing lands revealed that *Prosopis spicigera* and *Zizyphus nummularia* are the most important topfeed species. I. M. Qureshi and O. N. Kaul (Forest Research Institute & Colleges, Dehra Dun) described foliar constituents of some important tree species of the arid zone of India.

According to K. S. Sankhala (Forest Department, Rajasthan), the important fauna of Rajasthan desert are the Indian black buck, the Chinkara, the panther, the grey francolin, migrant Houbara and sand grouse. He indicated the possibilities of establishing various wildlife sanctuaries to protect some of the rare wild animals of the desert.

Pasture ecology — The selection of grasses and legumes for arid zone pastures was discussed by A. K. Chakravarty (CAZRI, Jodhpur). Among the 208 promising perennial species of grasses collected, *Cenchrus ciliaris* strain Nos. 357 and 358 have been selected for introduction in light sandy soil in low rainfall regions, and strain Nos. 226 and 362 for heavy soil in the high rainfall areas of the arid zone. Among the legumes introduced, *Dolichos lablab* var. *lingnosus*, *Atylosia scarabaeoides* and *Clitoria ternatea* have been found to be drought resistant to a large extent. From a population study conducted on *C. ciliaris* in different ecological habitats, the black-headed variety has been found on heavy, compact and flat soils, while plants with coloured stigma are associated with sandy loose soils. It has a bearing on future breeding and selection work. Studies on grasses and sedges of certain areas in Jhunjhunu district, Rajasthan, were described by M. C. Joshi and C. B. S. R. Sarma (Birla College, Pilani). Pasture ecology of grasslands in western Rajasthan was discussed by R. B. Das and C. P. Bhimaya (CAZRI, Jodhpur). Regression studies on height-weight relationship of the important range fodder species have indicated that the relationship between the percentage of height grazed and the percentage of forage utilized is of the quadratic nature for all the species studied. The dry-land seeded pastures studies at Pali, on sandy loam soil and 410 mm. average rainfall, showed that the relative performance of grass species, such as *Cenchrus ciliaris*, *C. setigerus*, *Panicum antidotale* and *Dichanthium annulatum*, was superior from forage production and utilization aspects as compared to those on sandy soils at the Central Research Farm, Jodhpur, with 330 mm. rainfall. However, the grazing capacity of *Panicum antidotale* was the same at both the places.

Animal grazing and management — V. Mahadev, P. S. Gupta and M. M. Jayal (Indian Veterinary Research Institute, Izatnagar) discussed the nutritive value of *C. ciliaris* from grazing areas of Rajasthan forests. This grass does not meet the maintenance requirement of grazing cattle without supplements of protein rich concentrates. To utilize the grass profitably it must be harvested or grazed at early stages. L. D. Ahuja (CAZRI, Jodhpur) discussed livestock management on range lands in western Rajasthan and outlined the work in progress in a range management project. Adaptation of livestock to arid climate was discussed by G. C. Taneja (CAZRI, Jodhpur). Under artificially created arid climate, the rectal temperature and respiration rate of animals exposed increase with rise in air temperature and are followed by depression of food consumption, milk production, blood carbon dioxide combining power, ascorbic acid, and increase in blood creatine. Cattle from arid region are able to vapourize relatively more moisture through the skin and have denser sweat glands as compared to European cattle in temperate climate. Lower basal metabolic rate of these animals results in less heat production with rise of air temperature and is due to decline in thyroid activity. The level of production is also considerably low. The depressing effect on milk production, food consumption and body weight becomes evident above 80°F. for European cattle and above 95°F. in arid climate cattle. Genetically, the tolerance to arid climate is weakly inherited. The crosses between the two breeds possessing the faculty for heat tolerance and high milk production should prove fruitful.

Ecology of Human Factors

A. B. Bose, S. P. Malhotra and P. C. Saxena (CAZRI, Jodhpur) discussed in their paper the problem of nomadism in the arid zone of Rajasthan. P. K. Misra discussed the nomadic Gadulia Lohars, their region of movement and composition of the bands. S. M. Ali's (Saugar University) paper was concerned with some aspects of the human geography of the Indian desert, with particular reference to the changing pattern of population, the forms and functions of typical settlements, and the effect of social and economic changes on the cultural landscape. A. B. Bose and S. P. Malhotra (CAZRI, Jodhpur) reported the results of a sample survey on land use, demographic features and sources of livelihood of the populations in the central Luni basin of western Rajasthan. A typical research programme in agricultural economics for arid lands was discussed by J. P. Hrabovszky (Indian Agricultural Research Institute, New Delhi). Specific problems recommended for research are: studies on input-output coefficients, uncertainty, institutional arrangement for water, and land use rights.

Pests and Their Control

D. R. Bhatia (Field Station for Investigation on Locusts, Bikaner) discussed the invasion and breeding regions of the desert locust and its seasonal migrations with downwind. The theory of phases and the factors leading to gregarization and initiation of fresh plagues were also discussed. Suppression of infestation in its initial stages and at the

time of outbreak have been suggested as the best approach to locust control. For direct offensive, BHC, aldrin and dieldrin have proved very effective. By consuming the annuals, locust loosen the soil resulting in its erosion. Some aspects of population dynamics of desert locust were outlined by M. L. Roonwal (Zoological Survey of India, Calcutta). The different morphological changes brought about during transformation from phase *solitaria* to *gregaria* were described.

V. C. Agarwal (Zoological Survey of India, Jodhpur) discussed rodent ecology and the morphological adaptations of the rodents as a result of desert conditions. Ishwar Prakash (CAZRI, Jodhpur) observed that the density of vegetation cover does not appear to influence the distribution of Indian desert gerbil but soil compactness is a limiting factor for their dispersal. The effect of water stress on the body composition and renal functions in small desert mammals was discussed by P. K. Ghose and K. G. Purohit (CAZRI, Jodhpur).

K. Gosswald (Institute of Applied Zoology, Würzburg University, Würzburg) described an apparatus used in studies on insect ecology, with special reference to temperature and humidity, and the influence of ecological factors on the metabolic functions of insects and the mechanism by which insecticides work. It has been shown that climatically sensitive species react intensively to weather changes, which is reflected by a decrease or increase in population density, while other species do not show this reaction. Experiments with ants have indicated that soil inhabiting species prefer a high relative humidity range while stone inhabiting species concentrate in the dry region of the humidity preference apparatus. Comparable differences are also found in toraites.

Mineral Resources and Their Exploitation

M. A. Hai, M. K. H. Siddiqui and E. R. Saxena (Regional Research Laboratory, Hyderabad) described the results of studies conducted on the utilization of some minerals from Rajasthan, such as gypsum, limestone, felspar and fullers' earth. A process for the manufacture of a versatile low-cost neutral desiccant from gypsum has been standardized. A superior quality white cement is obtained by heating together gypsum, limestone and feldspar. Potassium sulphate is obtained as a byproduct and can be used as a fertilizer. Mudh fullers' earth, after suitable processing, has been found to be a good bleaching agent for lubricating oils. Byproduct recovery from saline lakes and alkaline soils was dealt with by K. Seshadri and D. S. Datar (Central Salt & Marine Chemicals Research Institute, Bhavnagar). It has been estimated that about 25,000 tons of sodium sulphate can be obtained from the Sambhar bitterns after chlorination to destroy algae and by chilling to 0°C. Sodium carbonate can be recovered by carbonating the chilled mother liquor. By reacting the bittern with gypsum, sodium sulphate can be prepared economically; cost estimates for a 14-ton per day plant have been worked out. G. C. Jain, N. N. Sharma, R. L. Datta and D. S. Datar from the same laboratory enumerated the problems connected with the establishment of salt industry in Rajasthan. M. L. Sethi (Department of Mines & Geology, Udaipur) discussed the mineral wealth of Rajasthan. Large-scale deposits of limestone, gypsum, fullers' earth and feldspar exist in the State and their commercial utilization is underway. Eighty per cent of the country's requirements of talc and soapstone are being met and the state is the second largest producer of mica in the country. Forty miles from Bikaner, 60-80 million tons of lignite deposits have been located and are being mined by the open cast method. Processes for the utilization of silica sands in glass and abrasive industries are under examination.

Geography of the Desert

A. K. Sen (CAZRI, Jodhpur) discussed some geographic features of the Rajasthan desert. The physiographic, climatic and anthropographic conditions of Rajasthan are comparable to the identical phases encountered in other hot deserts of the world. There are evidences to show that Rajputana arid zone is a true desert and its location coincides with the global desert belt. However, the desert conditions have been accentuated in recent times due to over-exploitation of the resources. Mapping of arid lands of India by the National Atlas Organization was discussed by S. P. Chatterjee (National Atlas Organization, Calcutta). Maps depicting physical features, population distribution, hydrology, pedology, transport network, land use, administrative units, etc., are under preparation by the National Atlas Organization.

Macro- and Microclimatology

A new climatic index and its application to subclassification of arid zones were discussed by N. C. Majumdar (Defence Laboratory, Jodhpur). A simple nomogram, developed on the basis of the logarithm of the ratio of the annual average of the diurnal range of dry bulb temperatures and the average annual precipitation, has many promising features, such as reasonable linearity of the climatic scale, adequate sensitivity of the scale in arid zone to enable its sub-classification, a fair representation of the combined effect of the major climatic elements and a simple method of evaluation.

Energy from the sun in Rajasthan was considered by P. Jagannathan and H. R. Ganeshan (Meteorological Office, Poona). Maps have been prepared showing the distribution of solar intensity over Rajasthan for the winter, summer, monsoon and post-monsoon seasons. Minimum energy is received in winter and maximum in summer months and represents 319 billion W. during the year. The estimated energy figures may be usefully employed in assessing the efficiency of solar devices set up in the region. The results of a statistical study of the characteristics of the wet spells undertaken with the daily rainfall data for Jaisalmer and Bikaner, representing arid west Rajasthan and Kotah and Jaipur representing the semi-arid east Rajasthan, were presented by P. Jagannathan and V. K. Raghavendra (Meteorological Office, Poona). The frequency distribution of wet spells does not follow

the probability law, but their durations are related to sunspot characteristics. P. Jagannathan (Meteorological Office, Poona) discussed the climatic environment and its fluctuations in Rajasthan from data recorded over a number of years. A. Krishnan and K. A. Shankarnarayan (CAZRI, Jodhpur) discussed the criteria for the delimitation of arid zone of Rajasthan by applying the standard methods in use to find out how far different climatic classifications reflect the actual vegetation pattern of Rajasthan. These workers stressed the need for evolving a new method of classification to obtain a true picture of the vegetation pattern of the desert. N. D. Rege, A. R. Bhaskaran and N. S. Jayaram (Ministry of Food & Agriculture, New Delhi) described preliminary studies on the effect of hydroxyethylene docosanol (OED) on evaporation of water from soil surface. In arid zone, evaporation loss from soil surface is a major problem. Results from a drying cycle show that OED powder, when sprayed on soil, reduces evaporation from bare soil surface by about 15 per cent. Its disintegration in soil is very slow due to its waxy nature. The cost of treatment per acre is roughly Rs 40.

The question 'Is Indian desert a continuation of Sahara desert?' was examined by P. P. Sajnani (Institute of Tropical Meteorology, Poona). Temperature and moisture distributions in the lower and middle troposphere over Sahara and Thar exhibit a striking similarity of characteristics. With minor variations due to purely local conditions, the entire area stretching from Sahara to Thar appears to be a meteorologically homogeneous one and supports the view that the Thar desert forms a part of an extensive desert belt comprising the great Sahara and the arid regions of Arabia, Iran and West Pakistan.

S. K. Gupta (Soil Conservation Research, Demonstration & Training Centre, Kotah) discussed frequency analysis of rainfall data for use in soil conservation. Analysis of such data has been used to draw isopluvial maps useful in planning soil conservation programmes. Microclimate and vegetation of arid zone was dealt by I. M. Qureshi and B. K. Subba Rao (Forest Research Institute, Dehra Dun).

Climatic water balance of the Indian arid zone was discussed by V. P. Subramaniam (Andhra University, Waltair) who indicated the need for analysing data collected over a period of 30 years to obtain an idea of the departure of aridity index from the normal. A. R. Subramaniam (Andhra University, Waltair) presented results of comparative drought and aridity studies in the Indian arid zones. As remarked by E. Hovmoller (Meteorological Office, Poona), Chairman of the session, an important point to be borne in mind is that while macroclimate can hardly be influenced, every effort should be made to modify microclimate as this is particularly important for harnessing the resources of both arid and semi-arid regions.

Solar Energy, Wind Power and Fuel Energy

S. D. Gomkale, S. Y. Ahmed, R. L. Datta and D. S. Datar (Central Salt & Marine Chemicals Research Institute, Bhavnagar) dealt with the techniques and economics of conversion of saline water into fresh water. They suggested the adoption of either electrodialysis or ion-exchange method for the demineralization of brackish well water of low salinity available in certain regions of Rajasthan. A substantial part of the cost of desalination by distillation is taken up by power alone. The use of solar energy for demineralization of brackish well water in Rajasthan is recommended on account of the favourable conditions, such as low humidity, high wind velocity, high incidence of solar radiations, very few cloudy days, availability of cheap labour and materials of construction, etc., existing in this region. Discussing the present status of demineralization of saline water, M. L. Khanna (National Physical Laboratory, New Delhi) summarized the different methods employed at present in USA. Rajasthan offers vast scope for undertaking demineralization of brackish water on a commercial scale. On account of the favourable conditions available in the region, the chances of utilization of solar energy for large-scale demineralization of brackish well waters of Rajasthan are very bright. Large-scale trials of solar stills constructed on the ground with indigenous materials were recommended. Examination of dual-purpose desalting and electric power plants run with nuclear energy and their integration into local utility systems is also considered worth while. R. Natarajan (Defence Laboratory, Jodhpur) suggested that electrodialysis alone or in combination with ion-exchange appears promising for brackish water treatment in Rajasthan region. Need for the development of indigenous ion-exchange materials and their use in desalting waters of the region was emphasized.

The use of solar energy for household heating was discussed by J. P. Gupta and D. Krishnamurti (Defence Laboratory, Jodhpur). In the sub-zero ambient temperatures of cold arid region of Ladakh, where neither electric power nor the conventional types of fuels are available, living space heating was effected by radiative and convective heat losses from the surface of hot water tank and not by forced circulation of air. The NPL type solar water heater employed to heat water by thermo-siphon action showed an overall efficiency of 28 per cent in heating a room of size $12 \times 9 \times 8$ ft with solar energy, the maximum room temperature rise being 21·5°C. for an ambient temperature of 3°C. B. M. L. Saksena *et al.* (Defence Research Laboratory, Delhi) described a solar cooking device comprising a concentrator and a thermally insulated oven. The plane-glass mirror concentrator, identical to that previously used by M. L. Khanna and his coworkers (National Physical Laboratory, New Delhi) for concentrating sugarcane and palm juices, is used to concentrate solar energy on to the oven. Sub-zero ambient temperatures and wind do not affect its performance.

Reviewing the field of solar energy and its application, H. Tabor (National Physical Laboratory, Israel, Jerusalem) indicated that no commercial firm was at present concerned with large-scale utilization of solar energy, because of its diffused nature, requiring a large collection area. However,

possibilities exist today for exploiting solar energy for developing refrigerators in the hot and non-electrified regions of the world. He surveyed the present status of our knowledge with respect to materials, solar radiation measurements, solar distillation, refrigeration and air-conditioning, solar cookers, water and air heaters, and large-scale power units. Only the solar pond appears to have reasonable chance of success in large-scale exploitation of solar energy, specially for the manufacture of salt in arid zones, as the process is independent of weather on account of its large heat capacity and effective use of sunshine. In conclusion, he stated that solar energy utilization is attractive only in areas where the supplies of conventional energy are scarce and expensive or cost is of little consideration.

General Discussion

During the session, general discussion was held on the following subjects: Aerial photo-interpretation for survey of natural resources; Productivity of arid lands for farming and animal husbandry; Aridity — its causes, criteria and control; and Impact of man on arid land — retrospect and prospect.

Plenary Session

Eighteen resolutions were passed during the plenary session. It was recommended that an overall appraisal of arid and semi-arid regions throughout India should be conducted to determine future investigational programmes and their order of priority at the Central Arid Zone Research Institute, Jodhpur, and other institutions in the country.

Methionine Biosynthesis*

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ALTHOUGH methionine has long been known as an essential amino acid and lately as a methyl donor in biological transmethylations, its biosynthetic pathway in animals and microorganisms has only recently attracted attention. The discovery of du Vigneaud and coworkers[1,2] that both D- and L-isomers of homocysteine could support the growth of rats in the absence of methionine sparked investigations on the biosynthetic pathway of methionine. Homocysteine supplementation of certain methionine deficient diets was effective only when methyl donors such as choline and betaine were incorporated in the diet. This type of a direct transfer of the methyl groups from or to methionine *in vivo* was termed ' transmethylation '[3].

The development of the knowledge of *de novo* formation of the methyl groups from active formaldehyde helped in the resolution of methionine and thymidilate biosynthesis. These appear to be the only two compounds in which the methyl groups are so formed. Study of these reactions has led to the understanding of the biochemical function of folic acid and vitamin B_{12}. This has also led to the discovery of N_5-methyl FH_4†, a new intermediate in the biosynthesis of methyl groups[4].

*This article is based on a lecture delivered during the Summer School on the Current Trends in Biochemistry sponsored by the University Grants Commission at the Indian Institute of Science, Bangalore, during May 1964.

†Following abbreviations are used: FH_4, tetrahydrofolate; FH_2, dihydrofolate; ATP, 5'-(pyro)-adenosine triphosphate; AM, 5-adenosyl methionine; AH, 5-adenosyl homocysteine; PALP, pyridoxal phosphate; DPNH, reduced nicotinamide adenine dinucleotide; DPN, nicotinamide adenine dinucleotide; FAD, flavin adenine dinucleotide; and FMN, riboflavin-5'-phosphate.

Biosynthesis in Animals

The observation that a diet devoid of methionine and other methyl donors, but containing folic acid, homocysteine and vitamin B_{12} could support the growth of rats, modified the earlier concept of the indispensability of methionine. In rat growth experiments, du Vigneaud *et al.*[5] occasionally encountered animals capable of growing on homocysteine diet without added choline. This suggestion that the labile methyl groups were being synthesized *de novo* was verified by the appearance of deuterium in the methyl groups of choline isolated from rats whose diets contained D_2O and an adequate source of the labile methyl group[6].

Sakami and Welch[7] obtained further evidence that rats can synthesize the labile methyl groups *de novo* by demonstrating the incorporation of formate-1-^{14}C to methionine methyl groups. This incorporation could also occur in rat liver slices and the *in vitro* process was stimulated by folic acid. Such an involvement of folic acid and vitamin B_{12} was earlier suggested by Bennet[8] from his nutritional studies. Berg[9,10] was the first to observe the formation of methionine from homocysteine and formate by the cell-free extracts of pigeon liver homogenates. The incorporation of ^{14}C-formate into the methionine methyl proceeded better anaerobically than aerobically. The components and cofactors required for the methionine biosynthesis were suggested to be folic acid, ATP, Mg^{2+}, DPN and homocysteine by Doctor *et al.*[11] investigating with chicken liver acetone powders and by Nakao and Greenberg[12] in a sheep liver system. Nakao and Greenberg[13], measuring isotope incorporation

from serine-3-^{14}C and formaldehyde-1-^{14}C, verified the requirements reported by Doctor *et al.*[11] and established that FH_4 could substitute for folic acid. Traces of methionine greatly stimulated the synthesis of methionine. When serine was the carbon donor, there was an absolute requirement for serine hydroxymethyltransferase and PALP. AM severely inhibited the incorporation of the label. AM perhaps was a component of the system whereby its inhibition of the isotope incorporation into methionine could be explained as being due to isotope dilution. Stevens and Sakami[14], using an ammonium sulphate fraction of pig liver homogenates, confirmed the cofactor requirements suggested by Nakao and Greenberg[13], but reported that AH could replace the requirement of homocysteine but not ATP. Wilmanns *et al.*[15] isolated a folate intermediate believed to be the methyl acceptor. These results, however, have been disputed by Larrabee *et al.*[16] and Kisliuk[17].

Mangum and Scrimgeour[18] demonstrated the requirement for AM in catalytic amounts for the transfer of methyl groups from N_5-methyl FH_4 to homocysteine. The requirement for ATP and Mg^{2+} was abolished when AM was used in the reaction mixture. Kerwar *et al.*[19] have shown the involvement of vitamin B_{12} in this process. Dickerman *et al.*[20] have purified the chicken liver enzyme 10- to 30-fold and reported an anaerobic stimulation by cobamide and suggested that cobamide maintained an essential —SH group on the enzyme in the reduced state.

Biosynthesis in Microorganisms

The work on methionine biosynthesis in animal systems has been parallelled by similar and in some instances more fruitful studies in the mutant strains of *Escherichia coli*. Hatch *et al.*[21] have separated the methionine synthesizing system into three partially purified fractions: (i) serine hydroxymethyltransferase, (ii) a fraction that reduces N_5,N_{10}-methylene FH_4 to N_5-methyl FH_4, and (iii) a protein fraction containing vitamin B_{12}. The combination of the three fractions carried out methionine biosynthesis in the presence of the following cofactors and substrates: serine, homocysteine, PALP, FH_4, ATP, DPNH and FAD or FMN. When N_5,N_{10}-methylene FH_4 was used as the source of C_1 donor, the requirement for serine, PALP and serine hydroxymethyltransferase was abolished. These studies were carried out using two mutant strains of *Esch. coli*, one requiring either B_{12} or methionine for growth, and the second requiring PABA and methionine. The important finding of this group of workers was that N_5-methyl FH_4 acted as an intermediate in the reaction and could methylate homocysteine directly[16].

Guest and collaborators[22,23] and Woods[24] suggest that two pathways exist for the formation of methionine in *Esch. coli*. In one of these, there is a requirement for vitamin B_{12} protein and in the other there is no requirement for vitamin B_{12} protein but only for triglutamate FH_4 as a cofactor. The second pathway is inhibited by monoglutamate FH_4 and appears to operate in *Esch. coli* PA-15, which has been grown on methionine in the absence of vitamin B_{12}. On addition of vitamin B_{12}, the bacterium changes over to the first pathway which is obligatory in *Esch. coli* 121/176. The methyl group transfer involves AM, a vitamin B_{12} enzyme and a pyridino-flavoprotein. They suggest that the oxidation level of cobalamin cofactor remains unchanged throughout the reaction.

Mechanism of Methionine Biosynthesis

Nakao and Greenberg[13] were the earliest to suggest a possible mechanism of methionine biosynthesis based on the incorporation of formaldehyde-1-^{14}C. Stevens and Sakami[14] postulated that AH could replace homocysteine and proposed a condensation of formaldehyde, FH_4 and homocysteine to an intermediate of the type $-S-CH_2-N<$, i.e. where the methylene group is bridged between the sulphur of homocysteine and the N_5-nitrogen of FH_4. The final step in the methyl biosynthesis required the reductive cleavage of the $-CH_2-N<$ bond to yield FH_4 and methionine. Alternatively, they suggested that the reducing power might emanate from the reduced pyrazine ring of FH_4 to yield FH_2 and methionine. The FH_2 formed is converted to FH_4 by dihydrofolate reductase. This latter type of cleavage has been shown to occur in the case of thymidylate synthesis[25].

Wilmanns *et al.*[15] proposed a mechanism for methionine biosynthesis similar to that of thymidylate synthesis and presented isotopic incorporation data in support. They also reported a folate intermediate, N_5-methyl-7,8-FH_2, and postulated AH as the methyl acceptor. This system was inhibited by antifolate compounds such as aminopterin and amethopterin. The third hydrogen for the methyl group originated from the hydrogen atom attached to carbon 6 of FH_4. These findings have, however, been severely criticized[16,17]. It is now reported that both the experimental finding and the mechanism have been withdrawn[15].

Biosynthesis of N_5-Methyl FH_4

The initial steps in the biosynthesis of methionine are now fairly well established. Serine and formaldehyde have been the most commonly studied precursors of the methyl group of methionine. Studies with animal and bacterial systems have established the requirements for formation of methionine to be serine, serine hydroxymethyltransferase, PALP, FH_4, ATP, Mg^{2+} and pyridine nucleotide. The enzymic conversion of serine to glycine has been demonstrated in a variety of systems[26–30]. These studies have indicated that β-carbon of serine is released as enzyme bound C_1 unit at the oxidation level of formaldehyde. PALP, Mg^{2+} and pyridine nucleotides have been implicated as cofactors in this reaction.

Blakeley[30] and Hatch *et al.*[21] have shown that formaldehyde reacts with FH_4 to form N_5,N_{10}-methylene FH_4. The latter workers have demonstrated the appearance of this intermediate in their reaction mixtures, and the enzymic formation of this compound from serine in *Esch. coli* extracts. They have shown that N_5,N_{10}-methylene FH_4 can serve as a precursor for methionine biosynthesis. When formaldehyde is used as the carbon source,

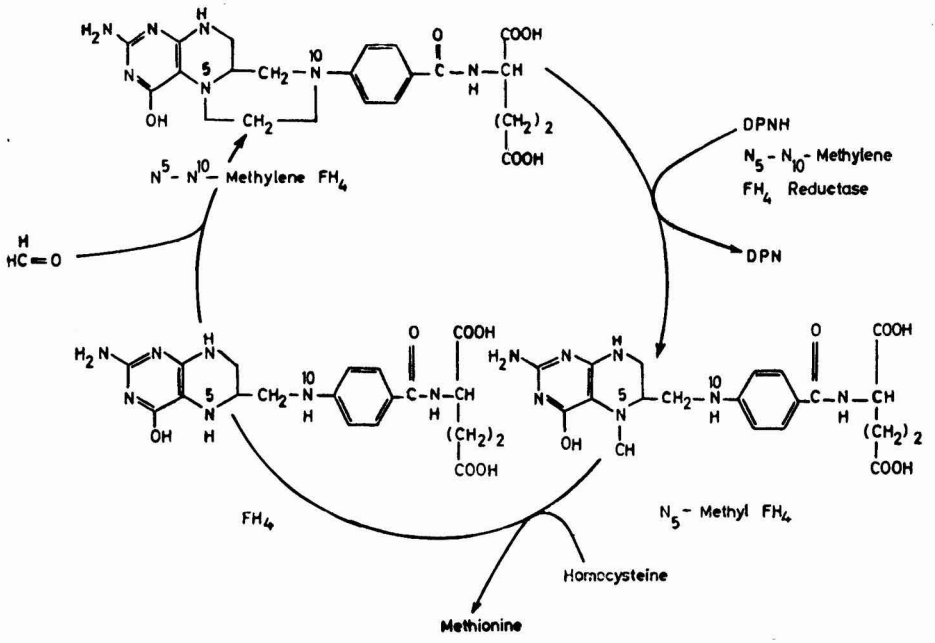

Fig. 1 — Biosynthesis of N_5-methyl FH_4 (after Larrabee *et al.*[16])

the requirement for serine, serine hydroxymethyltransferase and PALP is abolished.

The N_5,N_{10}-methylene FH_4 is converted to N_5-methyl FH_4 in the presence of an enzyme isolated from bacterial source[4] and mammalian system[31-33]. The enzyme is a flavoprotein requiring pyridine nucleotide and was missing in *Esch. coli* 113/3 mutant requiring methionine for growth. N_5-methyl FH_4 was identical with the folic derivative 'prefolic A' isolated earlier from horse liver[34]. The enzymatic reduction requires reduced pyridine nucleotide, usually DPNH. Keresztesy and Donaldson[35] and Sakami and Ukstins[32] prepared this compound by reducing the N_5,N_{10}-methylene FH_4 with potassium borohydride. Kisliuk and Sakami[27] studied the origin of the hydrogen atom involved in the reduction of N_5,N_{19}-methylene FH_4 to N_5-methyl FH_4. When tetradeuterofolate was used as a cofactor in the methionine synthesis, the methionine methyl group did not contain any deuterium. When the reaction was carried out in D_2O there was an incorporation of two atoms of deuterium per molecule of methionine synthesized. One of this was located in the methyl group and the second in the C_2 atom of methionine. These results suggested that the hydrogen atoms of methyl group arose from the solvent and not from the pyrazine ring of FH_4. The biosynthesis of N_5-methyl FH_4 from C_1 units is summarized in Fig. 1.

Transfer of Methyl Group from N_5-Methyl FH_4 to Homocysteine

Requirement for AM — The requirements for the transfer of a methyl group from N_5-methyl FH_4 to homocysteine have been studied[16,17,36-38] in view of the requirements for NADH, FAD and vitamin B_{12} protein for the methylation of homocysteine. Originally, it was assumed that the abolition of the lag phase by preincubation with ATP and Mg^{2+} was due to the formation of an unknown intermediate or cofactor resulting from the interaction of ATP, Mg^{2+} and homocysteine. de La Haba and Cantoni[39] observed that AH is formed from adenosine and homocysteine by a rat liver system and Nakao and Greenberg[13] observed AH formation in the sheep liver preparation. AH or enzyme system synthesizing AH were tried by these workers but they were unable to abolish the lag phase. It was earlier reported that AM stimulated the biosynthesis of methionine[37,38]. It was thought that ATP and Mg^{2+} could react with the endogenous methionine in crude enzyme systems to form AM which might abolish the lag phase[36]. The transfer of the methyl group from N_5-methyl FH_4 to homocysteine was catalysed by AM abolishing the lag phase[18,20,40]. Stevens and Sakami[14] had concluded that free AM was not an intermediate in methionine biosynthesis. Nakao and Greenberg[12], on the other hand, reported that addition of AM severely inhibited the transfer of label from formaldehyde-1-^{14}C to methionine-^{14}C and suggested that AM might be involved in methionine biosynthesis. That the synthesis of AM represented a rate limiting step in methionine biosynthesis was established by replacement of ATP and Mg^{2+} by AM resulting in the immediate formation of methionine and preincubation of AM with other components of the system (except N_5-methyl FH_4) when no enhancement of the rate

of methionine biosynthesis was observed. Mangum and Scrimgeour[18] and Foster *et al.*[41] showed that AM acts catalytically in methionine biosynthesis. The latter workers observed that 200 moles of methionine were synthesized per mole of AM, which is rather unusual. It would appear that this compound could function as an intermediate in methionine biosynthesis in which the sulphur atom is successively methylated and demethylated or it would have to methylate some compounds one at a time and is not directly involved in methylation reactions. The latter possibility finds favour since it has been impossible to demonstrate the incorporation of a radioactive methyl group from ^{14}C-methyl labelled N_5-methyl FH_4 into AM (ref. 36). The formation of AM from ^{14}C-methionine and ATP from a crude enzyme preparation has been reported[41]. AH, adenosine, 5-methyl thioadenosine, Barker's coenzyme B_{12} and methyl vitamin B_{12} were unable to replace AM.

Requirement for a reducing system and vitamin B_{12}—Another requirement for methionine biosynthesis is a reducing system which is met by a DPNH flavoprotein dehydrogenase. Catalytic amounts of $FADH_2$ can replace this protein fraction. The function of this system appears to be to reduce the cobalt atom of vitamin B_{12}. Using the vitamin B_{12} enzyme and a reducing system, it has been shown[23,37,40,42] that vitamin B_{12} has to be reduced before the enzyme can catalyse the transfer of methyl group from N_5-methyl FH_4.

Brady *et al.*[43] and Peterkofsky and Weissbach[44] studied the synthesis of adenosyl vitamin B_{12} enzyme. Although they have reached different conclusions, their data could be interpreted to mean that the reduction of the cobalt atom in vitamin B_{12} from Co^{3+} to Co^{+} is followed by the reaction of the reduced cobalt with ATP to form adenosyl vitamin B_{12}. This biosynthetic reaction has been shown to have cofactor requirements similar to the methyl transfer step in methionine biosynthesis, namely DPNH and FAD. Müller and Müller[45] and Smith *et al.*[46] found that fully reduced vitamin B_{12} reacts almost instantaneously with alkyl halides to form a new type of vitamin B_{12} derivative characterized by cobalt alkyl bond. Thus methyl vitamin B_{12} can be prepared chemically with methyl iodide or dimethyl sulphate, and recently Foster *et al.*[41] presented experimental evidence for the formation of methyl vitamin B_{12} from AM and hydroxy vitamin B_{12} by extracts of *Esch. coli*. This synthesis by *Esch. coli* requires an additional reducing system. A chemical synthesis of methylcobalamin from AM and hydridocobalamin has been reported[47]. Since the reaction mixtures contain —SH compounds which can convert vitamin B_{12} to a reduced form the requirements for additional reductants indicate that the product of the reaction is the more highly reduced form of vitamin B_{12}. This is possibly a form in which the positive charge on cobalt is one, i.e. a form reduced by two electrons below the level of hydroxycobalamin. It has also been shown that AM has no effect on the transfer of methyl groups from methyl vitamin B_{12} to methionine indicating that AM must be acting prior to the formation of methylcobalamin. Similar requirements have been demonstrated in avian and mammalian systems[48,49].

Loughlin *et al.*[48] have purified (250-fold) the enzyme from pig liver, catalysing the transfer of methyl group from N_5-methyl FH_4 to homocysteine. N_5-methyl FH_4-monoglutamate and triglutamate can be used as substrates. AM and reduced flavin are required as cofactors for the reaction. The vitamin B_{12} content was proportional to the enzyme activity at all purification stages. Dickerman *et al.*[49] have isolated from avian liver an enzyme system, catalysing the transfer of methyl group from methyl vitamin B_{12} and from N_5-methyl FH_4 to homocysteine. The two enzyme systems, however, could not be separated. For the methyl vitamin B_{12} transferase activity, only the methyl acceptor homocysteine was required, whereas as for the N_5-methyl FH_4 transferase activity, in addition to homocysteine, AM and β-mercaptoethanol as reducing compounds were required. Cyano-vitamin B_{12}, deoxyadenosyl vitamin B_{12}, methyl vitamin B_{12}, reduced form of cyano-vitamin B_{12}, all stimulated the methyl FH_4 transferase reaction. Nutritional studies indicated that cyano-vitamin B_{12} deficiency led to a marked reduction of both transferase activities and replenishment with cyano-vitamin B_{12} or Barker's coenzyme restored both the activities. When ^{60}Co-cyanocobalamin was injected to vitamin B_{12} deficient chicks, radioactive incorporation was observed in the fractions which coincided with methyl FH_4 transferase activity in blood. Incorporation was also observed when normal rats were injected with the radioactive vitamin B_{12} but to a less extent. Methionine supplementation resulted in reduction of methyl FH_4 transferase activity but not methyl vitamin B_{12} transferase activity.

The methyl analogue of dimethylbenzimidazole cobamide reacts non-enzymatically, spontaneously but slowly with homocysteine to yield methionine[50]. The reaction was increased tenfold by the addition of purified vitamin B_{12} enzyme and the rate of reaction was proportional to vitamin B_{12} concentration. No cofactors or substrates other than homocysteine were required. But the presence of air or cofactors needed for methyl transfer from N_5-methyl FH_4 decreased the rate considerably. A quantitative conversion of methylcobalamin to methionine occurred when an excess homocysteine was present. The methyl group of methylcobalamin that is transferred to homocysteine is linked to the cobalt atom. Direct confirmation for this was obtained using ^{14}C-methyl vitamin B_{12} and homocysteine[16,17,51].

Weissbach *et al.*[42], working with extracts of wild type *Esch. coli*, have provided evidence that methyl vitamin B_{12} can form an active vitamin B_{12} protein under conditions where cyano-vitamin B_{12} and deoxyadenosyl vitamin B_{12} are not active. Activity with vitamin or coenzyme could be obtained if they are converted to vitamin B_{12}, a vitamin derivative containing divalent cobalt. These results led to the postulate that the active species in methyl transfer from N_5-methyl FH_4 is a reduced vitamin B_{12} protein. Weissbach *et al.*[50] have observed that among the different alkyl derivatives tested

only ethyl vitamin B_{12} and β-propionyl vitamin B_{12} could replace methyl vitamin B_{12}. This is explained by assuming that the enzyme can cleave the alkyl cobalt linkage in those vitamin B_{12} derivatives that are active, yielding a reduced vitamin B_{12} derivative, very likely protein bound and the corresponding carbanium ion. The reduced vitamin B_{12} protein can then accept a methyl carbanium ion form N_5-methyl FH_4 and transfer it to homocysteine. Guest *et al.*[23] have also postulated that the active species in the methyl transfer is a reduced vitamin B_{12} protein. The alkyl derivatives which could replace methyl vitamin B_{12} were found to inhibit the methyl transfer from N_5-methyl FH_4 suggesting that they are bound to the enzyme but were not cleaved to yield active reduced vitamin B_{12} protein.

In considering this mechanism of methyl transfer, the possibility also exists that the postulated reduced vitamin B_{12} protein, active in methyl transfer from N_5-methyl FH_4, also acts in the transfer of methyl group from exogenous methyl vitamin B_{12} to homocysteine. However, the data of Weissbach *et al.*[50] do not support such a hypothesis. A 100-fold purified enzyme from an *Esch. coli* mutant catalysed the transfer of the methyl group from N_5-methyl FH_4 to homocysteine and also the transfer of the methyl group from methyl vitamin B_{12} to homocysteine[51]. If the same enzyme is responsible for these two methyl transfers, there is a possibility of two distinct catalytic sites on the same enzyme molecule.

One obvious possibility[23] is that the methylcobalamin is a free cyclic intermediate. It combines with the enzyme to form a complex from which the methyl group is transferred to homocysteine with a consequent liberation of the enzyme (which would be free to combine with another molecule of cobalamin) and a non-methylated cobalamin (possibly hydroxycobalamin or vitamin B_{12}), since the hydridocobalamin, if formed, would presumably suffer immediate oxidation. If this hypothesis is correct, then the vitamin B_{12} enzyme would in reality be the methylcobalamin complex and not an enzyme bearing a cobalamin derivative as a prosthetic group.

Another possibility (Fig. 2), which is also in accordance with the fact that the vitamin B_{12} derivatives are generally found strongly bound to protein, is that the prosthetic group of the vitamin B_{12} enzyme is a non-methylated cobalamin derivative and it accepts a methyl group from N_5-methyl FH_4 with transient formation on the enzyme of a methylcobalamin prosthetic group. The methyl group is then transferred to homocysteine and the vitamin B_{12} enzyme liberated in a different oxidation state. It is possible that the prosthetic group, in the state in which the methyl group is accepted, may be hydridocobalamin or vitamin B_{12s} stabilized by combination with the enzyme protein. In the second hypothesis, it is possible that a non-methylated prosthetic group takes up a methyl group from methylcobalamin and the cycle continues or the non-methylated vitamin B_{12} enzyme dissociates and the apoenzyme again can recombine with methylcobalamin.

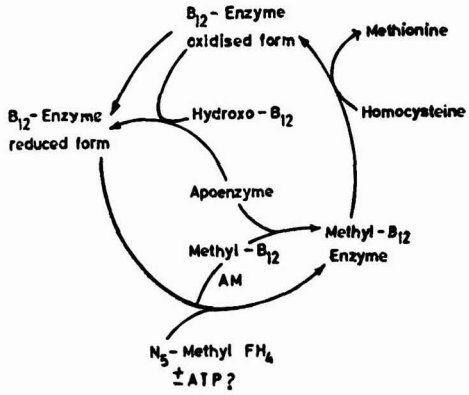

Fig. 2 — Possible mechanism for a function of methylcobalamin in methionine biosynthesis

The simplest interpretation would be that AH is the actual substrate methylated by vitamin B_{12} enzyme system and that the primary product is AM which then reacts with homocysteine yielding free methionine and another molecule of AH in a cyclic reaction in which catalytic quantities of AM would suffice. However, it has not been possible to demonstrate the methylation of AH and AH will not replace AM or homocysteine. It is possible that AM may act in its customary role of methyl donor and generate molecules of methylcobalamin thus initiating the cycle. It is, therefore, of interest that a chemical and enzymatic synthesis of methylcobalamin from AM and hydridocobalamin has also been described[41,47,50].

Kerwar *et al.*[19] have further studied the formation of methylcobalamin in a pig liver system. They have fractionated the system into two fractions, A and B, on DEAE-cellulose. Fraction A had a very low activity even in the presence of AM, but the activity was substantially increased by the addition of methyl vitamin B_{12}. Fraction B showed very little activity and was not stimulated by methyl vitamin B_{12} but it stimulated AM supplemented fraction A. These workers conclude that the ammonium sulphate fraction contains three forms of methionine synthetase, an active holoenzyme containing bound methyl vitamin B_{12}, an apoenzyme that can be reacted by added vitamin B_{12} and a second inactive one in which there is a bound breakdown product of methyl vitamin B_{12}. Reactivation of this last form via ancillary enzyme present in fractions A and B involves the following sequence:

$$B_{12} \longrightarrow B_{12r} \longrightarrow B_{12s}$$
$$B_{12s} + AM \longrightarrow \text{Methyl vitamin } B_{12} + AH$$

These workers believe that methyl vitamin B_{12} is not an intermediate in the process of methyl transfer and have experimental evidence to show that methyl vitamin B_{12} is a poor methyl donor compared to N_5-methyl FH_4. It is suggested that as methyl vitamin B_{12} does not appear to be the primary methyl donor in the transfer of methyl

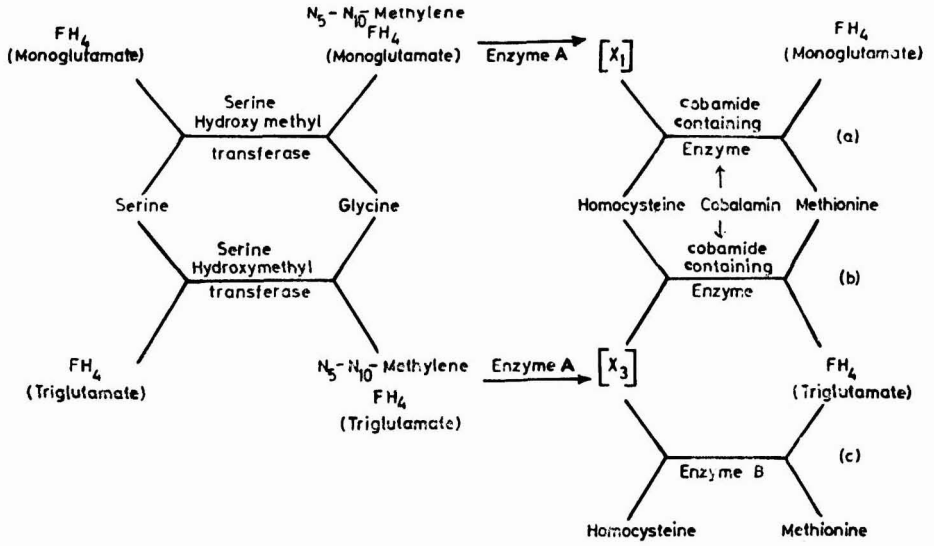

Fig. 3 — Possible enzymic mechanism for methionine from homocysteine and serine

group from N_5-methyl FH_4 to homocysteine, its function is probably related to labilization of the methyl group from N_5-methyl FH_4.

In conclusion, Foster *et al.*[52-54] have proposed the following mechanism of methionine biosynthesis, involving vitamin B_{12} and also a vitamin B_{12}-independent pathway (Fig. 3). In the absence of added cobalamin, C_3 of serine is converted into methyl group of methionine via serine hydroxymethyltransferase, enzyme A and an enzyme B with triglutamate FH_4 as cofactor (pathway C). When cobalamin is available, the second mechanism can operate by using the cobamide containing enzyme as an alternate to enzyme B (pathway B). Although in both the mechanisms the triglutamate can be used as cofactor, monoglutamate is effective only in the cobamide dependent mechanism (pathway A) and inhibits pathway C. Mutation to loss of the enzyme A gives an auxotraph responding either to methionine or cobalamin. This postulation of two pathways have found support in the experiments of Buchanan and coworkers[40]. On the other hand, in mammalian systems, there appears to be a specific requirement for vitamin B_{12}.

Foster *et al.*[41] have proposed a detailed mechanism for the enzymatic transfer of the methyl group to homocysteine involving AM and vitamin B_{12} co-enzyme (Fig. 4).

The first step in the reaction is the reduction of the enzyme vitamin B_{12} to a valency of one (I). This process is carried out by a pyridine nucleotide dependent flavoprotein. This reduced compound then reacts with AM to yield AM-vitamin B_{12} enzyme (II). Methyl vitamin B_{12} is directly formed on the enzyme by the migration of the methyl group from AM to vitamin B_{12} to form enzyme vitamin B_{12} methyl AH (III). The AH thus produced on the enzyme is methylated by N_5-methyl FH_4 so that when methyl group of cobalamin is transferred to homocysteine (IV and V), AM is available to remethylate the unstable vitamin B_{12} by reaction (II). This mechanism suffers from the following drawbacks: (i) AH does not meet the requirements for AM. One explanation suggested is that AM is tightly bound to the enzyme and externally added AH is hydrolysed by nucleotide pyrophosphatase. (ii) When substrate amounts of ^{14}C-AM are added, it does not appear in methionine. If substrate amounts of AM and the enzyme are incubated in the absence of homocysteine the enzyme should be labelled and on addition of homocysteine the label should be transferred to it. This experiment has not been reported. (iii) Kerwar *et al.*[19] have shown that the addition of N_5-methyl FH_4 severely depresses the transfer of ^{14}C-methyl groups from methyl vitamin B_{12} suggesting that N_5-methyl FH_4 is the primary methyl donor. (iv) There is no known biochemical reaction in which such a sequence occurs and it is difficult to explain the requirement for all these cofactors when AH gets directly methylated by N_5-methyl FH_4. It should be possible that AH should substitute for homocysteine which is not the case.

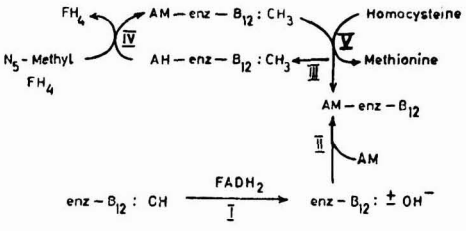

Fig. 4 — Mechanism of methionine biosynthesis

An excellent system to study such a reaction would be plants which are known not to contain vitamin B_{12}. It would be worth while to investigate in detail the mechanism of transfer of methyl groups from methyl FH_4 to homocysteine with the isolated enzyme systems in plants.

Summary

The biosynthesis of methionine in microorganisms, ovine and mammalian tissue, reviewed in this paper, has led to a clear understanding of interrelationship of folic acid and vitamin B_{12}. The study of the reactions involved has also led to the discovery of a new intermediate, N^5-methyltetrahydrofolate, involved in transmethylation reaction. Different routes for the synthesis of methionine have been observed in microorganism and in ovine and mammalian tissues depending upon the requirement of the form of folic acid derivative. The vitamin B_{12} coenzyme requirement in the final transfer of methyl groups from N^5-methyltetrahydrofolate to homocysteine has been demonstrated in the mammalian and in some *Escherichia coli* strains. Involvement of 5-adenosyl methionine in the transmethylation from N^5-methyltetrahydrofolate to homocysteine reaction is yet under active investigation and further substantiation is necessary to give a definite role for this compound in the reaction. It will be interesting to see whether similar biosynthetic mechanism also exists in plants which synthesize methionine. It is really fascinating that serine, a non-essential amino acid, donates a carbon atom to the synthesis of an essential amino acid, methionine, the reaction requiring both folic acid and vitamin B_{12} in coenzyme form.

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Molecular Basis of Viral Multiplication*

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WHAT is a virus? The definition always creates problems. So it is better to avoid a definition. There has been a good deal of controversy in the past and even today regarding the 'living' nature of the viruses. Since it is equally difficult to elaborate what 'life' is, the differences between the 'living' and the 'non-living' have to be shelved for a while. However, it cannot be denied that viruses have provided the maximum opportunity to analyse a number of complex situations which arise in the borderline of the living and the non-living. The parasitic nature of the viruses, i.e. their inability to grow without a host, indicates a low 'form' of life, but the viruses are more than parasites as they themselves know all the tricks of 'living'. They just need a platform to perform the tricks which are in no way less intelligent than those associated with the growth and reproduction of not only micro-organisms but also the giant mammals like elephants. It is not wise to engage oneself at the start in the solution of the problem of evolution, i.e. to decide whether viruses evolved before their hosts or whether they are derived from their hosts. But a question like the above raised in the very beginning keeps one alert about the problem to be faced in the course of discussion. The discovery of the viruses is as exciting as the discovery of life itself. Day-to-day experiences have helped us to understand the latter. In case of viruses, however, not only are the day-to-day experiences useless but also misleading in understanding the mechanism of their vegetative growth in parallel with the growth of the microorganisms. Both sexual as well as asexual reproductions are commonly encountered. But the vegetative growth of viruses is more than asexual reproduction. Most of our discussion will centre around this problem which can be designated as 'multiplication' to avoid controversial issues.

Historical Background

In the beginning of this century it was well established that the microorganisms are responsible for the pathogenic conditions. But a dramatic situation developed when Beijerinck in Holland was working with the mosaic disease of tobacco plant. It was already established by Pasteur that the bacteria were unable to pass through unglazed porcelain filter. What Beijerinck observed was that the pressed juice of the infected leaves of tobacco, after being filtered through unglazed porcelain, still retained the ability to infect fresh leaves. Thus the virus responsible for the tobacco mosaic disease (TMV) was the first filterable virus to be discovered. We are all familiar with the exciting achievement of Stanley in 1935 when he first crystallized the TMV which perhaps laid down the foundation of molecular biology. An organism appeared more as a molecule than as a complex myriad of organizations. But, after all, this was the disease of a plant. How about the animal diseases? The breakthrough came in 1900 when two German bacteriologists, Frosch and Dahlem, first found that foot and mouth diseases of cattle were due to an agent which could pass through the bacteria-proof filter. Then the claims poured in regarding the discovery of more and more filter-passing agents responsible for the diseases of the human as well as the animals in general. Some of these claims were eventually found to be baseless, but some stood the test of time. Unfortunately, or rather fortunately, difficulties were experienced with two important cases: one, the cow-pox of Jenner and the other, rabies dealt with by Pasteur. The causative agents in both the cases failed completely to pass through the filters. It took long time to realize the differences in terms of size. A virus may not necessarily be as tiny as to pass through the bacterial filter. It was merely by chance that the early viruses dealt with were filter-passing.

Bacterial viruses were first discovered in 1915 when Twort[1] isolated a filterable virus which produced a 'glassy transformation' of micrococcal colonies during the growth on the agar surface. This outstanding observation of Twort remained practically in darkness for two years till d'Herelle[2] in 1917 announced the discovery of a filterable agent, the 'bacteriophage', capable of lysing the growing cultures of enteric bacilli. The possibility of combating pathogenic bacteria with bacteriophages immediately created a sensation in the medical world. Soon after the discoveries of Twort and d'Herelle, a large number of bacteriologists got involved in studies on 'bacteriophages'. It took some time, however, to realize the actual nature and the mode of action of the bacteriophages. The tremendous knowledge we have acquired in last one or two decades regarding the mechanism of viral growth has been mainly through studies with the plant virus TMV and the bacteriophages. Naturally, most of our discussion will be centred around the bacterial viruses.

Composition and Structure of Viruses

Though viruses are unable to multiply on anything less complex than the interior of a living host cell, they themselves are very simple organizations. Essentially, they consist of a shell or coat of protein enclosing a core of nucleic acid. The core is usually deoxyribonucleic acid (DNA) which is the hereditary material for almost all the living entities so far known. Actually, the occurrence of DNA in the viruses indicates its involvement in living phenomenon. The unusual feature of some of the

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viruses like TMV, polio virus, encephalomyocarditis and bacteriophages like MS2, R17 and fr is the occurrence of ribonucleic acid (RNA) instead of DNA. Perhaps these RNA-containing viruses are the only instances which are dependent on RNA acting as the hereditary material. Most of the naturally occurring DNA exist in the double-stranded form which is referred to as the native state. Again some of the viruses like $\phi x174$ are exceptions which contain single-stranded DNA though the actual replicating form is double-stranded[3,4]. Same is found to be true for RNA-containing viruses[5,6]. Normally, they are single-stranded but during their multiplication the double-stranded form is the intermediate.

The shell or coat protein as already referred to serves as a protective jacket and also as a tool for breaking down the walls of the living cells which the viruses are able to attack. In many cases, like the T-even bacteriophages, there are tail-like structures which help them to sit on the bacterial wall and dissolve the cell-wall material with the help of the lysozyme contained in the tail. Usually, the sheath contracts and a spike-like structure protrudes out which helps the insertion of the hereditary material, in this case DNA, within the cell. The protein coat is left outside. This was unequivocally demonstrated by Hershey and Chase[7] using ^{32}P-labelled and ^{35}S-labelled viruses.

Most viruses fall in the size range between 10 and 200 millimicrons and naturally can be seen only under the electron microscope. As mentioned already, they are found to have different sizes and shapes. For instance, TMV look like slender rods with visible subunits. One RNA molecule (molecular weight of the order of 10^6) is coiled up in helical form and 1800 protein subunits are oriented along the spiral in perfectly organized fashion so as to give the overall rod-like shape. By osmotic shock it is possible to have RNA out of the protein sheath and leave the empty shell behind. Under controlled conditions, the reverse process, i.e. the recombination between the protein and nucleic acid units to make the fully formed virus, can also be carried out. Actually by this process, it was possible to demonstrate that the RNA is the hereditary material and the infection of the tobacco leaf is possible with this component only[8,9]. Protein coat is of no use in this respect.

Most small viruses, which look spherical under the electron microscope, are actually regular polyhedrons. Adeno virus, polyoma virus, poliomyelitis virus, etc., are the best examples. The structures of the T-even bacteriophages as revealed by the electron microscope have interesting features. They have hexagonal heads which contain the DNA in the tightly coiled form and tails which act more or less as injecting syringes. The distal end of the tail has some fine structures as well as lysozyme and some metal ions.

We have already mentioned about the mechanism by which the DNA is injected into the host cell through this tail. The viruses also exhibit remarkable specificity with respect to the host. One of the factors which must determine this specificity at the early stage of infection is the structure of the cell wall of the host.

Life Cycle of Bacteriophages

Let us consider the life cycle of T-even bacteriophages to illustrate viral reproduction. As soon as proper spatial relationship between the host and its virus is established, the DNA is injected into the host cell. Following this there is apparently a period of inactivity in the sense that the metabolic machinery of the host cell comes to a halt. This is the period during which the viral DNA takes control of the machinery of the host. Volkin and Astrachan[10], searching for new metabolites formed during this period, came across a new type of RNA whose base composition agreed more with that of the viral DNA than that of the host DNA and RNA. For some time the function of the viral-specific RNA was not understood. With the introduction of the messenger RNA concept by Jacob and Monod[11] and the discovery of the enzyme RNA polymerase (Burma[12]), it became clear that this T-specific RNA was the messenger RNA for the virus and was produced on the template of viral DNA. It was expected that the production of the host messenger RNA should be stopped at this stage and the viral messenger RNA should take its place in the dictation of protein synthesis.

Even before the concept of messenger RNA developed and the implication of limited RNA synthesis on viral infection was understood, the brilliant work of Flaks *et al.*[13,14] and eventually of Kornberg and his coworkers[15] showed the tremendous rate at which some specific enzymes were synthesized after the so-called 'eclipse' period. Even before these experiments, the general features of the viral metabolism were gradually being understood. For instance, Cohen[16] showed, for the first time with the use of ^{32}P as tracer, that the phage DNA is synthesized within the host with the help of the nutrients drawn from the medium by the bacterium after infection. It has already been mentioned that with the use of phage particles, labelled either in DNA with ^{32}P or in the protein coat with ^{35}S, Hershey and Chase[7] demonstrated that the coat was left outside and only the DNA invaded the host. There was some controversy in the beginning regarding the requirement of both the nucleic acid and protein of TMV for infection, but eventually the speculation was set at rest with the clear-cut demonstration that the infectivity was associated with the nucleic acid only[8,9].

Some time before the classical work of Kornberg and his coworkers[15] was published, Flaks *et al.*[13,14] had already shown that within several minutes after infection by T-even phages a new enzyme which hydroxymethylates deoxycytidine-5'-phosphate (dCMP) is produced. This was expected because T-even phage DNA contains hydroxymethylcytosine instead of cytosine present in the host DNA. This early observation was well supported by the finding of Kornberg *et al.*[15] who showed that several enzymes were induced in the phage-infected cells, one of which converts hydroxymethyl dCMP to hydroxymethyl dCTP, the proper substrate for DNA synthesis. Another newly synthesized enzyme

converts dCTP to dCMP, naturally to destroy the building blocks of the host DNA. There are also enzymes induced which are capable of glucosylating the hydroxymethyl groups of cytosine. T-even phage DNA has the characteristic feature of possessing glucosylated hydroxymethyl groups. Other than the level of these two enzymes, levels of several other enzymes already existing within the uninfected cell are also boosted up. These enzymes are involved in the phosphorylation of nucleotides to the triphosphates which are the true precursors of DNA synthesis. And all the more, the level of the DNA polymerase, which is responsible for the synthesis of DNA, is boosted up several fold. In the beginning it was not well understood whether the increased level of DNA polymerase was due to the accelerated rate of synthesis of host DNA polymerase or due to the synthesis of a new type of phage-specific polymerase. The latter view was found to be correct by immunological experiments[17]. This was found to be true not only for DNA polymerase but also some other enzymes, indicating clearly that phage genome has the informations for the syntheses of enzymes required for the syntheses of phage DNA and protein (Cohen[18] and Champe[19]). These enzymes, though may be similar in catalytic activity, may not necessarily be identical with the host enzymes. Subsequent to the report of Kornberg *et al.*[15], numerous reports have appeared which indicate the induction of a large number of enzymes in the host due to viral infection which are all connected with the metabolism of the virus. These observations have led to a clearer understanding of the series of events that take place after viral infection. The phage DNA that is injected in at first knocks the DNA of the host out of the position of control switch and starts producing its own messenger RNA. It has been shown by recent experiments that ribosomes which are normally involved in the formation of polyribosomes with host messenger RNA can be converted to polyribosomes bound with viral messenger RNA (Ames and Martin[20]). The next event is naturally the synthesis of the enzymes with the help of protein-synthesizing machinery of the host as dictated by the viral messenger RNA. These enzymes start synthesizing the building blocks of DNA at a fast rate and finally assemble those into DNA molecules. Within a short while after the DNA synthesis starts, the protein coats are also independently synthesized. At a later stage the combination between DNA and protein is initiated so that the fully formed phage particles are produced. Finally, a stage is arrived at when a large number of phage particles are formed (the number being defined as the 'burst size' of the particular phage) and the cell bursts open due to the lytic enzymes produced within the cell and the viruses come out of the host after completing their growth cycle and are ready to invade fresh host cells.

A few aspects of the vegetative growth of the RNA-containing phages may be worth mentioning here. It is quite evident that if RNA is the hereditary material of the RNA-containing phages, there should be naturally a mechanism for the duplication of RNA on its own template. Working with the RNA-containing phage MSϕ2, it was possible to demonstrate that the extracts of the host infected with this phage contain a new enzyme which can synthesize RNA from the nucleoside triphosphates[21,22]. The template requirement of this enzyme could not be demonstrated as the enzyme was associated with large amount of phage RNA though the indirect evidences indicated that this associated RNA acts as a template for RNA synthesis. This phage-specific enzyme is referred to as RNA-synthetase to distinguish it from RNA polymerase. Subsequently, it was demonstrated that the replicating form of these RNA-containing phages is double-stranded RNA[5]. As mentioned before, ϕx174 DNA is single-stranded, but after infection of the host the DNA becomes double-stranded and the complementary copy formed actually contains the messages for the production of the phage[23]. The infective RNA has been isolated and the X-ray diffraction studies indicate its double-stranded nature[5,6].

Lysogenicity

Sometimes the invading virus fails to lyse the cell and contrary to the general experience attaches itself to the bacterial chromosome. Now it cannot multiply as an autonomous body. The phage genome is duplicated along with the bacterial chromosome and in this integrated state it is transferred from one generation of the bacteria to the other. This stage of a bacterial virus or phage is called 'prophage' and the virus is classified as temperate virus. The prophages can also under certain conditions undergo the lytic cycle and then liberate new phages. Ultraviolet light, many chemicals and antibiotics act as inducing agents. The phages that the lysogenic bacteria produce on induction are in every respect similar to the infective phage. The best examples of the temperate viruses are λ phage of *Escherichia coli*, P22 phage of *Salmonella typhimurium*, etc. The history of development of our knowledge regarding lysogeny has been nicely elaborated by Jacob and Wollman[24].

How does a virus establish lysogenic relationship with the host? There is strong evidence (Luria[25]) that most of the prophages have definite positions on the host genome where they can attach themselves. Of course, there are exceptions as well. Working with the mutants of λ, Kaiser and Jacob[26] showed that there is a region called 'c' region in the phage genome which is responsible for the establishment of the prophage state. Different mutants in the 'c' region attach themselves to different positions of the host genome. A mutant of λ termed λba, which has 'c' region deleted (having 18 per cent less DNA content), cannot establish itself as a prophage (Kellenberger *et al.*[27]). It either multiplies vegetatively or establishes an abortive lysogenization, i.e. the phage does not multiply. Very recently, several workers have been able to demonstrate the complementarity of base sequence between the host genome and the viral genome by hybridization tests[28,29]. One should, however, remember that the genetic mapping clearly

indicates that the attachment of the viral genome does not disturb the linear sequence of the 'genes' and the spatial relationship must be of branched nature. The lysogenization is found to be intimately associated with the immunity of the cell to superinfection, i.e. a cell carrying a prophage cannot be superinfected by homologous phage. Regarding the immunity of a lysogenized cell towards superinfection, it has been established that if the superinfecting phage has the region responsible for lysogenization homologous to that of the prophage then the immunity operates[30]. This immunity may be either due to the steric hindrance or it may be due to some repressor produced within a cell which inhibits the vegetative multiplication of the phage.

The exact mechanism of lysogenization is still not known but some simple experiments performed by Jacob and Wollman[31] first showed that some repressor molecules are produced in the cytoplasm by the lysogenized cell and these cytoplasmic repressor molecules inhibit the vegetative production of the virus. Male and female strains of *Esch. coli* were chosen for these experiments. The media and the autotrophic strains were chosen in such a way that only the female or the recipient cells can grow. When the male or the donor cells containing no prophage were crossed with female lysogenized cells, the recombinants were still lysogenic as expected. Similarly, the cross between the male and female cells, both of which contained the prophages, led to the recombinants which were again lysogenized. But when the donor cells containing prophage were allowed to mate with recipients containing no prophage, the lysis of the latter took place. This zygotic induction clearly pointed out that the lysogenized cells must have some repressor(s) present in their cytoplasm. So any prophage transferred to a cell containing no repressor is expected to undergo vegetative multiplication.

It has already been pointed out that in case of virulent viruses the viral genome takes control of the bacterial machinery for protein synthesis. But what does happen in case of temperate viruses? The temperate viruses do not, under normal conditions, multiply autonomously and for their own duplication they need multiplication of the host cells as well. Naturally, any change in the biochemical activities of the host is not expected under these conditions. Though the mechanism of lysogenization is not very clear, some work has, however, been done at the biochemical level with the temperate viruses which points to at least partial influence of the viral genome on the host metabolism[32].

Conversion and Transduction

Cohen[18] and Luria[25] have discussed in detail the conversion of bacterial properties by the temperate phage genome. For example, the infection of *Salmonella* by the phage PLT22 initiates the production of somatic antigen 1 on the bacterial surface. A number of mutant phages termed as 'e' mutants have been isolated. Depending on the mutant, the nature of the antigen produced by the host varies. The somatic antigen normally contains a galactosyl-mannosyl-rhamnosyl sequence in which the galactosyl-mannosyl linkage is α. But if the mutant e^{15} infects the host the antigen produced has a β-linkage between galactose and mannose moieties. When a cell infected with e^{15} is superinfected by e^{34}, then the superinfecting phage induces the addition of glucose to the β-galactosyl residue of the antigen induced by e^{15}. But for this reaction to go on, the cell has to be previously infected by e^{15} because the enzyme produced as a result of e^{34} infection needs e^{15} antigen as its substrate. This situation is not understood at all as these antigens are not required by either the host or the virus. The developmental cycle of the phage is completely independent of the antigen production; for example, the production of e^{34} antigen needs superinfection by e^{15} but the life cycle of the phage e^{34} completes itself irrespective of the presence or absence of e^{15}.

The phenomenon of 'transduction' as discovered in connection with the studies with the temperate viruses not only helped tremendously in genetic analysis but also laid the basis of understanding of the interrelationship between the host and the phage genomes. In their classical work, Zinder and Lederberg[33] demonstrated that a temperate virus when induced may carry along with its own genome a portion of the genetic material of the host. This genote may be transferred to the new host which it lysogenizes. This phenomenon of transformation through the temperate virus was referred to as transduction. The biochemical changes occurring within a host cell as a result of viral infection, for instance, the antigen production, are due to the genes belonging to the phage genetic material. Biochemical changes are also expected due to the bacterial genome carried along with the phage genome. Luria and his coworkers[34-37] have done extensive biochemical analysis of the temperate virus P1. This virus can infect the enterobacilli. When it grows on *Esch. coli* it picks up the lac region (region responsible for the synthesis of β-galactosidase) of the host, looses some of its own genetic material and becomes defective in the sense that it cannot multiply as it has lost a part of its own genome. This defective virus is called P1dl i.e. P1 defective in lactose region. In order to study this temperate virus *Esch. coli* lac^- cells were infected with different types of P1dl phages. It was observed that the *Esch. coli* lac^- cells can behave as lac^+ as a result of introduction of the lac region by the virus. The study of the kinetics of β-galactosidase induction after the introduction of lac genes by P1dl into a lac^- host led to the observation that there was a lag period of about 15 min. after which there was an accelerated rate of enzyme synthesis which is then followed by a linear rate of production. The obvious question is "whether for the expression of the lac gene carried by P1dl has the phage DNA to be incorporated into the host genome?" It has been established that for the expression of the phage-carried lac genes the phage genome need not be incorporated into the host DNA. The structural gene (Z^+) for β-galactosidase introduced by the phage is not fully influenced by the regulator gene i of the host but can be controlled by the i of its own genome.

It takes about 60 min. for the repressor(s) to be produced and expressed. The same type of observation was made with the temperate phage λ as well.

What is the ultimate fate of the prophage? As mentioned before, either spontaneously or by physical and chemical agents like ultraviolet, mitomycin C, H_2O_2, 5-fluorouracil, etc., these prophages can be induced to start vegetative multiplication. Then DNA duplication starts, complete phages are produced and finally the host is lysed liberating free phages. As we know that after infection with virulent phages some new enzymes are induced and there is also an increase in the level of many enzymes. Naturally, it was interesting to look for similar changes when the prophages are induced to liberate normal virus particles. Weissbach and Korn[38] have shown that lysogenic induction of K12(λ) causes the appearance of a new DNAase activity, not previously observed in the uninduced cells. If *Esch. coli* carrying the prophage λdg or P1dl is induced by ultraviolet treatment to enter the lytic cycle, then the level of the β-glactosidase activity of the cell increases about 100-fold. It appears as if there is derepression of the lac genes[39-41]. There is something interesting in the phage P1dl which is different from λ. If a cell carrying the prophage P1dl is superinfected by λ and then treated with ultraviolet there is no derepression of lac genes, i.e. the level of β-galactosidase does not increase. The phage λ is more sensitive to ultraviolet induction than P1dl. Hence ultraviolet treatment induces rapid multiplication of the λ genome and, therefore, P1dl does not get any chance to multiply. But superinfection by the virulent phage P1KCvir has the same effect as that of ultraviolet.

Viral Genetics

Finally, a few words about the genetics of the viruses. We have already mentioned about the process of transduction which is due to the temperate phages carrying the genetic material from one bacterial strain to another. Studies on transduction have yielded much information regarding the genetic map of the host strains. Actually it is one of the most useful methods in carrying out genetic mapping. We have also mentioned about the process of 'conversion' which indicates the changes in the immunological properties of the cell wall of the host which is lysogenized. Considerable knowledge has been acquired regarding the genetics of the phage themselves. We are indebted to Seymour Benzer[42] for the fine structure analysis of the gene of a T-even bacteriophage. This was done mostly by recombination analysis. When a host is infected by two different mutant phages which are defective in two different regions, wild phage may be produced as a result of recombination. This has led to the possibility of genetic mapping in the case of viruses. The phenotypic expression of the genetic characters of the viruses may be visualized mainly in two different ways. First one is the host-specificity. For example, wild strain of T4 can grow on the wild strain of *Esch. coli* (*Esch. coli* B) as well as *Esch. coli* K (a lysogenic strain K12 containing phage λ) and *Esch. coli* S (a non-lysogenic variant of K12). All the three different types of r mutants of the phage T4 (rI, rII, rIII) grow on *Esch. coli* B as well as *Esch. coli* S. Though rI and rIII mutants can grow on *Esch. coli* K, rII mutants cannot grow on this particular host. Secondly, these mutants vary in plaque morphology as well. Wild type T4 produces small, fuzzy plaques (type w) on *Esch. coli* B whereas r mutants (all the three types) produce clear plaques (type r to indicate rapid lysis) on the same host. On the other hosts, however, the different strains may produce either 'r' or 'w' type plaques. rI mutants produce 'r' type plaques on *Esch. coli* S as well as on *Esch. coli* K, thus behaving in the same way on all the three hosts. rIII mutants, on the other hand, give 'w' type plaques on *Esch. coli* S as well as *Esch. coli* K in contrast to 'r' type on *Esch. coli* B. rII mutants give rise to 'w' type on *Esch. coli* S and as already mentioned do not grow on *Esch. coli* K at all. There are also so many different types of morphological variations, e.g. some may give rise to mottled plaques, others may give rise to small or large size plaques. These characteristics have enabled Benzer and coworkers to isolate a huge number of mutants of not only T4 but also of other phages. As mentioned before, Benzer has been able to carry out the fine structure analysis of the T4 genome and this is also being done in case of other phages.

In another area viruses have been extremely useful. We are all aware of the exciting development following the discovery of the 'genetic code' (Nirenberg and Mathaei[43] and Ochoa[44]). The mutants of TMV have substantiated the validity of the 'code' arrived at from the experiments with cell-free preparations. The RNA isolated from TMV may be subjected to limited alteration of chemical structure by treatment with HNO_2 as well as other chemical agents. These altered RNA molecules are able to infect tobacco leaves and give rise to mutant viruses, which vary not only in the nature of their nucleic acids but also the structure of the protein coats. Since the complete chemical analysis of protein chain of TMV has been carried out, it is easy to demonstrate the change in amino acid residues of the protein coat. It is possible to locate the change of amino acid residues of the protein by the finger printing method. Extensive data have been collected by Wittman[45] as well as Tsugita and Fraenkel-Conrat[46,47] which clearly indicate that the change in amino acid composition is in agreement with the genetic code proposed in terms of the base composition of the hereditary material, which in this case is RNA.

Voluminous literature has accumulated over the past one or two decades on the biochemistry of the host-virus relationship. In the present discussion no attempt has been made to cover all the areas and to catalogue all the facts. Anyone interested in details may refer to one of the recent excellent reviews published in this area[18,19,25]. In the present review only the salient points have been stressed to give an overall impression of the host-virus relationship to those who are interested in this borderline area which demands a knowledge of biochemistry, biophysics and genetics.

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Second Harmonic Studies in Alternating Current Polarography*

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THE first recorded observation of the distortion of the alternating current through the occurrence of a redox process was made by Müller *et al.*[1]. These workers found the distortion to be negligible at the half-step potential, a fact that could be used to locate this point. Later, van Cakenberghe[2] gave a mathematical analysis that showed the potential at which the second harmonic is zero to depend on the transfer coefficient of the process. Bauer and Elving[3] applied this method to the determination of transfer coefficients, but obtained results that differed from values obtained by other methods. Theoretically, van Cakenberghe's analysis[2] is incomplete since, in the derivation of the second harmonic current, three types of terms can be considered[4] as contributing to the magnitude of the current, but van Cakenberghe considered only one of these terms. An expression taking into account two types of terms was given in a general form by Aten[5] and in an explicit form by the present author[6]. This equation appeared to be supported by experimental data, but it has now been found that this treatment also is incomplete. The full analysis leads to an expression given in a general form in the earlier paper[4], and is shown in an explicit form in Eq. (2). For the particular case of limitingly low frequencies, where the kinetic parameters do not influence the process, the expression reduces to a relation given earlier in a general form by Senda and Tachi[7] and in an explicit form by Smith and Reinmuth[8].

In 1960, the author[9] pointed out that second harmonic measurements are a possible way of increasing the sensitivity of a.c. polarography by reduction of the background current which is due to the double-layer capacity. This was confirmed by the work of Smith and Reinmuth[8]. Delahay *et al.*[10] pointed out that this feature could also be of benefit in fundamental studies. Eq. (2) provides the basis for the realization of this possibility. The application of second harmonic measurements to analytical work has been considerably developed by the recent work of Neeb[11,12].

Experimental Requirements

In comparison with a.c. polarography, the second harmonic measurements require in addition that the detector be capable of measuring the second harmonic component in the presence of the larger fundamental component. The discrimination needed to accomplish this increases at higher frequencies and at lower concentrations of depolarizer due to the effect of the double-layer capacity. But increasing the amplitude of the alternating voltage decreases the required discrimination, since the total current (capacitive as well as faradaic) is proportional directly to the amplitude of the voltage, while the second harmonic component increases as the square of the amplitude of the voltage (for amplitudes that do not exceed a few tens of millivolts). For example, with a voltage of about 5 mV. amplitude the faradaic current of a fast process contains about 10 per cent second harmonic component at the potential where the latter attains a peak value. Consequently, discrimination would become difficult only if the capacitive current were greatly in excess of the faradaic, and this is not the case at concentrations and frequencies normally considered. In attempts to increase sensitivity in analytical work, discrimination as well as the small magnitude of the signals might become a problem were it not for the fact that, in such work, it is not important to keep the amplitude of the voltage low; for instance, Neeb[12] has used amplitudes of over 30 mV., which would be unacceptably large in fundamental studies. In the latter, on the other hand, there is no need to use such small concentrations of depolarizer.

The main experimental requirement that needs some attention is the freedom from second harmonic component of the applied voltage. It is obvious that if the applied signal contains second harmonic, then the second harmonic polarogram will show this as a background current which has the form of a conventional a.c. polarogram. Not only is it necessary that the source provide a pure signal, but the output impedance of the source needs to be low. The cell comprises a non-linear load of low impedance, so that an oscillator with the usual output-impedance of say 600 ohms may appear to be harmonic-free when fed into a 600 ohm resistive load and yet show appreciable harmonic distortion when fed into the polarographic circuit. One possible solution is to couple a suitable source to the circuit through a power amplifier that regulates the cell voltage via a feedback loop.

Given a cell voltage that is sufficiently free of second harmonic component, the background current observed will be due to electronic noise and to the non-linearity of the double-layer capacity; this offers a considerable decrease in background as compared with a.c. polarography, and makes it possible to carry out measurements that do not require base current corrections. However, in fundamental studies it is necessary to correct the observations for the drop of alternating potential across the ohmic resistances in the cell. The appropriate relation is given by Eq. (1).

$$V = \{(V_{cell})^2 - 2IRV_{cell}\cos\phi + I^2R^2\}^{\frac{1}{2}} \quad \ldots(1)$$

where V is the alternating voltage at the interface; V_{cell}, the voltage across the cell; I, the total alternating current; R, the ohmic resistance of the cell; and ϕ, the phase angle between I and V_{cell}. It

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is apparent that this correction requires measurement of I, R and ϕ; usually, however, the value of V is not critically determined by the magnitude of ϕ, and a simple and not accurate determination of the latter suffices, by direct comparison of current and voltage traces on a double-beam oscilloscope.

Thus, the experimental requirements of second harmonic studies are more comprehensive than those of faradaic-impedance work, but the extra complexity seems warranted by the freedom from background current.

Determination of Kinetic Parameters

The values of the kinetic parameters can be obtained by comparison of observed second harmonic current values with the theoretically derived relation between this current, the kinetic parameters and experimental variables. The relevant equation for a system involving a single electron-transfer step and associated mass-transport by diffusion is believed to be as follows:

$$\Delta i_2 = \frac{n^3F^3ACV^2k}{2R^2T^2\,(e^P+d^{\frac{1}{2}})^2} \times \left\{ \frac{(e^P-d^{\frac{1}{2}})^2Z^2+4(\alpha-\frac{1}{2})(e^P+d^{\frac{1}{2}})(e^P-d^{\frac{1}{2}})Z+8(\alpha-\frac{1}{2})^2(e^P+d^{\frac{1}{2}})^2}{(Z^2+2Z+2)(Z^2+2\sqrt{2}Z+4)} \right\}^{\frac{1}{2}} \quad \ldots(2)$$

$$Z = k\sqrt{2/\omega D_R}\,(e^P+d^{\frac{1}{2}})\exp[-\alpha P] \quad \ldots(3)$$

$$P = nFE/RT \quad \ldots(4)$$

and k and α are the kinetic parameters; Δi_2, the amplitude of the second harmonic current resulting from superposition of $V\cos\omega t$ at the electrode of surface area A in a solution containing concentration C of depolarizer whose oxidized and reduced species have diffusion coefficients D_0 and D_R ($d=D_R/D_0$); n, F, R and T have their usual significance, and E is the electrode potential referred to the formal standard potential of the process.

Eq. (2) describes curves of the form shown in Fig. 1. For a particular value of the transfer coefficient, the symmetry of this curve varies with frequency in a manner that depends on the magnitude of the rate constant; for a given value of $k/(\omega D)^{\frac{1}{2}}$, on the other hand, the symmetry of the curve depends on the magnitude of the transfer coefficient. By comparing the experimental and theoretical curves, it is possible, therefore, to determine the kinetic parameters of a process. The procedure is fully comparable with that described in detail by Barker[13] for determining the kinetic parameters from measurements of the redoxo-kinetic potential, even though the actual mathematical terms involved are different in the two cases.

Scope of Second Harmonic Studies

Thus, it can be foreseen that second harmonic measurements will find application in a general way in electrochemical work, in the same way as a.c. polarography. In particular, the absence of background current encourages the idea that systems in which adsorption occurs may be more amenable to such work than they have proved to be to faradaic-impedance studies. In the latter, the capacity current is not only present but also changes in magnitude — in a manner that is not *a priori* known nor readily measured — according to changes in the composition of the system studied.

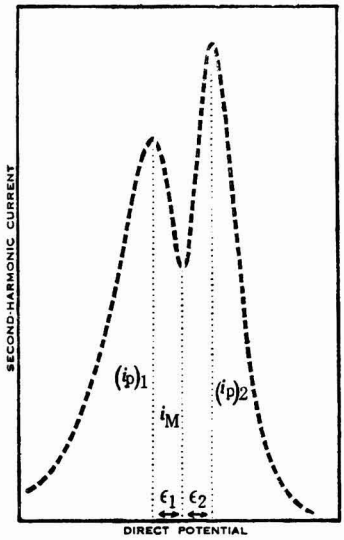

Fig. 1 — Variation of second harmonic current with polarizing potential [The magnitudes of $(ip)_1$, $(ip)_2$, i_M, ϵ_1, ϵ_2 depend on α, k, ω, D_0, D_R]

Another field of application is that of tensammetric processes. Just as the kinetic parameters of redox processes may be determined, one may be able to measure similar[14] parameters of adsorption-desorption processes. Preliminary studies (Bauer, H. H. & Foo, D. C. S., unpublished data) have indeed shown that second harmonic measurements are considerably more sensitive to the conditions under which surfactants are examined than are the observations made at the fundamental frequency.

In short, it appears to be worth while to apply second harmonic measurements in any situation that is amenable to a.c. polarography. The instrumental complexity is not great, and the absence of background current an advantage of quite considerable magnitude.

Summary

The superposition of a sinusoidal alternating voltage at the working electrode of a polarographic cell is accompanied by the flow of an alternating current whose waveform is, in general, distorted. When the amplitude of the alternating voltage is small (of the order of millivolts), the main component of the distortion is the second harmonic. The experimental requirements for the measurement of the second harmonic current are critically examined and the applications of the second harmonic studies in electrochemical work are indicated.

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Theorem of Corresponding States

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SINCE the classical experiments of Andrews[1], there has been a search for a theory of gases and liquids and continuum of state. Considerable success has been achieved in the case of low density gases, both along classical and statistical lines. As of today, there is no theoretical equation of state of fluids at moderate and high densities. The experimental data are generally fitted to an algebraic function — an equation of state — in closed form.

An equation of state and generalized properties have several advantages. An equation of state, whether theoretical or empirical, allows interpolation (possibly extrapolation also) and condenses P-V-T data to a large extent. Equations of state such as the van der Waals' equation also help in theoretical interpolation of such phenomena as critical state.

Although the analytical forms of the equations of state become very complicated if they have to represent the P-V-T data fairly accurately, graphical representation of several thermodynamic properties based on the theorem of corresponding states (TCS) is very useful. The generalized charts help in evaluating the properties with minimum amount of experimental data. Also, generalized methods are useful where the data are totally lacking. For engineering purposes, the generalized charts represent the thermodynamic properties with adequate precision. The errors involved in such a predication sometimes tend to cancel out if such charts are used consistently.

van der Waals' Theorem

The classical theory of equation of state first formulated by van der Waals[2] has not only shown that a universal relation among pressure, volume and temperature exists (perhaps a complicated one as compared to that of van der Waals), but also has stimulated thought and interest leading to a better understanding of the behaviour of fluids. Since the time of van der Waals several equations of state have been proposed that represent fairly well the P-V-T relationships, in certain limited ranges of temperature and pressure. A few equations used for such a representation are those of Dieterici[3], Berthelot[4], Wohl[5], Beattie and Bridgman[6], Benedict *et al.*[7] and Martin and Hou[8]. The most general form is the series representation of the compressibility factor Z either in pressure or in reciprocal volume form. All the above equations can be put into virial form. Convergence of the series[9] is an essential factor at higher density regions. The TCS first formulated by van der Waals can be stated as

$$f(P_r, V_r, T_r) = 0 \qquad \ldots(1)$$

The fact that the P-V isotherms show a point of inflection (possibly a saddle point) leads to Eq. (1) when combined with the van der Waals' equation.

The simple van der Waals' TCS is followed by inert gases like argon, krypton, neon and xenon, but is found to be not applicable in the case of molecules which exhibit characteristics such as polarity, low molecular weight, non-sphericity, association, dissociation, etc. The absence of interaction energy characterizes the ideal gas model. Real gases deviate from ideality because of (i) the attractive and repulsive forces they exhibit at low and high densities respectively, and (ii) the formation of coexisting phases. This leads one to formulate certain criteria to be satisfied by substances if they are to conform to the TCS. Such criteria are given by Pitzer[10] and later modified by Guggenheim[11]. These in short are: (i) the molecules exhibit

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spherical symmetry, (ii) the temperature is high enough so that classical statistics is applicable, (iii) the potential energy is only a function of the distance of separation and is of a universal type except for two characterizing constants, and (iv) the intermolecular degrees of freedom are completely independent of the molecular volume.

Su's Modification

van der Waals' TCS was first modified by Su[12] as

$$F(P_r, V_r', T_r) = 0 \qquad ...(2)$$

by defining a pseudo-reduced volume as

$$V_r' = V/V_{ci} \text{ where } V_{ci} = RT_c/P_c$$

on the lines suggested by Kammerlingh Onnes[13]. The modified TCS shows that

$$Z = F'(P_r, T_r) \qquad ...(3)$$

instead of

$$Z/Z_c = F'(P_r, T_r) \qquad ...(4)$$

which is an outcome of van der Waals' TCS.

Equation Based on Potential Parameters

Before going into the discussions of the other modifications of the TCS, it is appropriate to point out that pressure, volume and temperature can be deduced using not only the respective values at the critical point but also the potential parameters as

$$P^* = P\sigma^3/\epsilon;\ V^* = V/\sigma^3;\text{ and } T^* = kT/\epsilon$$

The above definitions indicate that a general law of force, to take into account the attractive and repulsive forces, exists. In other words, any two-parameter relation such as

$$U(r) = \text{energy parameter}\left(h\,\frac{r}{\text{distance parameter}}\right)$$

leads to a TCS[14] of the type

$$Z = F''(P^*, T^*) \qquad ...(5)$$

Lack of data and methods of evaluation of intermolecular potential field, except for helium and hydrogen, have led several workers to assume the functional form of the potential field. One of the most commonly used potential energy functions is that due to Lennard-Jones[15] who considers the attractive and repulsive forces as additive. These forces have been discussed by Heitler and London[16] and London[17]. London has shown that the attractive forces are proportional to the distance of separation and takes the form $-\sigma r^{-6}$. Lennard-Jones found that any power value between 9 and 14 is equally good in representing repulsive forces. For the sake of simplicity and mathematical convenience, the repulsive forces are taken to be proportional to the inverse twelfth power of the distance of separation. If such a universal potential energy function exists which differs only by the two constants σ and ϵ, it follows that there is a TCS in classical statistics, the form of which is as in Eq. (5).

On the other hand, the formulation of quantum statistics leads to a TCS of the form[18]

$$Z = F''(V^*, T^*, \wedge^*) \qquad ...(6)$$

where $\wedge^{*2} = h^2/m\epsilon\sigma^2 = \wedge/\sigma^2$.

It is shown that this correction or third parameter, $\wedge^*$, is necessary if the de Broglie wavelength is much larger than the dimension of the potential field, in such cases as hydrogen and helium at very low temperatures. We shall not discuss this point any further as our interest lies above such low temperatures and also we can exclude the two light quantum mechanical gases, hydrogen and helium. It is likely that a modified potential energy function, for example, of exponential type with two adjustable constants, might represent the behaviour of gases better than the Lennard-Jones potential function. This is claimed on the justification that the generalized compressibility charts[19] based on potential parameters show a better representation than those based on macroscopic properties.

Other Modifications

Although van der Waals' law does not hold good with adequate precision for gases having different Z_c values, it has been shown by several workers[12,19-25] that the modified Su's law holds good with adequate precision in engineering applications, and whereas van der Waals' law holds good at the critical point, Su's law holds good at regions outside the critical point. This, of course, is the case when gases of different Z_c are correlated. In a recent modification[26], this difficulty has been eliminated by defining a new function

$$V_r'' = (a^{\log Z/\log Z_c})(T_r/P_r) \qquad ...(7)$$

thus leading to a TCS of the form

$$f''(P_r, T_r, V_r'') = 0 \qquad ...(8)$$

For accurate work, the equation of state in general should be represented as

$$P = g(V, k, T, \sigma, \epsilon, \mu, Q, \beta, x, m) \qquad ...(9)$$

Applying the Buckingham PI theorem[27] the relation

$$Z = G(V/\sigma^3, kT/\epsilon, \mu^2/\epsilon\sigma^2, Q/\epsilon\sigma^3, \beta/\sigma^3, h/\sigma\sqrt{m\epsilon}, x) \qquad ...(10)$$

results. In order to find a functional relationship among the various dimensionless groups, one has to rely on 'simplifications and practical utility'. Because of the lack of data on molecular and electrical properties, and for purposes of achieving simplicity with reasonable accuracy, Eq. (10) is generally written as

$$Z = G'(P_r, T_r, K) \qquad ...(11)$$

where K is the third parameter defined by several workers in different ways. Some of the modifications[28-32] of TCS are summarized in Table 1.

Cook and Rowlinson[33] have modified the Lennard-Jones potential function for elliptical and polar molecules considering each case separately. In the case of elliptical molecules it is assumed that only the attractive forces are affected by orientation. A parameter, $\delta = a^2g^{-2}/T^*$, is defined where g^{-2} is the average of $[g(0_1, 0_2, \varphi)]^2$, 0_1 and 0_2 being the

TABLE 1 — SOME MODIFICATIONS OF TCS

Ref.	Formulation and concept	Remarks
Riedel[28]	$K=Y_c=\left(\frac{d \ln P}{d \ln T}\right)_{T=T_c}$ Slope of the vapour-pressure-temperature curve at the critical point	—
Meissner and Sefarian[29]	$K=Z_c=\frac{P_cV_c}{RT_c}$	A direct consequence of van der Waals' theorem of corresponding states
Pitzer and coworkers[30]	$K=W=-\log P_r-1\cdot0$ From the value of reduced vapour pressure at $T_r=0\cdot7$	Riedel's parameter and Pitzer's acentric factor are related
Bloomer[31]	$K=S=s/R\ d_c'$ Slope of the pseudo-critical isometric in dimensionless form	—
Hall and Ibele[32]	$K=\mu_r=\mu/\sigma\sqrt{\epsilon}$	—

angle between the axes of the molecules and the line joining the centre and φ the azimuthal angle.

Newton[34] suggested that, in the case of hydrogen, helium and neon, an empirical constant 8 be added to critical pressure and temperature before using them to get the respective reduced properties, so that the above gases can be fitted to the generalized charts. To improve the accuracy of the generalized enthalpy charts, York and Weber[35] proposed that the values from the two-parameter charts be multiplied by a constant ϕ, where ϕ is defined as

$$\phi = (T_c/470)^n \qquad ...(12)$$

They have tabulated the values of n for temperatures above the critical. A good accuracy is claimed for this procedure.

Another method of generalization of thermodynamic properties is due to Edmister[36] who defines a residual volume α as

$$\alpha = RT/P-V \qquad ...(13)$$

In recent years several workers have made use of one or the other modification of the TCS to achieve better accuracy in the representation of compressibility and other thermodynamic properties of gases. Riedel[28] has used his parameter extensively and has calculated the thermodynamic properties of many gases.

Brock and Bird[37] have used Z_c and Y_c as the third parameter in a study of the application of the TCS to surface tension. It can be concluded from their work that Y_c is superior to Z_c, if other experimental variables can be measured accurately. In another study, Lydersen *et al.*[38] have used Z_c as the third parameter to calculate the various thermodynamic properties. Although these workers give some reasons for the choice of Z_c, there are no reasons to show that Z_c is better than Y_c or W. It is also indicated that the effect of Z_c becomes negligible above a reduced temperature of 1·20.

Daub[39], in attempting a generalized correlation of the compressibility of gas mixtures, using pseudo-critical constants, has indicated that W is superior to Z_c. Kordbachen and Tien[40] have compared their calculations of the effect of pressure on enthaply with those of Lydersen *et al.* (using Z_c as the third parameter), Curl and Pitzer[41] (using W as the third parameter), and Hougen and Watson (using only two parameters). From a comparison of the values of carbon dioxide ($Z_c = 0\cdot287$), ammonia ($Z_c = 0\cdot234$) and methane ($Z_c = 0\cdot289$), they conclude that the method of Lydersen *et al.* does not give better results than the simple two-parameter methods, and further state that if the experimental values are reliable, Z_c might not be the ideal parameter to characterize the intermolecular effect.

van der Waals' equation, modified by Hirschfelder *et al.*[42], also involves the third parameter Z_c and is shown to hold good in the superheated regions only (where possibly the effect of Z_c is negligible) but not at high density regions. This casts further doubt on the use of Z_c as the third parameter.

Hobson and Weber[43] have applied the extended TCS, using Z_c as the third parameter, to saturated liquids and vapours, but have broken down the values of Z_c between 0·22 and 0·29 into four values: 0·220, 0·245, 0·275 and 0·290. The parameter δ, in the modified theory of Cook and Rowlinson[33], is chosen by trial and error. They list, for each substance considered, two values of δ, one from vapour pressure and the other from rectilinear diameter. In a later paper, Rowlinson[44] gives generalized compressibility as a function of T_r and d_r. But for gases other than inert gases, he resorts to the definition of a pseudo-reduced density ρ' defined as

$$\rho' = 0\cdot293(RT_c/P_cV) \qquad ...(14)$$

This coupled with δ is used to find the values of Z.

As mentioned earlier, in the case of two parameters, the adequate precision with which Su's theory holds good has been shown by several workers. Kobe and Murti[21] have shown the superiority of pseudo-critical volume on application to the McLeod equation of state. Gebhart[45] attempted an improvement of the TCS by grouping gases on the basis of molecular similarity. In conclusion, he says, " The satisfactory conformity of the generalized equations and compressibility charts to the actual behaviour of the diverse groups of gases indicates that the gain in accuracy to be obtained by grouping like molecules is perhaps not worth the inconvenience of multiple results ". In a recent work[25], pseudo-critical volume has been used in generalizing the Benedict-Webb-Rubin equation of state which is used in the evaluation of isothermal changes of enthalpy and entropy with pressure and fugacity coefficients. This shows a considerable improvement over that of others[38,40,41].

The extended TCS of Hall and Ibele[32] is based on Su's TCS. Using permanent dipole moment as the third parameter these workers have achieved a good correlation in the case of polar gases although there is no correlation beyond a pseudo-reduced density of 2·0.

The choice of Z_c as the third parameter is not superior to the use of only two parameters.

Although Z values vary, it should be noted that most of the gases have a value between 0·26 and 0·29. Seventy-three out of eighty-two gases listed by Lydersen *et al.*[38] have a value of Z_c between 0·25 and 0·30. Even in this narrow range is involved the highly uncertain factor V_c, which is not determined experimentally with the same accuracy as P_c or T_c. Generally, V_c is obtained either by extrapolation of PV isotherms or by the Cailletet and Mathias[46] law of rectilinear diameter. Extrapolation involves great uncertainties and this is particularly so around the critical region. This can be seen easily by looking at the compressibility or any other chart representing any thermodynamic property. Under these circumstances, the grouping of gases with Z_c of 0·25-0·30 under three Z_c values of 0·25, 0·27 and 0·29 does not seem to be an appropriate choice. If the reasons for different Z_c values of gases lie in experimental techniques and extrapolation of critical volume, then, even on theoretical grounds, the assumption that Z_c should be the same for all substances is justified by the theoretical model of Rowlinson[44]. In certain cases even if the substances having the same Z_c correlate better, it might be because of the molecular similarity. The conformity of carbon monoxide (Z_c, 0·294) and nitric oxide (Z_c, 0·256) with certain generalized charts[25] illustrates this point.

The Riedel parameter and Pitzer's acentric factor are very similar and are related. The Riedel parameter does not predict the critical point and also it is not an experimentally measurable value. The Benedict-Webb-Rubin equation of state, although very complex and cumbersome, is known to predict the volumetric data of gases, specially that of hydrocarbons, more accurately than hitherto known. It does this with eight adjustable constants. The acentric factor of Pitzer *et al.*[30] has been used by Opfell and coworkers[47] along with the Benedict-Webb-Rubin equation to predict the volumetric data. They found that the data thus predicted are less accurate than those obtained using the Benedict-Webb-Rubin equation alone. The reason possibly lies in the use of the acentric factor to characterize the intermolecular effect better than the eight adjustable parameters alone. Also, the number of parameters is increased from 8 to 16, on modification, using the acentric factor. This casts further doubt on the usefulness of the acentric factor as the third factor.

Large deviations are observed in the case of substances like ammonia and water, even when the acentric factor is used. The deviations here are attributed to hydrogen bonding and association effects. Also, in the case of polar gases, the authors suggest that the extended TCS be used up to a value of $\mu^4 P_c^2/T_c^4 = 0{\cdot}5 \times 10^{-6}$ e.s.u. The acentric factor has been used only for normal fluids. A normal fluid is defined by the relation

$$\frac{\sigma_0 V_0^{2/3}}{T_c^4} = 1{\cdot}86 + 1{\cdot}18W \qquad \ldots(15)$$

the quantities being expressed in dynes/cm., cc./mole and °K. respectively and W being the acentric factor.

In using thermodynamic charts for industrial purposes, Su's modification is quite adequate and the third parameter may not be required. However, from the microscopic viewpoint, some parameter other than the critical properties will yield better results. The Boyle point[48] has been suggested in this context. Future research should be directed to finding a potential energy function applicable to both polar and non-polar molecules and molecules of different shapes.

Conclusion

(i) Su's modification is superior to others.

(ii) Although the third parameters might improve the correlation in certain restricted regions of temperature and pressure, they cannot be used in predicting the data with greater accuracy in all the regions of interest. The calculations of the thermodynamic properties of gases are complicated and tedious and the third parameters do not give any better results. Moreover, in the application of the third parameter, information is needed which may not be available or experimentally determinable.

(iii) Generalizations based on potential parameters might be successful.

(iv) The Boyle point might be very useful in calculating the thermodynamic properties of gases.

(v) In the case of gas mixtures, the combination of the critical constants to get pseudo-critical constants itself presents considerable difficulty.

Summary

The various theories of corresponding state and their usefulness in predicting the thermodynamic properties of gases are discussed.

Nomenclature

a = a constant, characteristic of the substance, in Rowlinson third parameter
d = density
d'_c = P_c/RT_c, the pseudo-critical density
d_r = d/d_c, the reduced density
h = Planck's constant
K = a generalized third parameter
k = Boltzmann constant
m = mass
P = pressure
P_r = P/P_c, the reduced pressure
P^* = $P\sigma^3/\epsilon$, the reduced pressure
R = universal gas constant
r = distance of separation
S = Bloomer third parameter
s = slope of the pseudo-critical isometric
T = temperature
T_r = T/T_c, the reduced temperature
T^* = kT/ϵ, the reduced temperature
V = volume
V_r = V/V_c, the reduced volume
Vr_i = $V P_c/RT_c$, the pseudo-reduced volume
V^* = V/σ^3, the reduced volume
Y_c = Riedel third parameter
Z = PV/RT, the compressibility factor
δ = Rowlinson third parameter
ϵ = minimum potential energy
Λ = $h/\sqrt{m\epsilon}$, the de Broglie wavelength
Λ^* = $h^2/m\epsilon\sigma^2$, the reduced de Broglie wavelength
μ = permanent dipole movement
μ_r = reduced dipole movement
σ = collison diameter
W = acentric factor
ρ' = 0·293 (RT_c/P_cV), the Rowlinson pseudo-reduced density

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REVIEWS

THE HIGH TEMPERATURE ASPECTS OF HYPERSONIC FLOW edited by Wilbur C. Nelson (Pergamon Press Ltd, Oxford), 1964. Pp. xiv+786. Price £ 15

If one considers a body in continuum flying at hypersonic speeds, the air in the region between the shock wave and the body is drastically slowed down relative to the body in the compression process. The high kinetic energy of the stream is then almost entirely converted to heat, thus imparting a large amount of energy to the air molecules. The shocked molecules equilibrate their translational and rotational degrees of freedom within a few collisions. In order to achieve thermal and chemical equilibrium, it is necessary to excite vibration, dissociate molecules and produce new molecules, ions and electrons. Thus, the aerodynamicist has to determine the precise state of the gas surrounding the body, in addition to the usual requirements of specifying drag, moments and heating, so that radiation, communications and other observable effects may be calculated. The production in laboratory of these high energy flows is achieved through shock tubes, shock tunnels, hot shot tunnels, etc. The measurement of the properties of the flowing gas thus achieved, their interpretation and scaling to free flight conditions require the understanding of the complex interactions between the chemistry of high temperature air and the fluid dynamic expansions. Thus, the aerodynamicist is led to the field of high temperature physics, in particular the chemical kinetics and radiation physics.

The present volume is the proceedings of a specialists' meeting held in Belgium in April 1962 and deals with some of these problems. It begins with an article on 'The hypersonic aerodynamic problems of the future' and ends with a discussion on 'The assessment of the present status and future requirements for high temperature hypersonic facilities'. These are very interesting and useful and give an insight into the kind of problems one would encounter in the study of flight through planetary atmospheres from theoretical and experimental points of view. The papers presented are grouped under five headings: (i) Inviscid flows, (ii) Experimental techniques, (iii) Chemical kinetics and radiation, (iv) Radiation and wakes, and (v) Heat transfer and viscous flows.

Section (i) deals with theoretical studies of chemical, vibrational non-equilibrium for inviscid flows, (ii) with the production and measurement of flow properties of dissociated, ionized gases in non-equilibrium in shock tunnels and plasma jets, (iii) and (iv) with the theoretical and experimental studies of chemical effects and radiation in hypersonic flows, and (v) with the problems of heat transfer.

Even though the work presented in this book is already two years old, it is still quite useful to people working on the various aspects of high energy flows. The printing and get-up is excellent as all Agardographs.

D. TIRUMALESA

NEUTRON IRRADIATION AND ACTIVATION ANALYSIS by Denis Taylor (George Newnes Ltd, London), 1964. Pp. ix+185. Price 50*s*.

Neutron irradiation analysis involving either the examination of induced activities or of the prompt radiations emitted is an important technique for the determination of trace elements in many materials. Developments in instrumentation, such as in gamma-ray spectroscopy and in data-handling and control equipment, have led to the introduction of automated activation analysis which will eventually control the quality of many manufactured products. There are a number of excellent review articles on the subject but very few books have appeared. The present book thus fulfils a great need.

The book begins with a short introduction followed by chapters on neutron interactions and the detection of prompt radiation. The subsequent two chapters which constitute the main body of the text are devoted to neutron activation analysis proper and its applications. Brief accounts of automation, analysis of process streams and the use of activation analysis in prospecting are next given. The concluding chapter deals with some special applications and some likely developments in the future. There are appendices giving a glossary of terms, a summary of the elements of radioactivity, some useful tables, and an account of absolute activity measurements making use of the gamma-spectrometer.

The book deals with the principles of the subject and is addressed to the student possessing only rudimentary knowledge of radioactivity. Those who lack even this knowledge will find the short account of radioactivity given in Appendix II sufficient to follow the main text. There are lucid descriptions of the main measuring techniques and large numbers of applications are given. The illustrations are excellent. Although it is true that the practice of activation analysis cannot be entirely learnt from a book, the present work forms a useful introduction to the subject and will benefit the would-be activation analyst.

S. R. MOHANTY

ANALYTICAL INSTRUMENTATION — Proceedings of the Tenth National Analysis Instrumentation Symposium, 1-3 June 1964, San Francisco, California; edited by L. Fowler, R. J. Harmon & D. K. Roe (Instruments Society of America, *Distributors*: Plenum Press Inc., New York), 1964. Pp. x+340. Price $ 14.50

This volume presents a selected portion of the papers presented at the 10th Annual Symposium on Analysis Instrumentation held in June 1964, in San Francisco, and organized by the Instruments Society of America.

Instrumentation plays a major role in analytical chemistry of today, and since widely diverse techniques are used, a large body of scientists — chemists, physicists and electronic engineers — is constantly at work in devising instrumentation aimed at greater precision, accuracy and time-saving devices.

Though most of the papers are on instrumentation, there are also a few of the review type. The compilation is based on material originally prepared for oral presentations and, therefore, as the editors point out, the presentation is stylistically uneven. The purpose in allowing this was to present the material of sufficient novelty and/or utility to interested scientists with the shortest possible delay. This purpose has been served to a great extent.

The following are the major topics covered in the volume. The ' Optical method ' includes papers on versatile process polarimeter, flame photometric instrumentation at IPNPP and film coating monitoring by non-dispersive infrared. Papers on membraneless potentiometric analysis of dissolved oxygen in process stream, evaluation of *p*H measurement response, process polarograph for measuring p.p.m. H_2S, a new process analyser for hydrogen and electrochemical transducers for water quality have been included under ' Electrochemical and chemical methods '. ' Physical properties methods ' include papers on on-stream pour tests, haze point analysers, the design and performance of the piezoelectric sorption hydrometer, and boiling point analyser sampling system for closed-loop control of a fractionating tower. Presented under ' Radiation methods ' are papers on precision radiation gauging with a pulse-frequency computer and application of activation analysis in the petroleum and chemical industries.

Some good review papers on the role of EPR in free radical chemistry, present status of high resolution NMR, modern electroanalytical techniques, advances in emission flame photometry, atomic absorption instrumentation and technique, and an instrumentation paper on closed-loop liquid feeder have been included under ' Laboratory methods '. Other papers presented are on automatic attenuator for Perkin-Elmer 800 gas chromatograph and simultaneous analysis and B.t.u. determination of natural gas under ' Gas chromatography '; on instrumentation for sampling and analysing combustion particulates using electron microscope, collecting representative exhaust gas samples and instrument measurement of contamination in clear rooms under ' Air pollution instrumentation '; and on in-stream analysers aid in computer control, gas stream analysers in computer process control and chromatographs mated to digital computer implement process studies under ' Process stream analysers in computer system '.

What is covered in the volume is a very broad area. However, one can be certain of finding something useful in the subject of his interest.

V. T. ATHAVALE

PHOTOELECTRIC EFFECTS IN SEMICONDUCTORS by S. M. Ryvkin; authorized translation from the Russian by A. Tybulewicz (Consultants Bureau Enterprises Inc., New York), 1964. Pp. xv+402. Price $ 22.50

The volume under review presents in a connected way the theory and experimental methods relating to those phenomena in semiconductors in which carrier generation through photon interaction constitutes the primary event. Three broad aspects, viz. generation, recombination and transport of optically created excess carriers, are discussed individually and are preceded by three introductory chapters dealing with general description of photoconductivity, methods of measurement of steady state photoconductivity and the principal phenomenological parameters connected with it. A chapter covering 15 pages is devoted to the processes of generation. Recombination and trapping are dealt with in seven chapters occupying more than 150 pages. An almost equal space in the last four chapters is devoted to transport of excess carriers in bulk semiconductors and junction devices. Each one of the chapters is supplemented by a list of representative references. Clarity of exposition and thoroughness of treatment of the topics presented are the two outstanding traits of the volume. The simplest and the most restricted problem is often discussed first and this is used as stepping stone for discussion of topics of more general type — a feature that does not restrict the appeal of the volume to the specialist alone. The exhaustive treatment of recombination and trapping of various types is, to the reviewer's mind, a unique contribution of the volume to the subject. The author has taken care to point out in the preface that the volume does not claim to be a complete review of the field of photoconductivity. Thus, to mention a few, topics like effects arising out of free carrier absorption, field or pressure dependence of optical absorption and exciton formation do not find a place in the volume, while mentions of experimental results on typical semiconducting materials are rare — only a few finding stray and casual references. Printing errors are within modest limits. Excepting for these, the volume remains a highly useful contribution that bears in an abundant measure the proof of competent and authoritative handling. A copy of the volume should be indispensable for every worker in the field of photoconductivity.

S. DEB

DISLOCATIONS — International Series of Monographs on Solid State Physics: Vol. 3, by J. Friedel (Pergamon Press Ltd, Oxford), 1964. Pp. xxi +491. Price £ 6

The book is based on an earlier one written by the author in French in 1956. However, the English version is thoroughly revised and brought up to date. As no books on dislocations have appeared during the last ten years, a new book on the subject was needed and this first class book fulfils this need.

The book is divided into three parts: part one devoted to general properties of dislocations, part two to the dislocations networks and plastic properties of crystals, and part three to the interaction of dislocations with other defects. There are seven

chapters in part one, five in part two and five in part three. There are four useful appendices on fundamental relations of elasticity, some physical properties of elements at room temperature, common slipping and twinning directions for some crystalline systems and lexicon of technical terms. Principal works on dislocations are listed in the beginning of the book and a complete list of references to original literature is given at the end. The index is rather inadequate.

Although the book is written for metallurgists, the treatment of the subject is exhaustive and will interest all research workers in solid state physics also. The mathematical treatment is simple and clear which makes the book useful for university students also. The book is strongly recommended to all solid state research physicists and M.Sc. physics students of Indian universities. The diagrams are clear and the get-up is good.

S. C. JAIN

ULTRASOUND: PHYSICAL, CHEMICAL AND BIOLOGICAL EFFECTS by Isaak Efimovich E'l'piner; translated from the Russian by F. L. Sinclair (Consultants Bureau Enterprises Inc., New York), 1964. Pp. viii+371. Price $ 22.50

This book gives, for the first time, a comprehensive account of the physical, chemical and biological effects of ultrasonic waves. After giving the fundamentals of ultrasonic wave propagation in the first chapter, the author passes on in the second chapter to discuss exhaustively the cavitation phenomenon, which is responsible for all the effects of ultrasonics. The main features discussed in this and the next chapter are: (1) the high local temperature; (2) the high local pressure; (3) the electrochemical effect; and (4) sono luminescence at the site of collapsing as well as resonating cavitation bubbles. A complete critical account of these factors as well as the effect of nature of gas on these factors is given in Chapter 4. The importance of ultrasonic waves in breaking down macromolecules without, however, affecting the integrative character of the individual units is brought out clearly in Chapters 5-7, discussing, for example, ultrasonically depolymerized dextron used in medical practice, the action on collagen fibres resulting in the ease of stretching of tendons, formation of copolymers, denaturation as well as production of native fragments of DNA, and the degradation of fibrinogen, which prevents blood clotting. By far the best practical use of ultrasonics is in emulsifying, discussed exhaustively in Chapter 8. In Chapter 9, the author gives an account of the effect of ultrasonic waves on microorganisms. In the words of the author, the possibility of attenuation of virulence without alteration of the antigenic specificity of virus particles opens a promising field of application of ultrasound for the production of therapeutic biological preparations.

Experiments on the use of ultrasonics in the production of improved seeds which give high yields of potato, beet root tubers and peas shoots and pods have been conducted in Russia. These are discussed in Chapter 10 which gives an account of the treatment of brain tumours as well as destruction of cancerous tissues (ultrasonic neurology). The detection aspect of ultrasonic waves, known as ultrasonic biolocation and ultrasonic cardiogram, involves a study of velocity and attenuation of these waves in human and animal tissues, bones and nerves. A very good report of the fundamental studies in this direction is given in the last chapter.

Exhaustive references are given at the end of each chapter. This book not only gives, for the first time, a critical account of the practical uses of ultrasonic waves, but is stimulating for further research in this field.

For every student of ultrasonics and specially for those who do research in the applied field of ultrasonics, this book is a must.

S. S. CHARI

METHODS IN MICROANALYSIS: Vol. 1 — SIMULTANEOUS RAPID COMBUSTION, edited by J. A. Kuck; translated by Phyllis L. Bolton & K. Gingold (Gordon & Breach Science Publishers, New York), 1964. Pp. xv+560

An excellent compilation of the research work carried out by the Russian School of Microchemistry, this book presents valuable information about the methods developed as a result of extensive research in the field of rapid estimations of elements in pure organic and organometallic compounds. An additional attractive feature of these methods is the simultaneous estimation of several elements in a single determination which enables saving of time required for the individual estimations of the elements and also the precious research sample.

Of the fifty-six chapters, two-thirds are devoted to the modifications relating to the carbon and hydrogen determination, most of which describe simultaneous determination of other elements. New approaches to the estimation of nitrogen by hydrogenation, magnesium fusion, etc., are described in some of the ten chapters on the nitrogen determination, three of which also include simultaneous estimations of other elements. A relationship established between the length of the contact layer and the method of decomposition of the sample, and an empty tube technique are reported for the direct determination of oxygen in Chapter 36. Three other chapters give details about its simultaneous estimation with other elements. Only with a few exceptions, the methods for the estimations of sulphur, phosphorus, halogens, silicon and mercury appear as simultaneous estimations of carbon and hydrogen, nitrogen, etc. The techniques employed cover pyrolysis, pyrohydrolysis, magnesium fusion, vacuum combustion, measurements of thermal conductivity, electroconductivity, etc. A review of the advances in organic elemental analysis in USSR and other countries is given in the last chapter of the book.

The modifications used have impaired the simplicity of performance in certain cases. However, these seem to be specially designed to meet the needs of a typical class of compounds and researchers engaged in work relating to such compounds might find these quite useful.

The contributions of Miss Korshun and her co-workers constitute the major part of the book. Her systematic consideration of the possible chemical reactions and logical deductions of the solutions to the problems confronted have revealed significant and readily available data which will save for many the time for a thorough literature survey.

The editor and the translators have successfully fulfilled their object of helping the microchemists who are handicapped due to the language barrier in furnishing them the vast information of the microchemical research carried out in the past two decades in the USSR.

V. S. PANSARE

INTERNATIONAL CLAY CONFERENCE, 1963: Vol. 1, edited by I. Th. Rosenqvist & P. Graff-Petersen (Pergamon Press Ltd, Oxford), 1963. Pp. ix+376. Price £ 5

The International Clay Conference held at Stockholm in August 1963 was the result of the opinion expressed by several representatives of the CIPEA (Comité International Pour l'Etude des Argiles) at the Copenhagen meet in 1960 that since the meeting place of the International Geological Congress in 1964 in India was too far away for many European and American scientists, clay scientists should explore the possibility of arranging a meet before that date.

The international character of the symposium is reflected by the fact that nearly 13 countries contributed 35 papers for discussion. These were presented under six sections, namely (i) clay mineral structures and composition (10 papers), (ii) clay mineral genesis (8 papers), (iii) ion-exchange in clay minerals (7 papers), (iv) clay-electrolyte-water system (3 papers), (v) clay organic complexes (5 papers), and (vi) clay minerals in industrial applications (2 papers). Countrywise, the contributions are: USA (9), UK (5), Germany (4), Spain (4), Belgium (3), Australia and Cezechoslovakia (2 each) and one each from Japan, India, Italy, France, Sweden and Canada.

Of special interest in Section 1 is the recent concept of Radoslovich on clay mineral structures and the experimental verification of his predictions regarding the role of interlayer cations as affecting the 'b' dimensions of muscovite (Burns and White). Another interesting account of the role of crystal structure in solid state reactions of clays and related minerals is given by Brindley. Acid dissolution of biotite, the effect of temperature on diffusion mechanism and white rim formation are given by Gastiche, while the mechanism of acid attack on montmorillonite clays as that of uniform process leading to vacancies in octahedral position is presented by Newmann's imaginative approach through the thermal analysis of acid treated clays.

Section 2 has four papers in German, of which Goldschmidt's paper on diagenesis of clay minerals at depths up to 3000 metres may be of interest to petroleum technologists. Sudo's account of interstratified minerals of Japan as approached through theoretical concepts of crystal growth of mixed crystal as also the other papers on soil formation from different types of rocks under different environments as in Scotland, Italy or Czechoslovakia should contribute to our knowledge regarding the mechanism of rock weathering and soil formation.

Ion-exchange mechanisms in vermiculite and montmorillonite form the main theme in many papers in Section 3. The only paper from India (Sen and Guha) deals with the oft-discussed but highly controversial field of phase transformation in kaolin as studied through the changes in the base exchange capacity of kaolin on heat treatment. The authors have some modifications to suggest on the results of Brindlay and Nakahira on the composition of meta-kaolin. Cesium sorption reaction on clay minerals has been studied (1) as a useful property for detecting the presence of montmorillonite in interlayer mineral systems (Tamura), and (2) to utilize the sorption properties of vermiculite to decontaminate the radioactive effluents containing ^{137}Ce with high fission yield, which is a health hazard (Jacob).

Section 4 has three papers, one theoretical (Gast) dealing with the diffusivity of cations in clays as affected by the electrostatic forces and the increased viscosity of the 200-300 A. thick surface water. Of the other two with practical applicability, one suggests that through a simple test involving determination of the time of imbibition and the area wetted by a drop of ethelene glycol or water over a clay surface, its green strength and plasticity could be assessed (Konta and Borobec). The other paper is of importance to engineers and agriculturists concerned with soil erosion and shows how erosion is increased through waters containing dispersing agents.

The section on clay organic compounds contains five papers, mostly with technical applicability. Van Olphen has shown that very high pressures of the order of several thousands bars are needed to desorb hydrocarbons from some clay minerals that may be contained in source rocks, thus accounting for certain aspects of petroleum mining. Mechanism of soil stabilization by TBC (4-*tert*-butylpyrocatechol) and the removal of surfactants in detergents occurring in household waste water by the clay minerals in the soil are discussed in two more papers in this section (Hemwall and Wayman).

The last section on clay minerals in industrial application has two papers only, one giving compositional data for British clays used in fine ceramic industry (Holdbridge) and the other on the anisotropic thermal conduction due to the parallel alignment of clay particles.

The editors deserve congratulations for the able classification of the papers which perhaps is not as easy a job as it may appear. The usual high standard of the publication attributable to the Pergamon Press is maintained. The book is a useful addition to the library of those interested in clays.

The reviewer apologizes for not doing full justice to the papers written in languages other than English.

N. R. SRINIVASAN

BOOK NOTES

PATENTED INVENTIONS OF CSIR: 1940-64 (Council of Scientific & Industrial Research, New Delhi), 1965. Pp. 228. Price Rs 16 or 33*s*. or $ 5.00

This up-to-date compilation of patent specifications contains brief particulars of about a thousand inventions of the Council of Scientific & Industrial Research, arranged in chronological order with suitable indexes provided to facilitate subjectwise, authorwise and laboratorywise location. The patents relate to all subjects from agriculture, through chemical compounds and engines, to wood-working.

In an interesting foreword, Dr S. Husain Zaheer, Director-General, Scientific & Industrial Research, recalls the role of the Council in speeding up economic development through organized scientific research in industry.

The compilation is a valuable document for all those interested in the indigenous development of new products and processes.

PUBLICATIONS RECEIVED

MICROWAVE SCANNING ANTENNAS: Vol. I — APERTURES edited by R. C. Hansen (Academic Press Inc., New York), 1964. Pp. xvii+442. Price $ 16.00

CHEMISTRY OF LAC by P. K. Bose, Y. Sankaranarayanan & S. C. Sen Gupta (Indian Lac Research Institute, Namkum, Ranchi), 1963. Pp. viii+225. Price Rs 20.40

GROUP THEORETICAL CONCEPTS AND METHODS IN ELEMENTARY PARTICLE PHYSICS edited by F. Gursey (Gordon & Breach Science Publishers, New York), 1964. Pp. vii+425. Price: Regular edition $ 19.50; Professional edition $ 12.50

BRITAIN: AN OFFICIAL HANDBOOK, 1965 edition (Her Majesty's Stationery Office, London), 1965. Pp. ix+584. Price: Paper $ 3.20; Cloth $ 5.50

MOLECULAR REARRANGEMENT: Part 2, edited by Paul de Mayo (Interscience Publishers Inc., New York), 1964. Pp. viii+709-1244. Price $ 20.00

MAMMALIAN PROTEIN METABOLISM: Vol I, edited by H. N. Munro & J. B. Allison (Academic Press Inc., New York), 1964. Pp. xv+566. Price $ 18.50

PLASMA SPECTROSCOPY by Hans R. Griem (McGraw-Hill Book Co. Inc., New York), 1964. Pp. xi +580. Price $ 18.50

GROUP THEORY AND SOLID STATE PHYSICS: I, edited by P. H. E. Meijer (Gordon & Breach Science Publishers, New York), 1964. Pp. x+293. Price $ 5.95

MICROWAVE CIRCUITS by Jerome L. Altman (D. Van Nostrand Co. Inc., London), 1964. Pp. xxi +462. Price $ 15.00

POLYMERS: STRUCTURE AND BULK PROPERTIES by P. Meares (D. Van Nostrand Co. Inc., London), 1965. Pp. xi+381. Price $ 12.50

ELECTRODEPOSITION AND CORROSION PROCESSES by J. M. West (D. Van Nostrand Co. Inc., London), 1965. Pp. xii+189. Price 27.6*s*.

GOLD MINING INDUSTRY IN INDIA: Memoir 1 (Geological Society of India), 1963. Pp. 131

GENERALIZED HYPERGEOMETRIC SERIES by R. P. Agarwal (Asia Publishing House, Bombay), 1963. Pp. 109. Price Rs 5.00

ELECTROMAGNETIC ASPECTS OF HYPERSONIC FLIGHT: Second Symposium on the Plasma Sheath, Its Effect upon Reentry Communication and Detection, edited by Walter Rotman, Howard K. Moore & Robert Papa (Spartan Book Inc., Baltimore), 1964. Pp. ix+369. Price Rs 75.00

CARAKA SAMHITA by Priyadaranjan Ray & Hirendra Nath Gupta (National Institute of Science, New Delhi), 1965. Pp. viii+120. Price Rs 15 or 20*s*.

ADVANCES IN ORGANOMETALLIC CHEMISTRY: Vol. 2, edited by F. G. A. Stone & Robert West (Academic Press Inc., New York), 1965. Pp. ix +440. Price $ 15.00

EMULSIONS: THEORY AND PRACTICE, ACS Monograph No. 162, Second Edition, by Paul Becher (Reinhold Publishing Co., New York), 1965. Pp. xi+440. Price $ 22.00

VARIATIONAL TECHNIQUES IN ELECTRO-MAGNETISM by Laurent Cairo & Théo Kahan (Blackie & Son Ltd, London), 1965. Pp. xv+152. Price 27*s*. 6*d*.

EFFECTS OF RADIATION ON SEMICONDUCTORS by Viktor Sergeevich Vavilov; authorized translation from the Russian by A. Tybulewicz (Consultants Bureau Enterprises Inc., New York), 1965. Pp. xi+225. Price $ 15.00

CRYSTAL CHEMISTRY OF TETRAHEDRAL STRUCTURES by Erwin Parthe (Gordon & Breach Science Publishers, New York), 1965. Pp. xii+176. Price $ 9.50

THE NATURE OF INDUCTION MACHINES by Philip L. Algar (Gordon & Breach Science Publishers, New York, 1965. Pp. xiii+516. Price $ 25.00

FERROMAGNETIC RELAXATION THEORY by Marshall SPARKS (McGraw-Hill Book Co. Inc., New York), 1964. Pp. xii+227. Price $ 12.50

REFRACTORY TRANSITION METAL COMPOUNDS: HIGH TEMPERATURE CERMETS by G. V. Samsonov; Translation Editors: G. E. Gurr & D. J. Parker (Academic Press Inc., New York), 1964. Pp. viii +220. Price $ 9.00

RADIATION DAMAGE IN CRYSTALS by Lewis T. Chadderton (Methuon & Co. Ltd, London), 1965. Pp. xiv+202. Price 36*s*.

NOTES & NEWS

Phase-locking of lasers

Two lasers have been locked together in phase for the first time at the Bell Telephone Laboratories, where a feedback loop has been designed to lock the phase difference between the waves generated by two single frequency helium-neon lasers to within a third of a degree. The frequency of these lasers is approximately 5×10^{14} c/s.

Phase-locking at optical frequencies permits the use of homodyne detection — a technique that makes it possible to halve the transmitted power — in experimental light communication systems. In such a system a transmitter laser would be phase-locked with a receiver laser some distance away. Other possible applications include the combining of several low power lasers to produce one high power beam. The experiments also enable the study of the interactions of light waves from separate sources.

In these experiments (Fig. 1) the lasers are placed on a shock-mounted concrete table enclosed in a concrete vault to reduce sudden changes in frequency caused by acoustical and mechanical disturbances. The laser beams are combined by a half-silvered mirror, which sends part of the combined beam to a screen and part to a photomultiplier. The output current from the photomultiplier is proportional to the instantaneous power of the combined light waves striking the photomultiplier surface. The current increases when the two light waves interfere constructively and decreases when the waves interfere destructively. Thus, the value of the instantaneous current depends upon the phase difference between the two laser beams. The voltage produced by the current is fed back through a d.c. amplifier and a low pass filter to a piezoelectric transducer which supports one of the mirrors for the resonator of the receiving laser. The varying voltage applied to the transducer changes the resonator length and thus the frequency of the laser. This forces the receiving laser to track the frequency and phase of the transmitting laser. Phase-lock was maintained even when the transmitting laser's frequency was changed by ± 50 Mc/s. and when, in addition, its beam was attenuated by 50 db. The frequency over which the transmitting laser can be tracked could be extended by inserting a higher voltage d.c. amplifier in the feedback loop. Further improvement of the circuit has enabled the two beams to be locked together without the use of the vault.

That the lasers were truly phase-locked was proved by the following experiment. The path of one beam was lengthened until the beam's wavefront was essentially plane. The path of the other beam was kept short; thus its wavefront was curved. The combined beams from one of the output arms of the half-silvered mirror were directed to a screen. In the absence of feedback control, a spot of uniform intensity was formed on the screen. When the control was applied, the spot was changed to a pattern of light and dark rings caused by the stationary interference of the flat and curved wavefront beams, thus demonstrating that the beams were phase-locked.

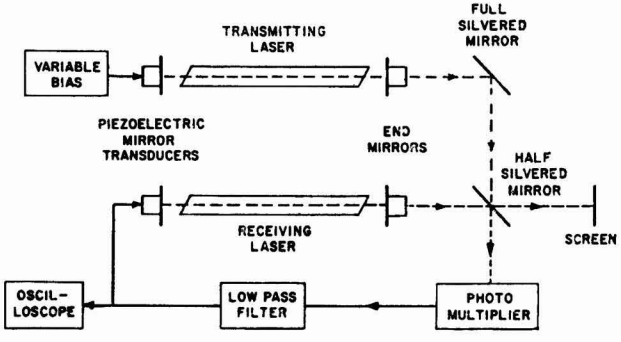

Fig. 1 — Phase-locking of lasers

Superconductivity in graphitic compounds

Superconductivity has been discovered in compounds of graphite and alkali metals by scientists at the Bell Telephone Laboratories, New York. This is the first time that carbon structures, rather than interstitial carbon atoms, have been directly involved in superconductivity.

The superconducting compounds consist of layers of either potassium, rubidium or cesium atoms interleaved with layers of carbon. For these compounds, the critical magnetic field — the field required to destroy superconductivity — depends on its direction through the material. Fields in the plane of the carbon layers must be stronger to quench superconductivity than fields cutting across the layers. This dependence of the critical field orientation with respect to the structure is greater in graphite compounds than in any other superconductor. The temperatures at which the compounds were found to become superconductive range from 0·02° to 0·55°K.

The strikingly large orientation dependence of the critical magnetic field is believed to result from the layer structure of the compounds. Graphite consists of closely packed layers of carbon loosely stacked on top of each other. Its well-known mechanical properties stem directly from this structure. Chemists have known for decades how to prepare compounds consisting of eight atoms of carbon with one of either potassium, rubidium or cesium. The discovery that superconductivity can exist in these systems now opens the way to the study of superconductivity in new circumstances.

The strong orientation dependence of the critical magnetic field is of fundamental importance

and bears on the basic nature of superconductivity. The experiments are likely to give more information about the nature of graphite itself.

New device for shock wave studies

A new device called the 'Phermex' (pulsed high energy radiographic machine emitting X-rays) has been designed at the Los Alamos Scientific Laboratory for fluid mechanics and chemical kinetics studies. Phermex is a high current standing wave linear accelerator which generates intense bursts of X-rays for flash radiographic studies of explosive driven metal systems. Hitherto there has been no satisfactory method of observing the characteristics of fluid flow subject to shock waves and this new device, because of the recent developments in high explosive technology enabling perfectly reproducible explosive systems, has made it possible to obtain a time sequence of mass distributions of bodies subjected to explosive waves. The device consists of a 1 MW. radiofrequency power amplifier and an electron beam injector. Target currents of over 20 amp. provide fluxes exceeding 9 roentgens per 0·2 μsec. pulse. Because of the nature of this accelerator a pulse of gamma rays consists of 10 sub-bursts, each about 6 nsec. long. The new device may also find application in several other fields. For example, the output electron beam may be used to generate exceedingly powerful bursts of Cerenkov radiation in the mm. wavelength and for studying the effects of electron or gamma radiation on properties of matter including biological tissues [*Phys. Today*, **17** (12) (1964), 19].

Fuel cell using methanol and air

A new fuel cell, which uses methanol and air as fuels and generates about 5 kW. of electricity with an overall thermal efficiency of 20-25 per cent, has been developed at the Thronton Research Centre of Shell Research Ltd. This requires a much lower working temperature (60°C.) compared to about 800°C. required in earlier fuel cells using hydrogen and oxygen as fuels and eliminates the need for heavy gas storage cylinders. In the cell, methanol is converted to hydrogen in a unit that forms part of the system and the complete unit fits easily on the back of a $\frac{3}{4}$ ton pick-up truck. In operation, methanol passes into the converter where it reacts with steam in the presence of a catalyst to form hydrogen and carbon dioxide and the hydrogen is further purified by passing over a palladium/silver diffuser. The design of a commercial fuel cell that uses methanol directly without having to convert it to hydrogen is also contemplated [*Chem. Engng News*, **42** (52) (1964), 31].

A new solid electrode for voltammetry

Carbon electrodes are used in voltammetry since long, but their applications have been widened by the recent studies on the polarographic behaviour of graphite and other solid electrodes, the pyrolytic graphite electrode and 'glassy carbon' which is gas impermeable, highly resistant to chemical attack, electrically conductive and is obtainable in relatively pure state. The use of glassy carbon electrode for voltammetry has been reported by Zittel and Miller [*Anal. Chem.*, **37** (1965), 200].

The electrode is prepared by a simple method. An epoxy resin (Shell. Co. Epon. 820) cylinder is prepared by pouring the molten resin between two sections of a glass tubing, the outer section of which is removed. The glassy carbon material is taken and the ends of the above cylinder are sealed. A small amount of mercury is poured into the central glass tube and contact is made by means of a copper wire, inserted into it. Since the glassy carbon is brittle and hard, the electrode is to be shaped by grinding or cavitation.

As in the case of all solid voltammetric electrodes, this electrode has also a fairly large background current, but this does not affect the applicability of this electrode.

The usable potential of this electrode in acid systems is determined by studying the effect of different mineral acids; it is found to be +1·3 to −0·8 in phosphoric acid solution and intermediate in sulphuric acid and perchloric acid solutions. This electrode is relatively insensitive to changes in *p*H. The polarographic behaviour of several ions at this electrode has been studied and in almost all cases the waves are well defined and reproducible.

The glassy carbon is manufactured in four grades depending on the 'proof-temperature', of which grade G.c.30s is the purest and best for the electrodes. Since this is isotropic, it does not require definite orientation. This electrode has the advantage of being insusceptible to poisoning or film formation like other stationary solid electrodes, and thus requires no pretreatment or treatment between uses. The cathode limit of this electrode may be extended up to −1·6 in basic media, unlike other electrodes.

The ease of fabrication and maintenance of the glassy carbon electrode compares favourably with all other solid electrodes. It has also the advantage of being inert to strong acids and oxidizing agents.—A. L. J. Rao, *Punjabi University, Patiala*

A general reaction for synthesizing heterocyclics and carbocyclics

A general reaction for synthesizing heterocyclic and carbocyclic ring compounds has been developed which may prove to be of some value for the pharmaceutical industry. The synthesis is, to some extent, a combination of both Michael and Wittig reactions. The key intermediate in the synthesis is the vinyltriphenylphosphonium salt which is prepared by reacting triphenylphosphorene with 2-phenoxyethyl bromide using phenol as the solvent. The resulting bromide is then decomposed by heating in ethyl acetate to give the desired vinyltriphenylphosphonium bromide in 90 per cent yield. In the general reaction, an anion reacts with vinyltriphenylphosphonium salt by a Michael type of reaction to produce an intermediate phosphorus ylid. The ylid then attacks the carbonyl by an intramolecular Wittig reaction to form a carbon to carbon double bond. This final step is irreversible, producing either heterocyclic or

carbocyclic compounds and triphenylphosphine oxide. Some compounds, viz. 3,4-chromene and 2,5-dihydro-2,3-dimethylfuran, have been prepared. In the synthesis of 3,4-chromene, vinylphosphonium bromide is reacted with the sodium salt of salicylaldehyde, using acetonitrile as solvent. Yields as high as 71 per cent of 3,4-chromene and 51 per cent of triphenylphosphine oxide are obtained. Similarly, 2,5-dihydro-2,3-dimethylfuran has been synthesized by reacting acetoin with the vinyltriphenylphosphonium bromide in the presence of sodium hydride [*Chem. Engng News*, **42** (28) (1964), 68].

Chlorella as animal feed

Chlorella, a unicellular green alga, distributed all over the world and with a faster rate of multiplication like bacteria, is attracting attention as one of the new food resources. The alga can be cultivated in large quantities using only air, water, sunlight and small amounts of waste materials such as livestock and poultry manure and sewage water. About 50 per cent of the dry matter of chlorella is protein of good quality. The alga also contains large quantities of lipids, carbohydrates, chlorophylls, vitamins and minerals. The nutritive value of chlorella is higher than the sum of the nutritive values of soybean and spinach; attention is presently being focused on the possibility of large-scale utilization of this alga for human consumption and as animal feed.

Mass cultivation of this alga requires shallow ponds lined either with concrete or vinyl lining. The form and size do not matter considerably, though a rectangular form appears to be more convenient than square and circular forms. The outdoor ponds with a depth varying from 4 to 8 in. are more common, since the solar energy radiating on a unit area of the pond is constant. As in vegetable cultivation the use of manure as a source of nitrogen, phosphorus and potassium is an important aspect in the cultivation of this alga. Nitrates such as potassium, ammonium and calcium are excellent sources to give immediate effect for the growth of the alga. However, natural fertilizers containing organic nitrogen like the decomposed viscerae of fish, faeces of livestock and oil cakes form excellent sources of nitrogen.

Chicken manure and sewage water form good sources for organic nitrogen and carbon dioxide. The problem of artificially supplying carbon dioxide required for photosynthesis can be solved by using chicken manure and sewage water as manures, since the microbes present in them produce large quantities of carbon dioxide, which dissolves in water, and could be used by the alga. Natural fresh water from sources like well, river, pond and lakes can be used in cultivation. When city water is used, care should be taken to reduce the chloride content either by boiling or by using crystalline hypo. The *p*H of the culture medium should be slightly acidic (*c.* 7·0) when the phosphate exists in the easily utilizable form as $H_2PO_4^-$ and HPO_4^{2-}. When the *p*H goes beyond 7·0, the phosphate is converted into the anion PO_4^-, and the carbon dioxide is converted to HCO_3 and CO which are not absorbed by chlorella and also the possibility of harmful microbial contamination of the medium increases. In a strong acidic solution, the phosphate combines with iron and aluminium to form insoluble complex compounds. The photosynthetic activity of the alga generally reaches a maximum when the light intensity is 5000-10,000 lux. At low light intensities the multiplication of the alga stops and at high light intensities the bleaching of the alga occurs. For effective mass production of chlorella, the production of an active strain is very essential.

Chlorella requires for its nutrition the micro (trace) elements. To increase the chlorophyll content to deep green tone, addition of manganese and iron is essential along with a simultaneous increase in the nitrogen source. Addition of enzymes accelerates the decomposition of organic compounds and facilitates the availability of nutrients.

Factors such as intensity of sunlight, stirring rate, temperature, *p*H, supply of nutrients, check against contamination, etc., excess heat removal and evaporation rate should be controlled properly to obtain good growth of the alga. Agitation of the culture improves light and aeration. Chlorella can be harvested once in every 3 or 4 days (90-120 harvests a year) and it can be accomplished without any special equipment and techniques. The harvested alga is carried automatically through a transport pipe to a tank. Chlorella is generally collected by means of a centrifuge, but if large quantities are involved the technique becomes ineffective. The algal cells can be rapidly flocculated and sedimented using chemical agents such as alum, calcium hydroxide and magnesium sulphate. The supernatant is decanted and the algal bodies are purified by repeated flocculation and washing. The chlorella paste sedimented on the bottom of the harvesting tank is processed as follows: The paste is treated by heat, dried in air, made to dry chlorella powder, stored in the silo and made to enriched silage (fermented fodder). The chlorella paste may also be treated with boiling water for 2 or 3 min. and moved to cold water as soon as possible; the heat treated chlorella paste is suitable for livestock feed.

Chlorella can be stored either after drying in sun or in a desiccator or after a fermentation treatment. The bacterial contamination from the stored chlorella can be eliminated by pasteurization and by fermentation. The pasteurized and fermented chlorella are easily digested and the latter is liked more by livestock because of its lactic acid smell. The autolysing enzymes in chlorella can be inactivated by bleaching and the alga can be stored for a long time.

The yield of chlorella per unit area is not very definite. However, an average yield of 1 lb./sq. yd in wet weight, which is nearly 30 times the yield of rice, has been achieved. The yield of chlorella varies according to season, being high in spring and low in summer and early spring. A Japanese farmer from a 10 sq. yd culture pond has obtained a daily yield of 15·4 lb. and a total annual yield of *c.* 6200 lb. (*c.* 2·8 tons).

The protein in chlorella is more like animal protein than vegetable protein. It has three amino acids,

cystine, methionine and tryptophane, not found in soybean. It has the same amount of protein as fish and the vitamins A and C are very high. The nutritive value of chlorella can be increased by mixing it with rough feed.

The digestibility of fresh chlorella is nearly 70 per cent and on drying its digestibility is lowered to 65 per cent. The digestibility can be increased to 80 per cent by blanching the alga with hot water for 2 min.

Feeding experiments conducted with poultry showed an increase in the size of chicken and the egg production. The size and weight of eggs increased and the yolk became deep orange. Hogs fed chlorella gained healthier appearance. When 1 lb. of chlorella was given each day to a cow there was an increase in milk yield. Production with regular feed was 53 lb. per day and with chlorella, 64 lb. Similar experiments conducted with other animals showed an increase in body weight. In birds the frequency of egg laying is considerably increased. Gold fish and tropical fish fed chlorella increased their resistance against fish diseases. From the economic standpoint the cultivation of chlorella is very inexpensive and the labour cost is very little. The cost of growing 1 lb. of chlorella would be about the same as that of rice, but its nutritive value is twice that of rice. Chlorella feed saves the expenses of animal husbandry to a greater extent. Except in a few regions, in many parts of the world like Asia, Africa, Middle and South America where the situation of animal husbandry is very critical, the production of a new feed such as chlorella is long awaited [*Chlorella feed for animal husbandry* by Hiroshi Nakamura (International Chlorella Union, Tokyo, Japan)].

Progress Reports

Chemical Research Laboratories, CSIRO, Australia

The annual report of the Chemical Research Laboratories of the Commonwealth Scientific & Industrial Research Organization (CSIRO), Australia, for the year 1963-64 records the achievements of the laboratories in fundamental and applied research during the period and the part played by the laboratories in the expansion and development of both primary and secondary industries in Australia. A brief résumé of the activities of the various divisions of the laboratories is given below.

The Division of Applied Mineralogy has been mainly concerned with the nature and industrial application of natural and synthetic mineral products, specially the non-metallics such as cements and concretes, refractories, engineering ceramics, non-metallic minerals and foundry sands. A method has been developed for determining thermal conductivity of refractories. The method consists in inserting a butt-welded thermocouple between two bricks and using it as a line heat source by the application of an alternating current. The thermal conductivity is calculated from the rate of temperature rise of the thermocouple after filtering out the applied a.c. The method is simple and relatively fast but at present is limited to a maximum temperature of 600°C.

In the Chemical Engineering Division investigations are being carried out on desalination of water, hydrogenation of brown coal, fluidization, comminution, reaction kinetics, transport phenomena in turbulent systems, boiling heat transfer and rheological properties of non-Newtonian fluids. A prototype domestic desalination unit for producing up to 100 gallons of fresh water per day from saline water and a commercial model of cyclosizer, a laboratory precision apparatus for the rapid and accurate determination of particle size distribution within the sub-sieve range are under fabrication.

The activities of the Division of Chemical Physics cover the fields of spectroscopy, crystallography and solid state chemistry. The projects on which work is in progress include the study of molecular structure and energetics by means of optical spectroscopy, determination of trace elements by atomic absorption spectroscopy and molecular structure determination of sulphur compound from Raman polarization data. A new type of atomic spectral lamp which gives atomic resonance spectra several hundred times more intense than the conventional lamps has been developed. A number of instruments have been constructed during the period; these include an evaporation metering device, a thermal conductivity bridge, furnace controller, etc.

The Division of Mineral Chemistry carries out investigations in the fields of electrochemistry including gold cyanidation and fuel cells; mineral treatment comprising mainly hydrometallurgical studies of extraction processes; process development including pilot plant testing of processes; and development of new techniques in analytical chemistry. A new method for preparing anhydrous aluminium sulphate which shows promise of providing cheaper pure alum for the paper industry has been developed and tested on the laboratory scale. The product is obtained by reacting conc. sulphuric acid with basic aluminium sulphate or alumina trihydrate; the process avoids expensive crystallization procedures. A unique process for the recovery of zirconia from zirconium sulphate solutions has resulted from an extensive survey of the conditions necessary for the formation of polymeric species in solution. The polymerized species can be quantitatively recovered by the addition of an organic reagent which yields a precipitate containing as much as 28 moles of zirconia per mole of precipitant; impurities are eliminated during the precipitation stage.

In the Division of Organic Chemistry work on organophosphorus compounds has been continued, specially with regard to the preparation of biologically active substances. The use of phosphines as coordinating agents in preparing novel derivatives of metal sulphides and mercaptides has been extended from the initial work with gold to include silver and copper. Numerous chemical reactions of both aryl and alkyl aluminium compounds have been explored, and considerable progress has been made in understanding the exchange reactions which take place in these complex systems by NMR spectrometry. Some organic compounds of zinc have also been included in these studies. A new method

for the industrial preparation of the cyclopentadienyl derivatives of certain metals has been developed and a small pilot plant is now under construction.

Work in the Division of Physical Chemistry has been directed towards the study of thermodynamic properties of liquid mixtures; influence of pressure on the electrical conductivities of solids; magnetic behaviour of some iron complexes, and rates of ionic reactions; aerobic oxidation processes catalysed by metal ions; and water desalting and reclamation. A mixing cell has been devised for the precise measurement of the heats of mixing of liquids.

An electron spin resonance technique has been developed for identifying certain of the free radicals which are formed in proteins as a result of hydrogen ion abstraction by oxidation or by irradiation. It has been shown that during oxidation of a protein in an aqueous medium the most readily formed free radicals are those of the aromatic amino acids, particularly phenylalanine, tyrosine, etc. This leads to the conclusion that a major function of the aromatic units is protection of the functional part of a protein against damage by oxidation. A new oscillographic system with a bandwidth of 75 Mc. and a resolution of 20 nsec. has been constructed in order to observe and photograph light pulses emitted during the nucleation of crystals. The apparatus is sensitive enough to detect the small amounts of light emitted from sources such as a human hand, or the phosphorescence of ice. It is being used to examine the phenomena of crystalloluminescence and triboluminescence in some detail.

Dr A. R. Verma

Dr Ajit Ram Verma, Professor and Head of the Physics Department, Banaras Hindu University, Varanasi, has been appointed Director, National Physical Laboratory, New Delhi.

Dr Verma (*b.* 20 Sept. 1921) took his M.Sc. degree in Physics from Allahabad University in 1942 and Ph.D. from London University in 1952. He was Lecturer in Physics, Delhi University, during 1947-50 and British Council Scholar and later ICI Research Fellow, London University, during 1950-55. He became Reader in Physics, Delhi University, in 1955. In 1959, he joined Banaras Hindu University, Varanasi, as Professor and Head of the Physics Department, where he continued till he took up the present post.

Dr Verma's special fields of study are: crystal growth, imperfection in crystals, and X-ray and optical studies. He has done research work in collaboration with late Dr K. S. Krishnan, FRS, Prof. S. Tolansky, FRS, and Prof. J. D. Bernal, FRS, and has published over 40 research papers. He is the author of two books, *Crystal growth and dislocations* and *Polymorphism and polytypism in crystals*. He is a Fellow of the National Institute of Sciences of India.

Announcements

▪ *A Symposium on Radioactivity and Metrology of Radionuclides* will be held at Trombay during 13-17 December 1965 under the auspices of the Atomic Energy Establishment, Trombay. The symposium will cover various aspects pertinent to the problems of radioactivity measurements in fields such as radiological protection, studies of natural radioactivity, applications of radioisotopes in physical and life sciences and industry, and radioisotope standardization. The object of the symposium, the first of its kind to be held in India, is to help in the collation and exchange of information among scientists working in various laboratories in the country and provide information regarding developments in radiation measuring techniques, equipment and radioactivity standards.

Separate technical sessions will be devoted to: (1) Units of radiation and radioactivity, radioactivity standards and international intercomparison; (2) Radioactive decay chains, activity build-up, isotope ratio in heavy elements and disequilibrium studies; (3) Environmental radioactivity measurements; (4) Radiation dosimetry and radioactivity measurements in radiological safety evaluation; (5) Techniques of measuring radioactivity in bulk specimens and problems related to interpretation of results; (6) Radioactivity measurements for miscellaneous applications of radioisotopes in life sciences; (7) Radioactivity measurements for miscellaneous applications of radioisotopes in physical sciences; and (8) Techniques and instrumentation for radioactivity measurements.

Those desirous of participating in the symposium should communicate with Dr U. C. Gupta, Secretary, All India Symposium on Radioactivity and Metrology of Radionuclides, NIS Section, Electronics Division, Atomic Energy Establishment, Trombay, Bombay 74 AS. Two copies of papers along with abstracts should reach the Secretary on or before 1 October 1965.

▪ *A Symposium on Liver Metabolism* will be held in Bombay during 10-11 September 1965 under the auspices of the Society of Biological Chemists of India. Papers relating to experimental studies on structural, functional and biochemical aspects leading to current understanding of liver functions will be considered for presentation at the symposium. Broadly, these papers may deal with: (1) Factors influencing liver function — nutritional deficiencies and experimental injuries; (2) Biosynthesis and metabolism of lipids and steroids; (3) Metabolism of amino acids and proteins; (4) Carbohydrate metabolism and hormonal regulation; (5) The liver and vitamins; (6) Nucleic acids and protein biosynthesis; (7) Regulation of enzyme synthesis and other control mechanisms; and (8) Specialized functions of the liver. Further details regarding the symposium can be had from the Honorary Secretary, Society of Biological Chemists of India, Indian Institute of Science, Bangalore 12, or Dr Shanta S. Rao, Reproductive Physiology Unit, Indian Cancer Research Centre, Bombay 12.

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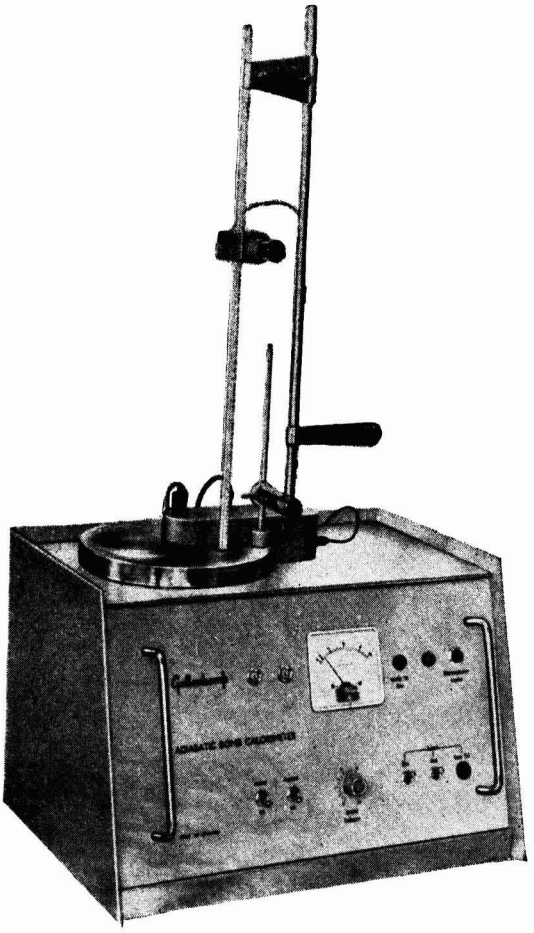

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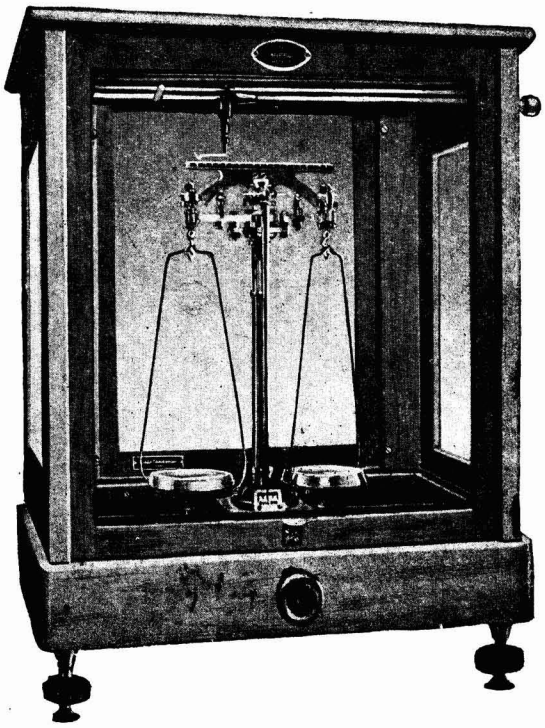

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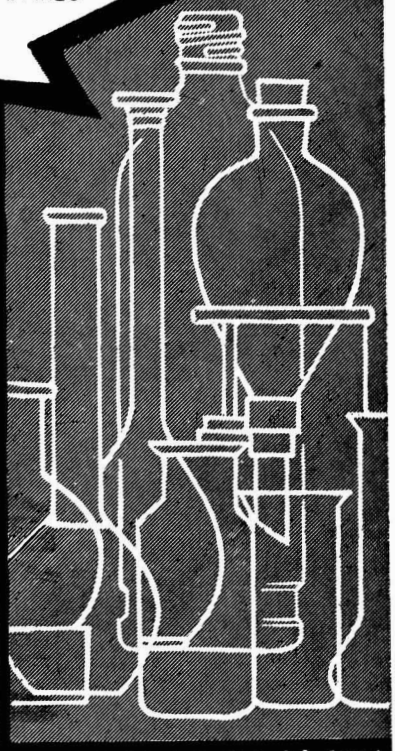

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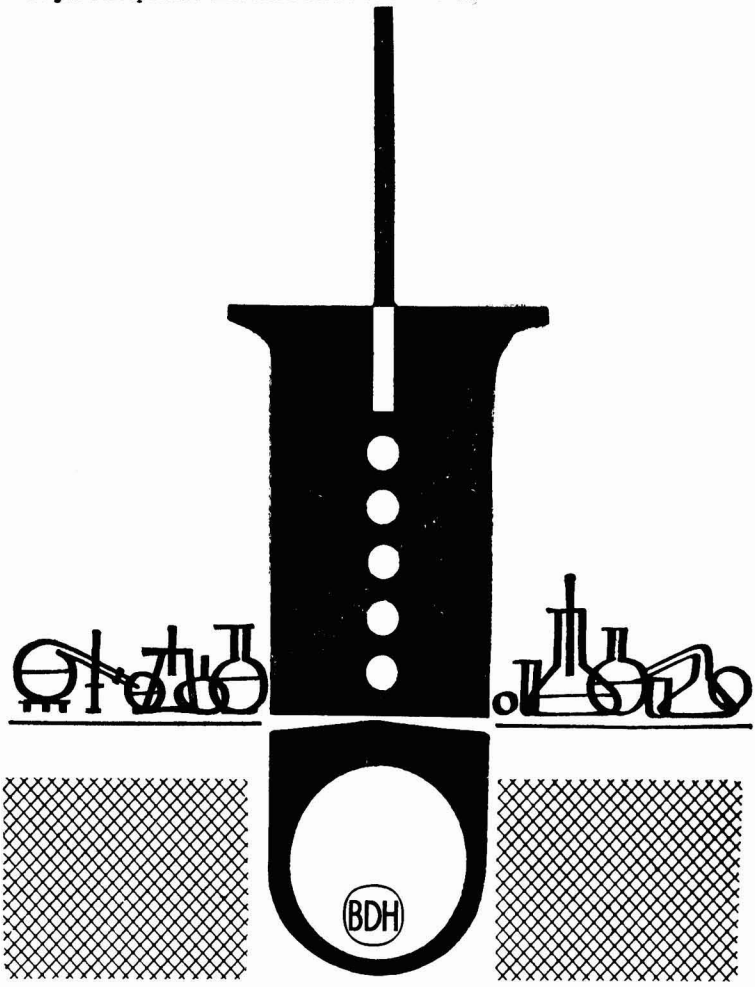

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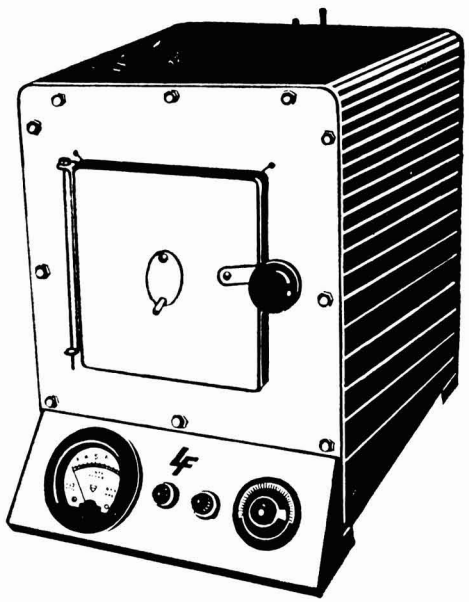

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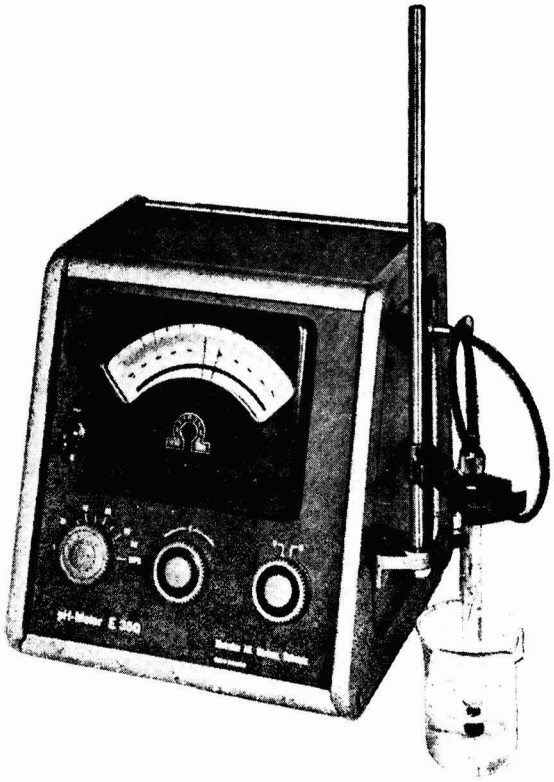

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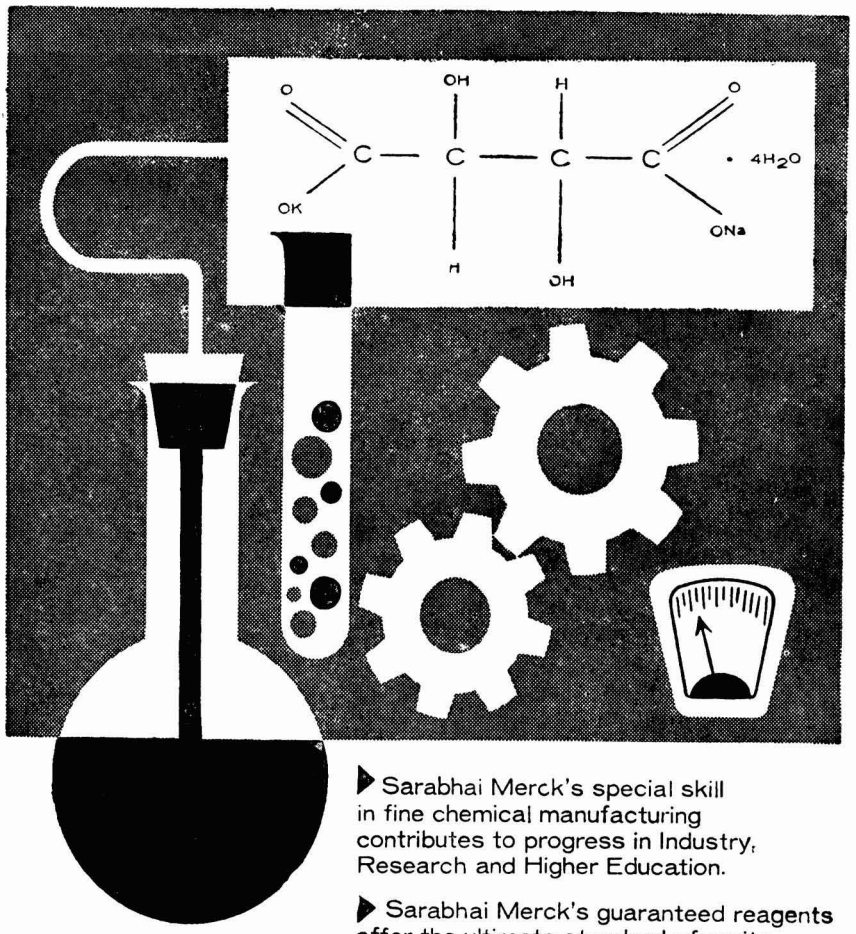

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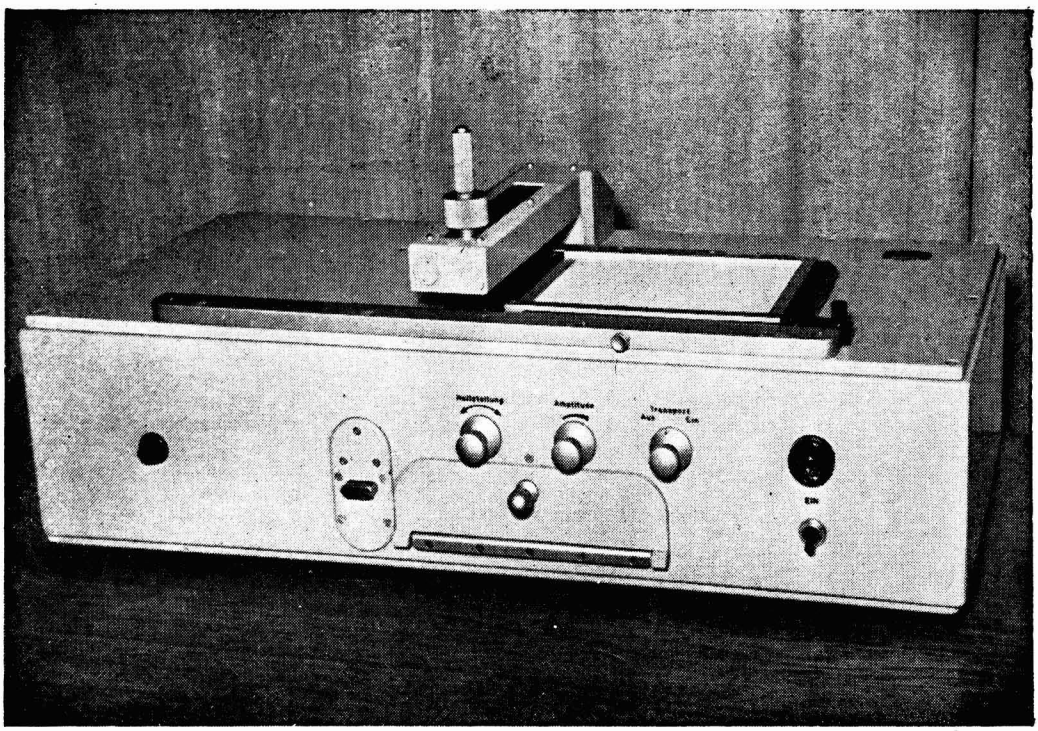

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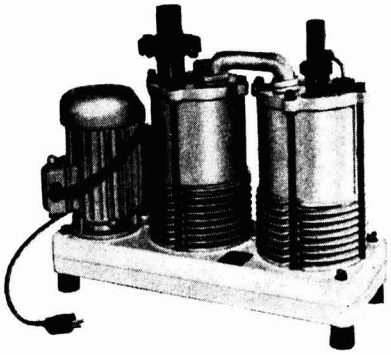

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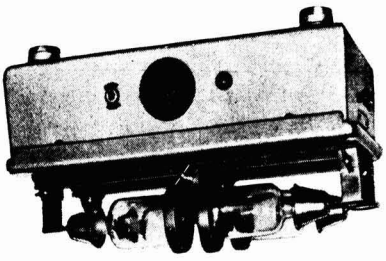

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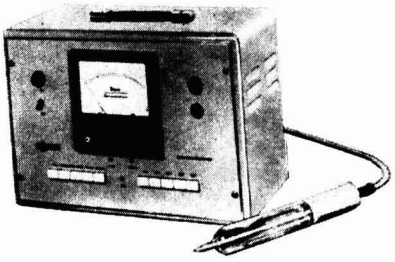

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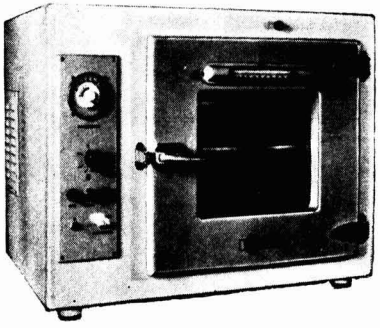

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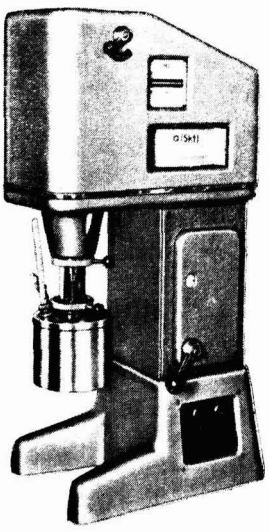

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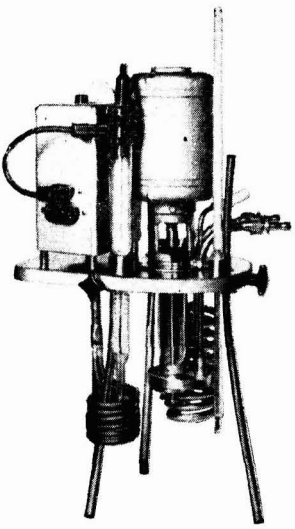

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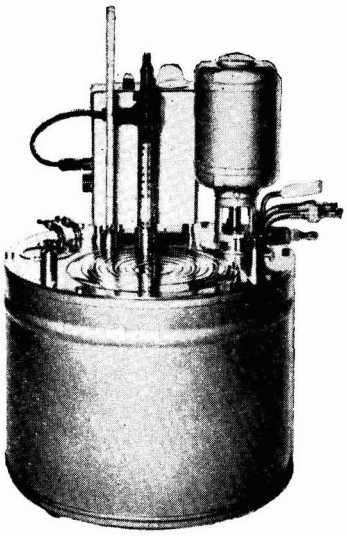

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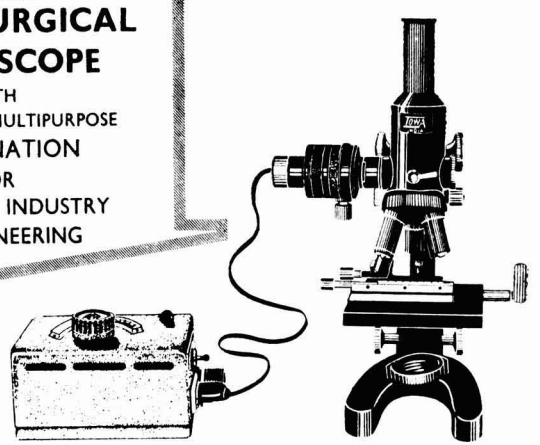

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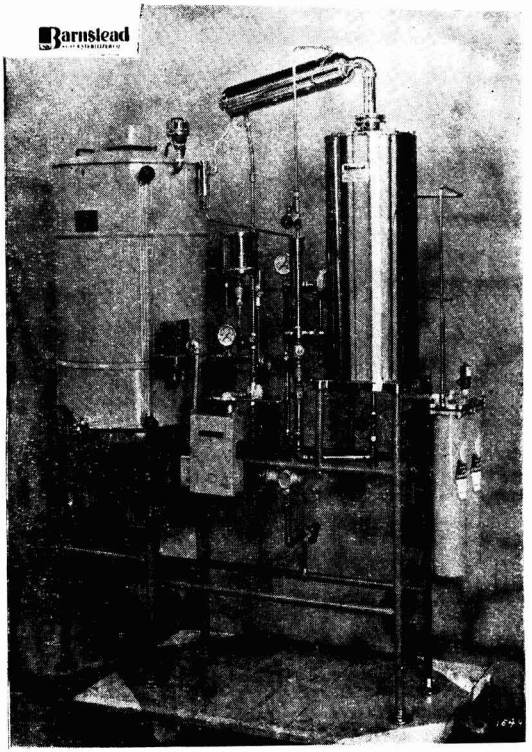

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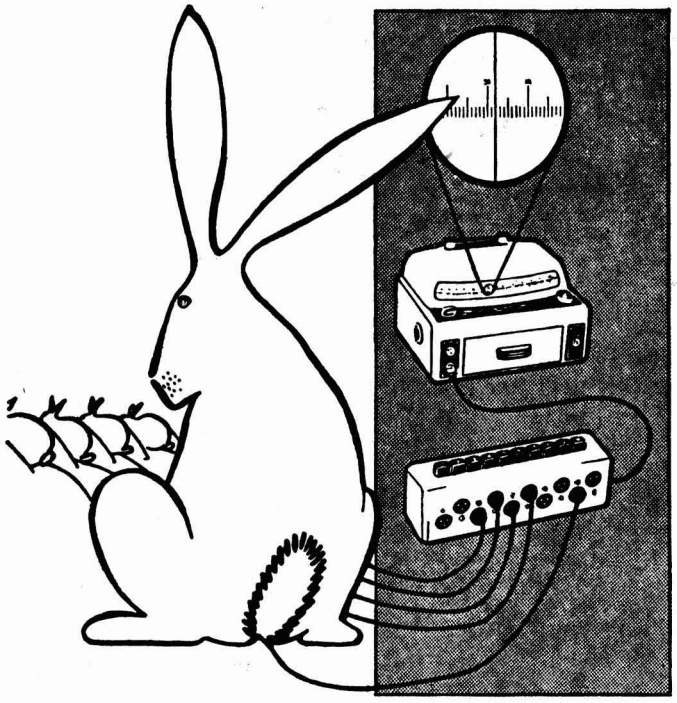

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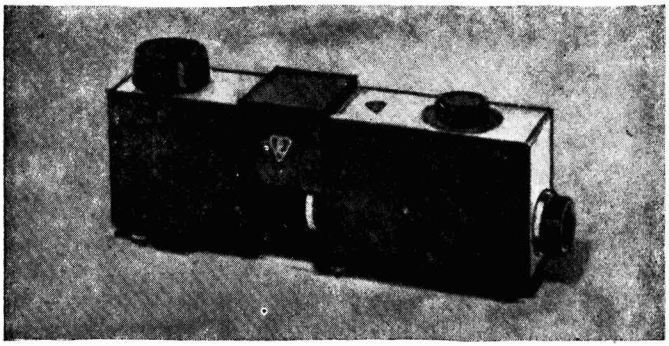

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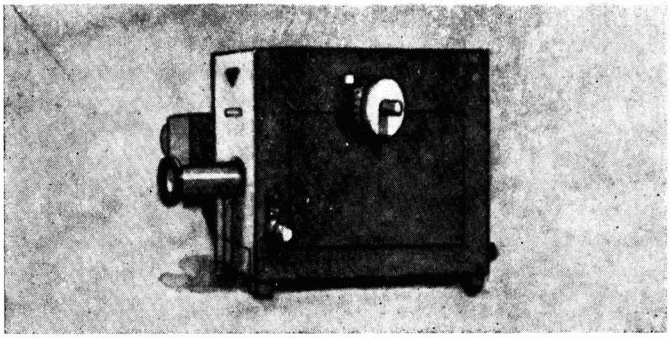
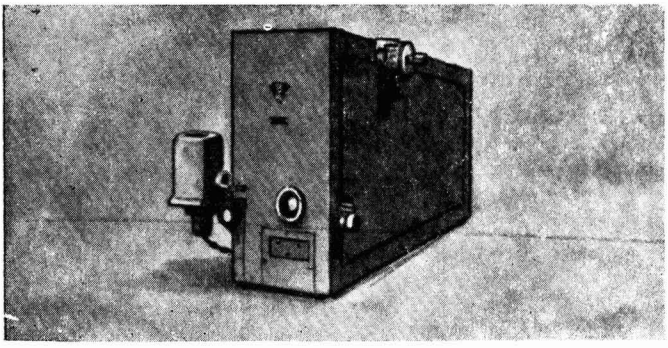
250 MM. MONOCHROMATOR





500 MM. MONOCHROMATOR

BAUSCH & LOMB

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