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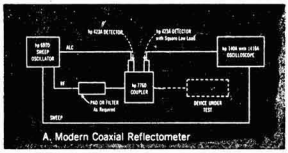
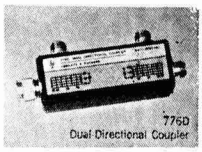
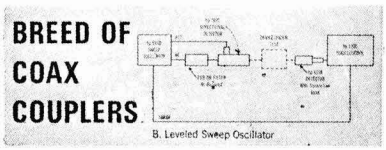
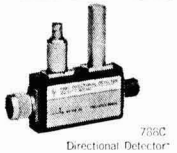
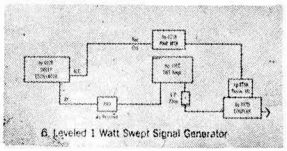
NEW BREED OF COAX COUPLERS


A. Modern Coaxial Reflectometer


770D
Dual-Directional Coupler


B. Leveled Sweep Oscillator




C. Leveled 1 Watt Swept Signal Generator

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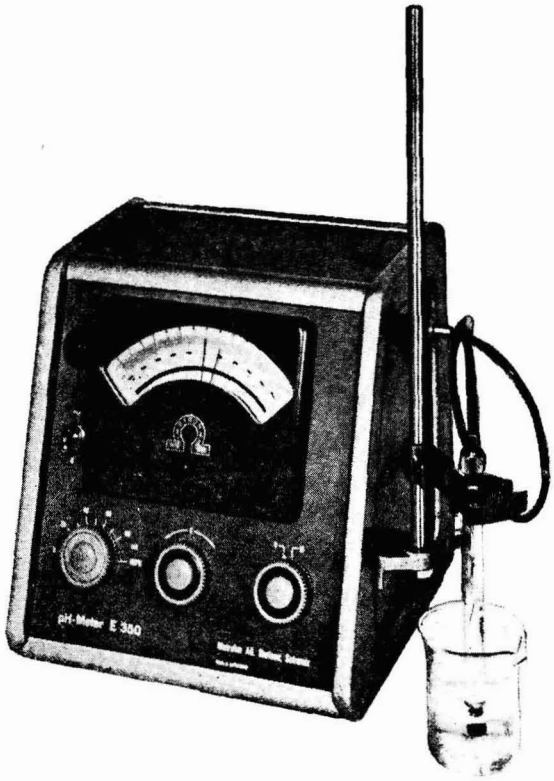

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INDEX TO ADVERTISERS

Homi Jehangir Bhabha

IT is with deep regret we record the tragic and untimely death of Dr Homi J. Bhabha on 24 January 1966 in an air crash near Geneva while on his way to attend a meeting of the International Atomic Energy Agency. He was 56.

Dr Bhabha held many important positions in national and international scientific organizations, and was the recipient of many honours. He was the Director and Professor of Theoretical Physics, Tata Institute of Fundamental Research, Bombay; Chairman, Indian Atomic Energy Commission; Secretary, Department of Atomic Energy; and Director, Atomic Energy Establishment, Trombay. He was a member of the Scientific Advisory Committee of both the United Nations and the International Atomic Energy Agency, and was the Chairman, First International Conference on the Peaceful Uses of Atomic Energy. He was the Chairman of the Indian National Committee for the International Council of Scientific Unions and Chairman of the Scientific Advisory Committee to the Cabinet, Government of India. Dr Bhabha was associated with the Council of Scientific & Industrial Research since its early days as a member of its Governing Body.

A distinguished theoretical physicist, Dr Bhabha achieved recognition for his pioneering work on cosmic radiation, theory of elementary particles and quantum mechanics. His cascade theory, developed with W. Heitler in 1937, is basic for an understanding of the soft component of cosmic radiation, and the behaviour of high energy electrons and gamma rays. He established the existence of a new elementary particle, the meson, which owes its name to him. His prediction of the slowing down of the rate of decay of μ-mesons with increasing velocity in accordance with the theory of relativity and its subsequent observational confirmation remain one of the best proofs of the correctness of Einstein's special theory of relativity. His work received wide recognition, and in 1941, at the early age of 31, he was elected a Fellow of the Royal Society, London. In 1955, he predicted the possibility of harnessing energy from fusion reactions and the taming of hydrogen bomb providing limitless source of power; it is only a man with his gifted ability to look far into the future who could do so.

To measure in concrete terms the many and varied achievements and contributions of a versatile scientist and engineer like Dr Bhabha is indeed futile. What he accomplished during the past three decades is evident in an abundant measure in the magnificent monuments — some completed and others in the blueprint stage — he has left behind, but what he could have achieved in the

years to come in the cause of world science and peace is beyond estimate. He was at his zenith when death suddenly snatched him away. With his vast and cumulative wisdom and experience in handling vital national and international affairs of science and technology, his contributions during the next decade or two would have dwarfed his earlier contributions. His was a dominant and convincing voice, and his views were much valued in international scientific circles. The world of science has lost his humanizing influence and his ardent championship of the developing countries.

To India, which is on the threshold of launching herself on a self-sustaining and self-generating economy, Dr Bhabha's death is an immeasurable loss. He had many fruitful and active years of life ahead, and with his unusual insight into and grasp of India's problems and needs in respect of scientific and technological development, he would have seen his dream come true — a modern India, whose progress, based on fruits of science and technology, would bring her people a decent standard of living, and earn her a valued place in the world. Dr Bhabha has well and truly laid the foundations for his dream to come true by his atomic energy programme and all its implications. The atomic energy programme is not merely a means to an end; it has many far-reaching effects. There

were many basic issues for which Dr Bhabha sought to find solutions even before he actually embarked on an active programme of work in the field of atomic energy. The development of a scientific and technological base and of the human resources of the type essential for scientific progress that will have a lasting effect on the economic development of the country was one of his principal objectives. The main accent on whatever programmes he undertook was self-sufficiency in every way. Thus he was instrumental, with his usual foresight, in setting up the Tata Institute of Fundamental Research, Bombay, which formed the nurturing bed and storehouse for trained personnel and know-how needed for meeting the demands of future developments in atomic energy, radioastronomy, space research, molecular biology, radiation medicine, etc. Electronics, basic to modern technology, made rapid strides under Dr Bhabha's stewardship and today India is almost self-sufficient in this advanced field.

Dr Bhabha was keenly conscious of the deleterious effects of leaning heavily on foreign know-how. By developing indigenous know-how in many sophisticated technologies he inculcated a strong sense of self-reliance among his scientists and technologists. The way he selected able and young scientists and moulded them to handle the difficult tasks of nuclear energy research and development provides a unique lesson and a pointer as to what can be done by similar methods in other areas. This he achieved by inspiring confidence among his workers and creating necessary climate to acquire it. Dr Bhabha saw to it that his workers developed contacts with eminent scientists. Not only had his workers ample opportunities to attend international scientific conferences abroad, but he also ensured that many such meetings were organized in India and invited prominent scientists to take part in them.

Dr Bhabha will be remembered by future generations as one of the architects of modern India. He has shown the way to progress in many areas of national endeavour, and the methods he successfully employed to earn India a prominent place in the field of nuclear energy should be followed and diligently applied to other areas of national activity. Dr Bhabha has laid true and solid foundations for national development, and there is every reason to hope that his unfinished work will be taken up and successfully completed by those in whom he has instilled a sense of purpose and dedication.

To conclude, we cannot do better than to quote from the resolution adopted by the workers of the Department of Atomic Energy at a meeting to pay tribute to the memory of Dr Bhabha.

" The death of a great man does more than put an end to a scientific career. It destroys an accumulation and synthesis of knowledge, skill, judgement and experience that cannot be transmitted and preserved in its entirety because it is incommunicable. His contributions to research will perpetuate his scientific memory. But as a unique personality, who sparked the development and advancement of atomic energy, and as one who, more than any other single individual in this country, recognized the importance of science in its manifold aspects for the progress of civilization, and as one who ardently advocated the progress of scientific progress in developing countries, Homi Bhabha was one of the greatest experiences in the life of the country and in this sense he will never die unless science itself ceases to exist.

" The greatest tribute we can pay Homi Bhabha is to dedicate ourselves to the ideals for which he stood and to do our utmost to contribute to the growth of scientific knowledge and its application for the betterment of the people of India."

Liver Metabolism—A Symposium

A. R. SHETH & U. S. V. ACHARYA
Reproductive Physiology Unit, Indian Cancer Research Centre, Parel, Bombay 12

THE Society of Biological Chemists, India, organized a Symposium on Liver Metabolism in the auditorium of the Cardiovascular and Thoracic Centre, Parel, Bombay, on 10 and 11 September 1965. It was perhaps one of the well-attended symposia, as delegates from various parts of the country got together in spite of the conflict on the borders of our country. A very cordial atmosphere prevailed during the sessions, which was brought about by Dr A. Sreenivasan (President of the Society), Dr (Mrs) Shanta S. Rao (Convener) and the Chairmen of the three sessions of the symposium, Drs H. R. Cama, V. Jagannathan and K. Sundaram. As many as 16 papers relating to experimental studies on structural, functional, biochemical and immunological aspects leading to the current understanding of liver functions were presented in three sessions. A fruitful attempt had been made in grouping these presentations in order to bring unity to the diverse subjects of study.

The morning session on 10 September was held under the chairmanship of Dr H. R. Cama of the Indian Institute of Science, Bangalore. A paper on the 'Intracellular localization and biosynthesis of catalase in rat liver' by T. M. Radhakrishnan and P. S. Sarma was presented by Dr Radhakrishnan. They reported that the catalase activity in rat liver was greater in the 100,000 *g* supernatant than in mitochondria. These results were confirmed by studying the *in vitro* and *in vivo* incorporation of labelled ^{59}Fe in the mitochondria and the supernatant.

Dr G. D. V. Van Rossum from the Christian Medical College, Vellore, read a paper on 'The control of respiration in slices of rat liver' which led him to propose that much of the respiration of liver slices is controlled by the availability of high energy acceptors other than ADP. Only 20 per cent of the respiration of the liver slice depended on ADP.

'Estimation of calcium in subcellular fractions of rat livers', a paper by Radhakrishna Murthy, N. Ramanna and M. V. Patwardhan, was presented by Dr Patwardhan from the Central Food Technological Research Institute, Mysore. The authors have modified a method for estimating calcium from biological material in general and liver mitochondria and homogenate in particular, by reacting the calcium with excess EDTA and titrating the excess EDTA with calcium chloride.

A paper on 'Gluconeogenesis from amino acids in rat *in vivo* and the regulatory factors' by L. Deshpande and G. B. Nadkarni was presented by Dr Nadkarni from the Biochemistry and Food Technology Division of the Atomic Energy Establishment, Trombay. Gluconeogenesis from amino acids *in vivo* has been assessed in normal conditions and in metabolic variations such as in different states of glycaemia, under hormonal conditions and after X-ray irradiation, using isotope tracer procedure.

Dr Sumati V. Bhide from the Indian Cancer Research Centre, Bombay, presented a paper on '*In vitro* effect of urethan on enzymes of intermediary metabolism'. She observed that in *in vitro* experiments, $10^{-1}M$ urethan inhibited ornithine transcarboxyle and carboxyl phosphate synthesis though aspartic transcarboxylase activity was not affected.

A paper on 'Lipoproteins of perfused rat liver supernatant fraction' by M. M. Bhargava and A. Sreenivasan was presented by Shri Bhargava. The paper dealt with the separation of lipoproteins from rat liver supernatant by paper electrophoresis. The chemical composition of these fractions with special reference to serum lipoproteins has been studied. They have also studied the changes in these lipoprotein fractions in fatty liver produced by administration of carbon tetrachloride.

Dr V. Jagannathan from the National Chemical Laboratory, Poona, took the chair during the afternoon session. The paper entitled 'Liver lysosomal enzymes in protein deficiency' by P. L. Sawant, S. Saroja, S. R. Padwal Desai and V. S. Kumta was presented by Shri Sawant. These authors reported that when rats were fed on a protein deficient diet there was a five- to six-fold increase in catheptic and acid ribonuclease activities of lysosomes indicating that the liver was damaged.

Miss Leelavathi from the Indian Institute of Science, Bangalore, presented a paper on 'Reversal of orotic acid fatty liver by nicotinamide' by D. E. Leelavathi and P. S. Sarma. They found that the fatty liver produced by feeding the pyrimidine precursor orotic acid to rat and mice could be reversed completely by intraperitoneal injection of nicotinamide at 12 hr intervals for four days. This was attributed to an increased rate of synthesis of adenine nucleotides as well as by reduced degradation. A paper by J. Amruthavalli and P. S. Sarma from the same laboratory on 'Orotic acid fatty livers-species specificity' was presented by Miss Amruthavalli. Induction of orotic acid in rat led to intense hepatic fatty infiltration, whereas the chick and the mouse failed to show fatty livers. Oral administration of orotic acid 6-^{14}C showed a decrease in adenine nucleotide following increase in uridine nucleotide levels in rats. In chicks, they found an increase in uridine nucleotides with no changes in adenine nucleotides whereas in mouse no change was observed. Further, intraperitoneal administration of 4-amino, pyrazolo pyrinodine — a potent inhibitor of adenine nucleotide synthesis, which is known to cause fatty liver in mouse and rat — caused no fatty liver in the chick.

Dr P. G. Tulpule presented a paper by P. G. Tulpule and K. Suryanarayan Rao from the Nutrition Research Laboratories, Hyderabad, on 'Changes in the composition of liver fat of aflatoxin fed animals'. In duckling, guinea-pigs and monkeys, oral

administration of aflatoxin (the toxic factor isolated from groundnuts infected with some strains of *Aspergillus flavus*) was shown to produce liver damage as evident by changes in the composition of liver fat.

A paper on ' The effect of Maharashtrian diets on liver enzymes of rats ' by A. B. Kamble and Kamala Sohonie was presented by Shri Kamble. With the exception of alkaline phosphatase, the activities of all other liver enzymes (xanthine oxidase, succinic and lactic dehydrogenase, aldehyde oxide, acid phosphatase, ATPase, transaminase and arginase) were found to be reduced with typical Maharashtrian diet as compared to those of casein diets.

Dr K. Sundaram from the Atomic Energy Establishment, Trombay, took the chair for the last session of the symposium. Dr H. R. Cama from the Indian Institute of Science, Bangalore, read a paper on ' Studies on the metabolic derivative of vitamin A ' by D. V. John, M. R. Lakshman and H. R. Cama. Their studies *in vivo* and *in vitro* on the β-ionone ring oxidized derivative of vitamin A (i.e. 5,6-monoepoxyretinal which has been demonstrated to be biologically as potent as the all trans retinyl acetate) showed that this compound is metabolized uniquely without giving rise to vitamin A. Further, 3-dehydro derivatives of vitamin A (i.e. 3-dehydroretinal which possesses a biopotency of 30-40 per cent of that of retinyl acetate) also metabolized in an identical way as retinal. A linear relationship between 3-dehydroretinal levels of blood and liver was detected irrespective of the dose. It was also found that the aldehyde oxidase from livers of different species metabolizes 3-dehydroretinal as efficiently as the retinal.

The next paper on ' Sulphite-cytochrome *c* reductase of rat liver microsomes — a cryptic enzyme ' by R. Ramasarma, V. C. Joshi and C. K. Ramakrishna Kurup, Indian Institute of Science, Bangalore, was presented by Dr Ramasarma. They found that sulphite-cytochrome *c* reductase was in a cryptic state in the microsomes of rat liver and could be liberated by treatment with deoxycholate, by ageing or by extracting the lipids with acetone when its activity increased 6-10 fold.

Dr G. P. Kamat from Haffkine Institute, Bombay, presented a paper by G. P. Kamat and S. S. Rao on ' Cytotoxic effect of rabbit anti-rat liver serum '. They found that the oxidative phosphorylation of rat liver mitochondria was uncoupled by rabbit anti-rat liver serum. The antiserum was shown to exhibit cytotoxic effect especially in young rats.

A paper on ' Biochemical studies with the liver of rats and mice fed with a progestational steroid ' by J. R. Padbidri, U. M. Joshi and Shanta S. Rao, Reproductive Physiology Unit, Bombay, was presented by Dr Rao. When a physiological dose of Enovid (a progestational agent widely used for contraceptive purposes) was administered, no change was observed in the activities of the enzymes studied (glutamic-oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase and succinic dehydrogenase). In the animals receiving a high dose of Enovid, succinic dehydrogenase and glutamic pyruvic transaminase activities of rat liver were considerably elevated.

Perhaps more effective and benefiting discourses could have been on some other general topics also related to the metabolism of serum proteins including lipoproteins and glycoproteins under normal and abnormal conditions of liver. Of course some of this inadequacy could be attributed to the inability of some of the delegates to attend the symposium due to national emergency. One of the interesting programmes of the symposium, a talk on ' Kwashiorkor and marasmus — A comparison of salient biochemical features ' by Dr C. Gopalan, Director, Nutritional Research Laboratories, Hyderabad, was cancelled at the eleventh hour for the same reason. However, to a large extent, the purpose of the symposium was well served since it enabled exchange of information and initiation of some new ideas to overcome some of the technical difficulties involved in the experimental studies and stimulated thought in the field which is of immense biochemical and biological importance.

The Seventh Biennial Conference on Carbon

B. R. PURI
Department of Chemistry, Panjab University, Chandigarh

THE Seventh Biennial Conference on Carbon, co-sponsored by the American Carbon Committee, and Research and Technology Division of the US Air Force, with the support of Union Carbide Corporation, was held at the Case Institute of Technology, Cleveland, Ohio, from 21 to 25 June 1965. Over 400 participants belonging to many disciplines, including physics, physical and organic chemistry, minerology, chemical and electrical engineering and rubber technology took part in the deliberation. The foreign delegates who participated in the conference were from Britain (24), France (21), West Germany (12), Canada (10), Japan (10), Italy (2) and one each from Belgium, Netherlands, Switzerland, Spain, UAR, Australia and India. The conference, therefore, had essentially an international character. The delegates were from many diverse institutions such as universities, industries, atomic energy establishments, air force and naval research laboratories, bureaux of mines, etc., of different countries.

The conference also provided a meeting ground and a forum for several formal and informal discussions. Over 200 technical papers were presented and discussed in 10 sessions. In addition, there were three night sessions for discussing specific topics. An idea of the vastness of the multifarious fields of research, both fundamental and applied, involving the versatile element carbon will be evident from the following topics discussed at the conference: (i) Electronic properties (33 papers); (ii) Oxidation studies (30 papers); (iii) Pyrolytic graphite (19 papers); (iv) Mechanical properties (23 papers); (v) Permeability studies and reactor materials (9 papers); (vi) Structural studies (22 papers); (vii) Fabrication technology (9 papers); (viii) Chemisorption and surface reactivity (9 papers); (ix) Thermal conductivity and emissivity (7 papers); (x) Lamellar compounds of graphite (9 papers); (xi) Physical adsorption (16 papers); and (xii) Nuclear radiation effects (19 papers).

Electronic Properties

Some of the papers on electronic properties related to studies of magneto-thermal effects in a pyrolytic graphite at temperatures below 4·2°K., and electrical resistivities of a series of graphites at temperatures from 4·2° to 1·3°K. as well as those of a series of carbon blacks at ordinary temperatures but under high pressures up to 160 kilobars. The resistivity in the case of graphite was found to be maximum at 4°K., while in the case of carbon black it was found to decrease with pressure and to vary in the presence of surface films. The development of a thermocouple consisting of a soft and a hard carbon for high temperature measurements was also described.

In some other papers the importance of spin-orbit interaction in graphite was brought out by accurate determination of the band parameters of graphite; optical properties of graphite were investigated and electron spin resonance (ESR) of the pyrolysis of aromatic hydrocarbons in liquid media and that of chars at liquid air and liquid hydrogen temperatures, as a function of the heat-treatment temperature, were reported. The ESR of chars heated above 500° was oxygen sensitive but was desensitized on pre-treating in an alkali metal vapour at 500°, the effect decreasing in the order Na, Cs, Rb and K. The ESR of different resins showed its dependence on heat-treatment temperature and on the kind of resin, in particular to the increase of methyl radicals. The ESR of graphitized P-33 carbon black in which variable amounts of boron were introduced at 1600-1700° showed a gradual shift of the ESR lines towards the free electron value g. The studies on ESR and diamagnetic susceptibilities of an ultrafine graphite as a function of heat-treatment temperature up to 3000° showed distinct increase in the latter value with increasing temperature. Similar findings were reported in another paper on carbon blacks. The diamagnetic susceptibilities of hydrolytic carbon samples deposited between 1600° and 2400°C. was found to depend on the apparent crystalline diameter and the degree of graphitization.

The effect on the diamagnetism, galvanomagnetic properties and structure of pyrocarbons doped with boron were described. The electronic properties of N-doped carbon prepared through pyrolysis of acridine and subsequent heat treatment from 600° to 2500°C. were also discussed. The effects of the adsorption of polar and non-polar molecules on the electrical conductivity of Saran carbon rods were reported.

Oxidation Studies

The electron microscopic observations of selected areas of graphite before and after exposure to CO_2 irradiated with ionizing and non-ionizing radiation reveal that the extent as well as rate of attack resulting in non-gaseous products depended chiefly on gas composition, temperature and the impurity content of the graphite. The presence of methane, acetylene and ketone vapours in concentration up to 1 per cent is found to reduce the rate of photolytic oxidation by carbon dioxide. The thermal reactions of a nuclear graphite with water vapour were discussed in a few papers. The effect of partial pressure of water vapour from 0·1 to 25·0 mm. was studied from 750° to 900°C. as such, as well as in the presence of hydrogen gas at a pressure of 1-8 mm. Two simultaneously occurring mechanisms were suggested. The inhibiting effect of hydrogen was shown to be due to its competition for active sites with the water vapour. The addition of half per cent of dichlorodifluoromethane reduced the rate of oxidation of certain nuclear graphites to about one-third.

The effect of oxidation on the pore structure of different graphitized carbon samples using low temperature adsorption and electron microscopic techniques were described. Thermal oxidation in air resulted in the increase in surface area and reactivity; radiolytic oxidation in oxygen having even a greater effect. The oxidation of carbon blacks was found to be a function of surface area and degree of deorganization. The kinetics of oxidation of graphite at temperatures 840-2200°C. and pressures 1·78-0·00178 atmospheres of oxygen were reported. The influence of solid catalysts on the etching of graphite single crystal was also studied. The effects of impurity content and BET area of graphites on the burn-off and rates of formation of CO and CO_2 were studied in the temperature range 500-640°C. The kinetics of the carbon-carbon dioxide reaction between 910° and 1007°C. were reported and a mechanism of the reaction proposed. In another paper the effect of hydrogen on the kinetics of the above reaction conducted at low pressure was described. A number of other papers also dealt with the same reaction.

Results of studies on the mechanism of carbon-oxygen reaction using ^{18}O as a tracer showed that both CO_2 and CO are primary products of the reaction and that both the oxygen atoms of the carbon dioxide produced do not necessarily come from the same oxygen molecule.

A number of papers dealt with oxidation of graphite by low concentrations of water vapour and carbon dioxide in helium and by mixtures of CO_2 and CO in helium. The rate of corrosion of graphite by CO_2 and the influence of doping highly purified natural graphite with iron carbonyl powder (300 p.p.m.) were also reported. The catalytic effect of 0·1 mole per cent of transition metals on the corrosion of graphite was the subject of study of another paper. A few papers discussed some of the above reactions on carbon blacks. Two combustion processes were shown to proceed simultaneously: a rapid attack on a limited number of active sites involving surface complexes and a slower first-order attack. The rates of the processes depend upon temperature, the pore size and the presence of the surface complex.

Pyrolytic Graphite

Pyrolytic graphite has assumed importance as a coating material for nuclear fuel particles since it is a relatively strong material with pronounced anisotropy. Some of the important papers contributed for this section described the structure (laminar, isotropic or granular) of the deposits in the fluidized bed and discussed the relation between the mechanical properties of the deposits and their structures. Electron microscopic and diffraction techniques, X-ray diffraction and small angle scattering data were used for determining the structure of deposits obtained by varying the temperature of the fluidized bed between 1500° and 1800°C. The average diameter of the layers was found to be in the range 50-150 A. and bulk density 1·40-2·088/cc.

In some other papers the effect of varying the conditions for the formation of deposits and heat treatment on the properties of pyrolytic graphite deposited from methane were described. The rate of the deposit was found to depend on the kinetics of the reaction, on the substrate area and the rate of transport of reacting gas to the substrate surface.

The kinetics of graphitization at temperatures up to 3000°C. was dealt with in 8 papers. It was shown that (i) the rate of growth of crystallite size was very high until a limiting size was reached and (ii) the size was heat-treatment temperature dependent.

Mechanical Properties

Measurement of moduli of elasticity, fracture stresses, fracture strains, strain energies and strain ratios, etc., were discussed in a number of papers in the section. The use of such tests in evaluating typical graphite was also discussed. In some other papers the mechanical properties of pyrolytic graphite which has been highly ordered by annealing were given. The possibility of using carbides, nitrides, borides and silicides of the transition metals with the object of improving mechanical strength of extruded graphites at elevated temperature was explored. These materials increased the tensile and flexural strengths of graphite rods in the temperature range up to 4000°F. The variations in hardness, mechanical strength, dynamic Young's modulus, thermal conductivity and thermal expansion coefficients with high temperature treatments of coal-carbon compacts were also investigated. Properties such as viscoelastic, rheological, lateral strain behaviour and stress strain graphs of different graphitic materials were also discussed.

Permeability Studies and Reactor Materials

The relationships which exist between different methods of permeability measurements in various grades of graphite having markedly different pore size distribution spectrum were discussed. It was possible to calculate Darcy permeability value from the cumulative pore size distribution spectra. The impregnation of graphitic samples with furfuryl alcohol and coal-tar pitch in reducing the permeability of graphite was described in some papers. The effect of gaseous impregnation of graphite samples by flowing in benzene vapour over samples maintained at temperatures 770-830°C. was also reported. It was also shown that graphites of high densities, low permeabilities, nuclear purity and suitable pore structures can be produced without the use of impregnation treatments of any kind but simply by using a chemical additive to the basic extrusion mix.

Structural Studies

The results of X-ray investigations on different natural graphites showed a close relation between structure and genesis. The results obtained after very short heat treatments on graphitizable carbons (cokes, pyrocarbons) were described. The kinetic studies on graphitization of three different samples of coke were made and the differences in the rate of graphitization was found to be due to different pre-treatment temperatures. The coke prepared by the carbonization of polyvinyl chloride and heated

at 700°C. graphitized very rapidly by the heat treatment at 1400-1500°C. under 10 kilobars.

The thermal polymerization of various hydrocarbons and heterocyclic compounds requiring the use of high pressures was also investigated. The polymers were heat treated at 3000°C. and X-ray diffraction and helium densities were determined. The kinetics of these polymerizations were also reported.

The structural changes undergone by cokes obtained from coals of various ranks on pyrolysis at 400-1000°C., the structural features of humic acid and the factors effecting the graphitization of carbons, as studied with the help of polarized light microscopy, formed the subjects of a number of papers. It was found that low temperature carbons show anisotropy if they are capable of subsequent graphitization at high temperatures (2700-3000°C.). Non-graphitizing carbons are isotropic throughout.

Fabrication Technology

The papers discussed in this section related to technological aspects of the breakdown of carbon powders in an air-driven fluid energy mill, critical rate of heating for baking and shaping carbon articles, preparation of graphite by isostatic pressure baking, preparation of carbon bodies from demineralized anthracite, graphitization of spectroscopic carbons, production of dense carbon aggregates and graphite carbide materials by hot working.

Chemisorption and Surface Reactivity

In this section special techniques for obtaining infra-red spectra for surface complexes on carbons were described and the formation of surface oxides on active charcoals, carbon blacks and diamonds were discussed. The formation of different types of complexes on treatment of active carbons, charcoals, carbon blacks and diamonds with oxygen at 400°C. and with oxidizing solutions at room temperatures were discussed. The presence of oxygen in the form of carboxyl groups, hydroxyl groups and complexes capable of evolving carbon dioxide and their influence on surface properties, such as acidity, polarity, cation exchange capacity, catalytic activity, dispersibility, hydrophobicity, wettability, combustibility, heat of immersion, preferential adsorption, etc., were discussed. In some other papers, fixation of gaseous chlorine by anthracite and that of chlorine, bromine and iodine on carbon blacks were discussed. Certain electrochemical properties of graphite anodes and the anode over-voltage of aluminium cells were also described.

Thermal Conductivity and Emissivity

The discussion in this section was confined to the papers dealing with thermal conductivity, thermal diffusivity, normal spectral emissivity of different types of carbons, of different grades of carbon composites, and isotropic and anisotropic material.

Lamellar Compounds of Graphite

It was shown that all lamellar compounds exhibit a transmission 'window' of half width about 0·2 μ in which the absorption coefficient is 3-5 times lower than that of graphite. In another paper, several hypothesis regarding the state of atoms or molecules distributed between the carbon planes in the lamellar compounds of graphite were discussed.

The preparation of graphite compounds of samarium, erbium and ytterbium by the reaction of graphite with rare earth solutions in liquid ammonia was reported. The thermodynamic properties of the cesium-graphite lamellar compounds were discussed in another paper.

Physical Adsorption

The monolayer physical adsorption of simple gases on graphitized carbon blacks with particular attention to the work on the uniform surface was discussed. The monolayer films are probably two-dimensional fluids. The properties of isolated molecules on the surface were reported. A radioactive tracer technique for the determination of adsorption isotherms at a very low vapour pressure of xylenol on a graphitized carbon black having a homogeneous surface was described. Adsorption of carbon dioxide on carbons at 0°C. at low coverage was used to differentiate between different adsorption sites while adsorption isotherms of *n*-butane at −78·5°C. and 0°C. on ultra-pure mineralogical graphite was suggested as a method for surface area determination. Another method of determining surface area, particularly for carbonaceous fibres, based on the use of electron microscopy and modified BET technique was described. In another paper the validity of the determination of surface area of non-porous carbons by gas adsorption methods was discussed and the utility of BET method for this purpose was shown to be doubtful. A method for determining helium densities for polymer carbons was described in another paper. Some other aspects discussed were molecular sieve properties and adsorption of nitrogen and carbon dioxide on closely sieved fractions of coconut shell charcoal.

The surface area of graphite calculated from adsorption isotherms of and heats of wetting in different liquids such as methyl alcohol, isopropyl alcohol, benzene and toluene were discussed.

The cumulative surface area technique for calculating the surface area of carbon blacks was discussed and the values compared with those obtained by BET technique. A fairly good agreement in several carbon black samples was obtained.

Nuclear Radiation Effects

The papers contributed in this section discussed irradiation induced plasticity in graphite, strength of irradiated graphite, the elastic moduli of reactor graphites, radiation effects at high temperatures and threshold energy for the displacement of atoms in graphite.

Gases with High Electric Strength

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GASES are widely used as electrical insulating materials. Air is the most important of all gaseous dielectrics because of its universal presence. It very often forms part of an electrical device whether we want it or not, acting as insulator in addition to solid or liquid materials specially provided. In certain places, e.g. along spans of overhead transmission lines between adjacent poles, air is the only insulation between the conductors.

Air is a good insulator when voltages are not very high. It is chemically quite stable and can withstand very high temperatures without change of its properties. Current leakage across air is insignificantly small, and dielectric loss angle of air is also extremely small. However, when the voltage across the air gap between electrodes is increased substantially, electric discharges readily occur in the air. In other words, the electric strength of air is far less (at normal pressure) than that of high quality solid and even liquid electrical insulating materials. Therefore, to ensure reliable operation of electrical devices, the distance between bare live parts at high voltage and also the distance from these parts to grounded conductor objects should be much greater than that between live parts which have solid or liquid insulation.

This shortcoming can be avoided in two ways. It is possible to use air at elevated pressure (or, on the contrary, at very low pressure) because the pressure of air, as in the case of other gases, has a considerable influence on the electric strength. This fact is used in air capacitors under pressure, in some designs of electrical cables, in vacuum capacitors, etc. Besides air other gases can also be used.

Other things such as the pressure, temperature, shape of electrodes and distance between them being equal, the electric strength of different gases is not the same. Nitrogen has electric strength approximately equal to that of air; hence nitrogen is used sometimes instead of air because of the absence of oxidizing effect on organic insulation. The electric strength of carbon dioxide is 0·9 of that of air. Hydrogen, which is of special interest in electrical engineering as a coolant for rotating electrical machines, has an electric strength only 0·6 of that of air. The electric strength of inert gases, such as argon and neon which are used for filling gas-discharge lamps, etc., is even less.

But there are some special gases which possess electric strength considerably greater than that of air. These so-called electronegative gases which are comparatively stable so as to be unaffected by collision ionization, have comparatively high molecular weight and density, and contain the halogen (usually fluorine and/or chlorine) atoms in their molecules.

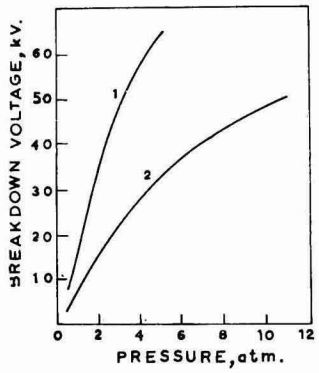

Fig. 1 — Dependence of breakdown voltage (a.c. peak values, or d.c.) upon absolute pressure at constant temperature of 20°C. [The gap between the electrodes is equal to 3·8 mm. Curve 1, in air; and curve 2, in elegas]

Prof. B.M. Hochberg[1-6], an eminent Soviet physicist, has observed that the electric strength of sulphur hexafluoride[7,8] is approximately 2·5 times that of air. Prof. Hochberg has called this gas with high electric strength ELEGAS (abbreviation from words ELEctricity and GAS). Pure elegas is not toxic to human beings and is 5 times heavier than air, quite stable and does not decompose when heated up to 800°C. Therefore, it is quite suitable for use in various electrical devices, capacitors, cables, etc. The advantages of elegas are most striking at high pressures. Fig. 1 shows how the breakdown voltage between two metallic discs with rounded edges in air and in elegas depends on the absolute pressure of the gas.

Elegas has a boiling point (at normal pressure) of −64°C. and can be compressed (at normal temperature) up to 20 atm. without being liquefied. These thermodynamic properties of elegas are very favourable because it may be utilized in its gaseous state at considerably high pressures, thus achieving very high electric strengths.

Dichlorodifluoromethane (CCl_2F_2) is one of the family of freons†. It has an electric strength very near to that of elegas. However, the boiling point of freon is only −28°C., and it can be compressed (at normal temperature) only up to 6 atm. Also, it may be pointed out that a freon can act harmfully on some organic electrical insulating materials, a fact that must be borne in mind in the design of electrical refrigerators.

Of great interest are also some perfluorinated hydrocarbons, i.e. hydrocarbons in the molecules

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†Freons have the general chemical formula $C_xCl_yF_z$; the name 'freon' has originated from the use of these gases in refrigerators.

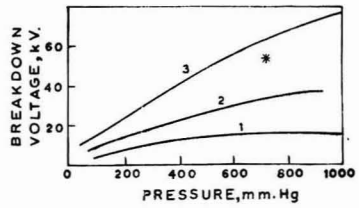

Fig. 2 — Dependence of breakdown voltage (r.m.s. values at 50 c/s.) upon absolute pressure [Electrodes are spheres 12·5 mm. in diameter spaced 5 mm. apart. Curve 1, in air; curve 2, in elegas; curve 3, in vapour of perfluoromethylcyclohexane. For comparison, the approximate breakdown voltage of transformer oil of mean refinement degree at 760 mm. of mercury column for the same electrodes, shape and gap between them, is indicated by the star]

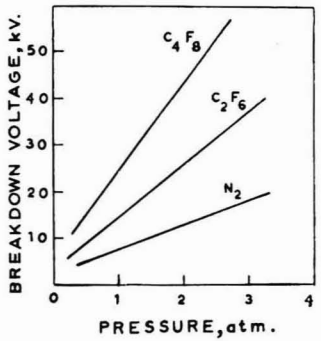

Fig. 3 — Dependence of breakdown voltage upon absolute pressure for N_2, C_2F_6 and C_4F_8 [Electrodes used are (1) steel sphere with a diameter of 19 mm. and (2) brass plate; gap between electrodes is equal to 2·5 mm.]

TABLE 1 — PROPERTIES OF PERFLUORINATED HYDROCARBONS

Property	Gas				
	CF_4	C_2F_6	C_3F_8	C_4F_8	C_4F_{10}
Boiling point, °C.	−128	−78	−37	−6	−2
Melting point, °C.	−184	−100	−183	−41	−80
Critical temperature, °C.	−47	−24	71	115	113
Critical pressure, atm.	40	33	27	28	23

of which all the hydrogen atoms are replaced by fluorine atoms. Several of these substances are gaseous at normal temperature and pressure; also a number of them are liquids.

Recently, it has been reported[9-12] that electric strength of perfluorinated gases and vapours of perfluorinated liquids may be 6-10 times greater than that for air (Fig. 2).

Basic physical properties of some perfluorinated hydrocarbon gases, namely (1) tetrafluoromethane (CF_4), (2) hexafluoroethane (C_2F_6), (3) octafluoropropane (C_3F_8), (4) octafluorocyclobutane (C_4F_8) and (5) decafluorobutane (C_4F_{10}), are given in Table 1.

The gases listed in Table 1 are not toxic. Their specific thermal conductivity is 3-5 times higher than that of nitrogen. Action of an electric arc or corona discharge may cause their chemical decomposition with liberation of toxic compounds.

Electric strength of perfluorinated gases increases with increasing molecular weight as will be seen from Fig. 3. It may also be noted that even a little admixture of electronegative gases or vapours to air (or nitrogen, etc.) may increase the electric strength of air substantially. This fact may be utilized in some cases to reduce the cost of electrical devices. A closed device filled by air with such an admixture could be much smaller in size and should be much cheaper than similar devices without this admixture. It also permits the designing of what may be termed ' boiling insulation '[13]. That is, small-sized electrical and radio devices are filled partly with a suitable fluoro-organic electrical insulating liquid having large latent heat (e.g. cyclic $C_8F_{16}O$). To improve heat transfer, the liquid is evaporated, then condensed in a cooler and returned to the device as a liquid. At the same time, the presence of the fluoro-organic liquid vapour under elevated pressure in the space over the liquid level improves considerably the electric strength of the gaseous (air or nitrogen) medium in the hermetically sealed device.

Summary

Data on the electric strength and other important characteristics of some gases and vapours, in particular those containing fluorine (sulphur hexafluoride, freons and perfluorinated gases) having electric strength comparatively higher than that of air, are given. The possibilities of using these gases in electrical engineering are indicated. In some cases, these gases at elevated pressure can provide electric strength even greater than that of transformer oil or other liquid dielectrics.

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Certain Characteristics of Thermal Diffusion Factor of Binary Gas Mixtures

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RECENTLY, Saxena and Joshi[1] have reviewed the various formulae for the binary thermal diffusion factor α_T, and demonstrated their relative accuracies by performing numerical calculations for the two representative systems He-Xe and Ar-Xe. Saxena and Mathur[2] utilized this information and studied the dependence of α_T on the temperature and composition for a large number of binary gas systems. They considered the familiar Lennard-Jones (12-6), and the modified Buckingham exp-6 potentials. A similar investigation has also been carried out by these authors for the isotopic mixtures[3]. Both theoretical and experimental studies on α_T are relatively laborious and tricky, and the characteristics of α_T which manifest themselves only under somewhat unusual experimental conditions have been hardly understood or even known till recently. During the past few years, many laboratories engaged in studying the phenomenon of thermal diffusion in detail have provided evidences on such features like the pressure dependence of α_T, its sign inversion under varying experimental conditions and the existence of a positive minimum or a slow decrease with increasing temperature, etc. Hence, it is considered of interest to attempt a review of all evidences available on such aspects of α_T with a view to presenting a consolidated and clear picture. In this review an effort is also made to examine the success of Chapman and Enskog theory, as given in the excellent monograph of Chapman and Cowling[4], to explain the observed behaviours of α_T. In conclusion, this critical review outlines the nature of experiments to be planned and the directions in which theory must be developed to make our understanding of this property (α_T) of thermal diffusion more complete.

Pressure Dependence of α_T

The existing theory[1,4] predicts that the thermal diffusion ratio (k_T) is independent of pressure. This is because k_T is the ratio of two types of diffusion coefficients, viz. thermal diffusion (D_T) and mutual diffusion (D_{12}), both being identically dependent upon the pressure. However, this conclusion strictly applies to dilute gases consisting of elastic spherical molecules. Thus, this prediction can be expected to hold only for noble gases in the pressure range much above the Knudsen gas, but at the same time below the pressure value where ternary and higher order encounters begin playing any important role. What one should expect if polyatomic gases are involved or if the pressure is high enough for ternary collisions to become important is not known with any reliance. On both these points, the status of the rigorous kinetic theory, in its present stage of development, does not permit even a guess. The formal kinetic theory of polyatomic gas mixtures given by Monchick *et al.*[5] suggests α_T to be seriously affected by the presence of internal degrees of freedom. The kinetic theory of even a moderately dense gas is still in the stage of development[6-8]. Robb and Drickamer[9] have also given an expression for k_T of dense gases based on the earlier developed irreversible thermodynamics.

Ibbs *et al.*[10] were probably the first to report an experimental investigation on the pressure dependence of k_T. They found k_T to be independent of pressure in the range 0·3-1·0 atm. for H_2-CO_2 and H_2-N_2 systems in the temperature range 288-373°K. Schmahl and Schewe[11] reported measurements on H_2-CO_2 and H_2-H_2S systems in the pressure range 0·15-3·5 atm., and temperature range 20-350°C. They found k_T to fall by more than a factor of 2 though the variation in the range 0·2-1·0 atm. is small being 6 per cent approximately. Thus, there is an approximate agreement between the results of Ibbs *et al.*[10] and Schmahl and Schewe[11]. Some doubts have been raised about the accuracy of the data of these latter workers[11]. Grew[12] earlier pointed out that this pressure dependence might be due to the fact that steady state was not reached in the experiments. Computations according to the theory of Saxena and Mason[13] do not support this view as the calculations indicate a value of only 15 min. for the relaxation time while in the experiments 10 hr were provided for reaching equilibrium. Grew and Ibbs[14] later attributed convection to be responsible for the observed dependence of α_T on pressure. This also does not seem very likely from the geometry of the apparatus used by these workers and more so on comparing it with the set-up employed by Saxena and Mason[15]. However, the experimental results of Grew[12] are in serious contradiction with that of Schmahl and Schewe[11]. Grew[12] found α_T to be independent of pressure for H_2-CO_2 system in the range 0·5-3·6 atm. and for the hot-bulb temperatures at 220, 330 and 350°C., the cold-bulb being at 20°C. The composition of the mixture was also not much different, being 32 per cent H_2 in the experiments by Schmahl and Schewe[11] and 44 per cent H_2 in the experiments by Grew[12]. The present authors feel that the discrepancy is quite serious and should be resolved by careful experiment rather than by any guess.

In this context it is interesting to quote the results of Becker and Schulzeff[16]. They tried equimolar mixtures of several binary systems in the pressure range 1-80 atm. and with the two bulbs at 20° and 160°C. In most of the cases α_T was found to increase with pressure[17]. The increase was by a factor of 3 for H_2-CO_2, 8 for N_2-CO_2 and Ar-CO_2 systems, and 5 for CH_4-CO_2 system; a relatively small pressure dependence was found for H_2-N_2 system while it actually decreased with pressure for N_2-CH_4 system. Becker[18] has also advanced a theoretical explanation

for this pressure dependence, basing the argument mostly on the imperfect nature of the gases. Here again we find for the H_2-CO_2 system an entirely different pressure dependence of α_T which is in complete disagreement with the two previous views[11,12] mentioned above.

Reference must also be made to the work of Kotousov[19] who investigated, in addition to H_2-CO_2 system, N_2-CO_2 and He-Ar systems. He has utilized the conventional two-bulb apparatus with the principal difference of a packed connecting tube. His measurements refer to equimolar mixtures and to an average temperature of about 315°K. He covered the interesting and yet unexplored pressure region 0·01-0·8 atm. He found α_T to increase with pressure, the magnitude of change is large in the range 0·01-0·1 atm., while it is only 10-20 per cent of that in the above pressure range in the range 0·2-0·8 atm. Waldmann[20] attributed this strange dependence of α_T on pressure to an additional mechanism of thermal effusion (thermal transpiration) going on side by side in addition to the normal thermal diffusion under such experimental conditions, though he also points out that part of the effect may be due to the errors in sampling. L. S. Kotousov also agrees with the latter view and is repeating measurements in the pressure region $< 0{\cdot}05$ atm. (private communication). Recently, Mason and Malinauskas[21] have studied the phenomenon of the flow of gases through porous media in the presence of temperature gradients on the basis of the so-called 'dusty gas' model. They have succeeded in quantitatively explaining the results of Kotousov[19] reasonably well, thereby establishing that a mechanism somewhat different from what is responsible in the conventional two-bulb apparatus is important in these measurements. Another similar investigation of thermal diffusion through porous media has been reported by Goshchitsku and Izrailevich[22] and Beckey and Groth[23] who considered equimolar mixtures of H_2-Kr, He-Kr and H_2-Ar.

The investigation of Drickamer and Hofto[24] is of particular importance in this regard for these workers investigated the Ne-Ar system for which Chapman-Enskog theory can be regarded as satisfactory. Working in the pressure range 0·5-2·0 atm. and varying the temperatures of the two bulbs between 197° and 474°K., Drickamer and Hofto found α_T to be independent of pressure. W. E. J. Neal has also obtained similar results for H_2-He and H_2-D_2 systems in the pressure range 0·3-0·9 atm. in a two-bulb apparatus (private communication). The accuracy of the analysis of the samples was about 5 per cent though the precision was about 1-2 per cent. Van Ee *et al.*[25] and Van Itterbeek and de Rop[26] have studied the H_2-N_2 and H_2-He systems in the pressure range 6-20 atm. in a two-bulb apparatus. They found α_T to increase with pressure, the change being more pronounced for H_2-N_2 system than for H_2-He system.

Lastly, mention may be made of a single investigation in this direction using a swing separator or trennschaukel of Fischer[27]. He worked with equimolar mixtures of H_2-CO_2 system in the pressure range 20-70 cm. Hg with the two end temperatures at 285° and 348°K. He found α_T to be independent of pressure. A much more careful planning is essential with this instrument for verifying such an effect than the conventional two-bulb apparatus. However, as more and more theoretical[28-31] and experimental[32,33] studies are being done on trennschaukel, it is hoped that in future it may be possible to employ this device with certain advantages for such investigations where the separation under a temperature gradient may be small and the cascaded separation of trennschaukel may prove to be of great help.

Combinations of rare gases are the only systems which one should investigate in verifying Chapman-Enskog theory. Unfortunately, only one measurement of this type has so far been reported[24] on Ne-Ar system and this confirms the prediction of theory, viz. α_T is independent of pressure as long as only binary collisions are important. The results of Kotousov[19] on He-Ar refer to a different physical situation, but even then these results confirm the theory, an approximate version of which has been given only very recently[21]. Measurements on monatomic gas pairs should be examined in more detail to elucidate the implications of the theory.

Results on isotopic mixtures are also not available except one involving hydrogen isotopes (Neal, W. E. J., private communication). The results on hydrogen isotopic mixtures, however, are in accordance with the expectations of theory and investigations on more and more isotopic mixtures of rare gases are worth undertaking, as the theoretical interpretation in the case of such mixtures is relatively easier and thus may prove of some significant help in developing the theory.

As already mentioned, the mixtures involving polyatomic molecules are likely to show a different behaviour than that expected from the theory strictly applicable to monatomic molecules only. Here again, unfortunately, a large number of systems tried so far involve H_2 as one of the components and it has been most difficult[34] to account for the rotational and vibrational nature of molecules of these systems. The experimental evidences are also inconclusive. Thus, for example, H_2-CO_2 system has been investigated by six different groups[10-12,16,19,27] and each worker differs in his conclusion from the others. Out of these, four measurements[10,12,19,27] can be regarded as consistent as they predict α_T to be independent of pressure. There are two reasons to take the view that the evidences available at the moment are more in favour of this conclusion. Firstly, the measurements of Grew[12] were performed by changing the temperatures of the hot bulb and thus these measurements may be regarded as more correct and free from any systematic error unlike in the case of the lone set of data by Schmahl and Schewe[11] which may be in error. Secondly, Weissman *et al.*[35] have shown that for this system Chapman-Enskog theory can be applied with reasonable success. Consequently, it is desirable to repeat the measurements for this system to resolve the discrepancy shown by the existing experimental evidences. The measurements of Becker and Schulzeff[16] and of Becker[17,18], which indicate α_T to increase with pressure, were carried out over a fairly large pressure range, where certainly collisions other than binary ones play an important role and the simple Chapman-Enskog theory cannot be

regarded to hold good. In the low pressure range, 1-3 atm., the data[16,18] of Becker and Schulzeff and of Becker also predict α_T to be approximately constant, within the precision of the data reported. This again confirms the view upheld above and indicates that the data of Schmahl and Schewe[11] may be in error.

The only other system studied involving a monatomic gas is H_2-He and for this the available evidences[25] (also Neal, W. E. J., private communication) suggest α_T to be constant up to 1 atm. and above this value α_T increases slowly with pressure. The H_2-N_2 system also behaves in the same way, except that the rate of change of α_T with pressure is more pronounced in this case than for the H_2-He system. The N_2-CO_2 system exhibits the same trend in the α_T variation with pressure. The values[16,18] remain constant up to about 3 atm. and thereafter rise with pressure. Kotousov's[19] limiting value at high pressure also agrees well with the other data[16,18], thus confirming the theory of Mason and Malinauskas[21]. The results on these three systems suggest a common trend, viz. initially α_T is independent of pressure and increases in value as the pressure increases, the rate being characteristic of the system. It is desirable that more elaborate and reliable data of this type on properly chosen systems be compiled by the experimentalists and a theory of moderately dense gas be developed to explain this behaviour. As the pressure is considerably increased most of the systems exhibit a very pronounced increase in α_T values with pressure[17,18]. However, a decrease in the α_T value with pressure is also found; sometimes it may even become zero. Reliable measurements should be attempted in this pressure range for the development of, and also to serve as checks for, a dense gas theory of non-spherical molecules. This, however, presents a combination of two difficult situations.

Temperature and Sign of the Thermal Diffusion Factor

The phenomenon of thermal diffusion means the partial separation of the components of a gas mixture under a temperature gradient. In most of the cases, the component of the binary mixture which is heavier in mass and larger in size (or more precisely it has a larger extension of its force field) is preferentially enriched at the cold bulb while the lighter and smaller molecules are enriched at the hot bulb. The net result is that there is an increase in the proportion of the heavier component down the temperature gradient. Under these conditions α_T is conventionally assigned a positive sign. In accordance with the theory, as the temperature increases, in most of the cases α_T is found to increase[2,3]. However, several interesting exceptions to this expectation have been observed and α_T is found to behave in many more different ways. For instance, for certain systems α_T is found to be negative at all temperatures, in others a positive minimum has been reported at high temperatures, while in some a sign reversal is observed at low temperatures or with varying proportions of the components at the same temperature.

Classically, for spherically symmetric molecules which have equal masses and identical force field extensions, α_T should be zero. This prediction cannot be tested directly, but many interesting cases have been examined. Thus, isotopic molecules of rare gases have identical sizes and the observed thermal separation is entirely due to the difference in masses. The theory has been found to be reasonably satisfactory[3] in explaining the observed effects. In the case of diatomic and polyatomic molecules the success has been only moderate[3] and is likely to be improved only when a theory for polyatomic molecules is developed. Hydrogen isotopes have provided many checks for the theory in view of the availability of a large number of its isotopes both stable and radioactive. Systems having equal masses are also very important for thermal separation in them is entirely due to the difference in their sizes. Many such systems are possible, viz. N_2O-CO_2, N_2-CO, He-D_2, He-HT, D_2-HT, etc. Such studies are also discussed here.

It should be possible to explain the observed effects mentioned above and many more to be described later which follow from the theory of thermal diffusion. Considerable effort in this direction was made as early as in 1940 by Chapman[36]. His investigations and the predictions, a few of which have since been experimentally examined, regarding the characteristics of α_T were based on the first approximation expression[4] and for a simple inverse power type molecular interaction. As this kind of force field is now known to be unrealistic and it became possible to consider more realistic and appropriate molecular potentials, Srivastava and Saxena[37] re-examined certain aspects of the thermal diffusion factor. They based their discussion on the familiar Lennard-Jones (12-6) potential and confined themselves to the binary combinations of rare gases. During the last ten years, studies[38-40] on higher approximations to α_T have clearly indicated that the first approximation expression, $[\alpha_T]_1$, will not be adequate for any reliable and accurate work. Therefore, the earlier works become premature and somewhat uncertain in certain of their conclusions. Consequently, we re-examine the situation particularly with a view to seeking the theoretical justification for the various observed effects in the second approximation expression. Detailed calculations by Saxena and Mathur[2] on a large number of binary systems have shown that the fairly reliable and dependable expression, to start with, is

$$[\alpha_T]_2 = \left[(6C^*-5) \frac{S_1X_1-S_2X_2}{Q_1X_1^2+Q_2X_2^2+Q_{12}X_1X_2} \right] \times(1+K_1)+K_2 \quad \ldots(1)$$

where the notation is the same as was used by Mason[40]. It may be seen that in Eq. (1) the quantity within square brackets is $[\alpha_T]_1$. The remainder after the term $(6C^*-5)$ will be referred to as (S, Q) factor. Though the contribution of K_1 and K_2 is small in many cases still it is large enough to be considered. The factors K_1 and K_2 depend also upon the relative proportion of the components. Thus, the two familiar arguments based on the first approximation expression for the thermal diffusion factor, α_T, will change sign when either $(6C^*-5) = 0$ or $S_1X_1-S_2X_2 = 0$ is invalidated because of the occurrence of a second factor K_2. The first reversal

occurs only with temperature and the second, for appropriate binary systems, under suitable conditions of X_1 and X_2, as explained by Chapman[36] (also Grew and Ibbs[14]). This is briefly discussed below.

Generally, when the mass difference of the two components is large S_1 is positive, S_2 is negative, $(6C^*-5)$ is also positive; though K_1 and K_2 are sometimes negative and sometimes positive, still the relative magnitudes of these quantities are such that α_T assumes the conventionally defined positive sign and the heavier molecule diffuses down the temperature gradient. Most of the binary mixtures fall in this category. The heavier molecule is invariably larger in ' size ' and this fact also enhances the thermal separation such that α_T is positive.

If, however, the mass difference of the two components is small and the heavier component is larger in size, the relative magnitudes of the various quantities in Eq. (1) are such that α_T is once again positive. On the other hand, if the heavier molecule is smaller in size, S_1 and S_2 change their signs such that now S_1 is negative while S_2 is positive so that the first term on the right-hand side of Eq. (1) becomes negative at all compositions. Further, if K_2 is either negative or has a small positive value, α_T will have a net negative value for all positive values of $(6C^*-5)$. Under these conditions the heavier component will diffuse up the temperature gradient. Cases of this type have been confirmed by experiments also. Leaf and Wall[41] found this situation in the case of carbon dioxide-cyclopropane mixtures using a thermal diffusion column. A similar conclusion has been derived by Clusius and Huber[42] for ^{20}Ne-ND_3 system, and is at least qualitatively confirmed by the theory of Mason and Monchick[43]. Using trennschaukel α_T is found to be negative for CD_4 and CHD_3 mixtures with ^{20}Ne throughout the composition range[27]. These results have also been explained satisfactorily[27] by theory.

A fourth possibility also exists[14,36]. This visualizes the heavier component again to be smaller but now S_1 and S_2 instead of having opposite signs, as in the third case, have the same signs, i.e. either both are negative or both are positive. Under such conditions depending upon the relative magnitudes of S_1 and S_2, and of K_2 a reversal in the sign of α_T may occur for a particular value of X_1. Such a possibility was first pointed out by Grew[44], though in an inconclusive manner[45], on normal neon-ammonia system. Later Clusius and Huber[42] have performed an unambiguous experiment using ^{20}Ne and ^{22}Ne in combination with ammonia on a thermal diffusion column. They confirmed the prediction inasmuch as they found the reversal of the sign in α_T for ^{20}Ne-NH_3 system at $X_1 = 0{\cdot}75$, and for ^{22}Ne-NH_3 system at $X_1 = 0{\cdot}4$. These results have been confirmed qualitatively by the theory of Mason and Monchick[43]. The quantitative agreement is not good both as regards the actual values of α_T as well as the value of X_1 at which reversal occurs. Clusius and Flubacher[46] have found a similar reversal for the two systems of ^{40}Ar in combination with $D^{35}Cl$ and $D^{37}Cl$ also. Mason and Monchick's theory has failed to confirm this reversal even qualitatively for these systems. This is an important indication for the development of theory involving polar gases.

The above four cases form interesting exceptions to the normal behaviour regarding the composition dependence of α_T encountered in thermal diffusion experiments, viz. an increase in the value of α_T with increasing percentage of the lighter component[15]. The theory of course agrees with this most general observation[2]. Interesting exceptional cases are also encountered in non-polar gases. One such example is that of a mixture of helium with hydrogen[47].

Let us recall a few other peculiar experimental results which are in serious contradiction with the expectations of theory. Clusius and Flubacher[46] studied the four isotopic systems ^{40}Ar-$D^{37}Cl$, ^{40}Ar-$H^{37}Cl$, ^{40}Ar-$D^{35}Cl$ and Ar-$H^{35}Cl$. The oddest feature found in their results was that the expectation of α_T to increase steadily as the mass of hydrogen chloride decreases, from 39 to 36, was not fulfilled. In actual practice this trend was not confirmed, the two middle masses (37 and 38) forming an exception. The order of increasing α_T is 39, 37, 38, 36. Normally speaking α_T should have zero value for the D_2-HT system. Experimental evidences[48,49] are contrary to this expectation. He-HT and He-D_2 are two other examples of systems of this type[49,50]. Another interesting effort of this type lies in the work of Becker and coworkers[51,52]. They tried combinations of $^{16}O^{12}C^{16}O$ with $^{16}O^{13}C^{16}O$ and $^{17}O^{12}C^{16}O$. The latter two varieties of carbon dioxide have the same mass but differ only in the isotopic substitution and consequently in the distribution of mass which alter the characteristic frequencies and moments of inertia. The symmetry numbers of the two molecules are also different. The two systems were found to behave differently in a thermal diffusion column. De Vries *et al.*[53] carried out a similar investigation using carbon monoxide. They found $^{14}C^{16}O$ to be enriched by a factor of 1·15 more than $^{12}C^{18}O$ against $^{12}C^{16}O$ in both the cases. The influence of asymmetry effects has also been investigated by Slicker[49] on systems HT-^4He, DT-^4He, HT-H_2, DT-D_2 and D_2-HT.

All these studies have led to a definite conclusion that Chapman-Enskog kinetic theory of elastic spherical molecules cannot explain the phenomenon and behaviour of thermal diffusion in polyatomic molecules. The assumption of a central or quasi-central intermolecular force model is certainly not adequate and the solution lies only in the development of a formal kinetic theory of non-spherical molecules. The presence of internal degrees of freedom of vibration and rotation is known to play an important role in the process of energy transfer and as thermal diffusion also involves such a process we should expect an identical situation in this case also. Indeed, the formal theory of polyatomic molecules[5] does show this promise. Mention may be made of the approach of Schirdewahn *et al.*[48] who gave an expression for α_T on the basis of dimensional analysis and involving moments of inertia of the molecules. This approach has been further discussed by Slicker[49]. It is still quite premature to assess these empirical approaches except with the impression of a very limited success.

The temperature dependence of α_T is essentially controlled by the factor $(6C^*-5)$ in Eq. (1), the (S, Q) factor is essentially independent of temperature[54].

The factors K_1 and K_2 besides being small (though not negligible) have a very feeble temperature variation. The value of $(6C^*-5)$ depends rather sensitively on the choice of the force model. Consequently, the temperature variation of α_T, as given by Eq. (1) on the basis of a realistic intermolecular potential, and also confirmed by experiments, is of three types: (1) α_T increases with temperature, and (2) will tend to a constant value at high reduced temperatures or (3) may even slightly decrease. Almost all common systems, where masses are widely different and sizes are monotonic functions increasing with mass, are examples of type 1. Examples of type 2 are found in binary mixtures of He with other rare gases, etc., while one familiar example of type 3 is He gas. However, there are a few departures from this general trend and these are discussed below.

It is interesting to observe that in general it is not possible to merge K_2 in the K_1 term of Eq. (1). Further, the magnitude of K_2 also varies in an irregular manner both in sign and magnitude from system to system, though for a particular system it usually increases with temperature. Thus, both (S, Q) factor and K_1 do not change much with temperature but $(6C^*-5)$ changes much more relatively. Also at sufficiently low temperatures numerical calculations for actual systems reveal that K_2 is usually negative and at such temperatures due to the small value of $(6C^*-5)$ it is possible that α_T becomes zero at a particular temperature; at still lower temperatures it will be negative instead of being positive. It may by pointed out that at low temperatures the factor $(6C^*-5)$ also becomes negative and, therefore, it is not essential for K_2 to be necessarily negative for an inversion in the sign of α_T. In fact, the binary mixture of H_2-D_2 is one such example where the K_2 term remains positive even when the first term is negative[55]. Contrary to the usual expectation, all that the accurate expression for α_T, viz. Eq. (1), indicates is that the reversal of the sign of α_T does not occur at the temperature where $(6C^*-5)$ is equal to zero, but that it occurs where the first term of Eq. (1) becomes equal to K_2 in magnitude but opposite in sign. This reversal in the sign of α_T which occurs in a system at sufficiently low temperatures is different from what is mentioned earlier with the changing proportion of a component at a particular temperature. Let us now study the systems where experimentally such a reversal has been observed in the sign of α_T at low temperatures.

Systems Showing a Reversal in the Sign of α_T

Watson and Woernley[56] were the first to observe such a reversal of α_T in the polar gas mixtures of $^{14}NH_3$-$^{15}NH_3$ in a two-bulb apparatus. The effect was found to occur near about 293°K. In isotopic molecules such a reversal was later observed by de Troyer *et al.*[57] for H_2-D_2 mixtures using again a two-bulb apparatus at 30°K. This has since been confirmed also by Grew *et al.*[58]. Ghozlan[59] has also observed such a reversal for H_2-HT and D_2-DT systems, though because of the experimental conditions it is not conclusive and it is advisable to repeat these measurements for confirmation. In no other isotopic molecule such a reversal has been reported. Work at very low temperatures in isotopic molecules stated above which may elucidate the position further has to be planned.

Grew *et al.* observed such a sign reversal in the value of α_T for Ar-O_2, Ar-N_2 and N_2-CO_2 mixtures while working on a two-bulb type of apparatus. Ghozlan[59] reported such an effect in He-^{85}Kr system at 80°K. and in mixtures of DT, HT and T_2 with ^{4}He. Grew and Mundy[60] report a reversal in the sign of α_T for Kr-Xe and Kr-N_2 systems. Recently, Cozens and Grew[61] have also confirmed the existence of such a behaviour in the systems CO_2-^{85}Kr, $^{14}CO_2$-Xe and CO_2-^{133}Xe.

Waldmann[62] has also demonstrated the change in sign of α_T from his studies on the phenomenon of diffusion thermo-effect. He found that the systems O_2-Ar and N_2-Ar exhibit this effect as do the systems N_2-CO_2, N_2-C_2H_4, O_2-C_2H_4 and Ar-C_2H_4.

The existing theory in conjunction with all the three molecular potentials, viz. (1) L-J (12-6), (2) modified exp-6 and (3) Morse, predicts such a reversal. Even for polar gases the extension of (12-6) potential by Monchick and Mason[63] has confirmed the existence of such an effect. Though in a few cases good quantitative agreement is also obtained we feel that neither can it be generalized nor can even be expected to be so. The reason is twofold. Firstly, in most of the cases the effect is observed at such temperatures where quantum effects are likely to be important, and secondly, most of the cases studied so far involve non-spherical polyatomic molecules. The only interesting case in which both these limitations are overcome is the Kr-Xe system. Indeed here a good and even a quantitative agreement is achieved as can be seen from the fact that the inversion temperature which has been found to be 155°K. (ref. 60) is in good agreement with the earlier predicted value of 149°K. by Srivastava and Saxena[37] on the basis of L-J (12-6) potential.

An interesting experimental investigation has been reported by Van der Valk[64,65] on the measurement of α_T for He isotopes in the temperature range 12·7-700°K. No sign reversal was observed; on the contrary, a strange temperature dependence specially at low temperatures came into light. In the low temperature range, initially α_T was found to decrease rapidly. Detailed calculations[66] based on the classical theory cannot explain this strange dependence and it is hoped that quantum mechanical calculations may provide some clue.

Theory predicts a slight decrease in α_T value as the temperature increases in isotopic mixtures[67]. This behaviour is observed in many gas mixtures also, the magnitude of decrease in α_T being much more pronounced than can be explained on the existing theory of thermal diffusion. Slicker[49] has presented the results of such a study involving DT-^{4}He, T_2-^{4}He and HT-^{4}He systems. These effects, if real, are yet to be explained by a suitable theory to be developed.

A similar qualitative effect was found by Waldmann[62] from studies on the diffusion thermo-effect. He found that for Ar-CO_2 system α_T continuously decreases as the temperature increases from 194° to 372°K. Weissman *et al.*[68] found a similar behaviour for Ne-CO_2 system in a two-bulb apparatus. Saxena

and Mason[15] have earlier reported measurements for He-CO_2 system and did not observe such strange temperature dependence and the results could also be explained by theory[35]. Weissman *et al.*[68] could, however, correlate these results of the three systems by a treatment based on the theorem of corresponding states.

Grew and Mundy[60] while working with Ar-Kr system found a rather unique dependence of α_T on temperature. They found α_T to steadily decrease with temperature in the range 940-149°K. and thereafter to start increasing as the temperature is further lowered up to 80·8°K. Thus, a definite positive minimum is detected. Measurements on Ar-CO_2 system reported by Cozens and Grew[61] also indicate a similar dependence. It is interesting to note that earlier measurements of Waldmann[62] are in good agreement with these data[61] and with two different types of mixtures. In the first 50 per cent CO_2 was used while in the second only 2 per cent $^{14}CO_2$. Both the mixtures were tried by Cozens and Grew[61].

This type of α_T dependence on temperature, namely exhibiting a positive minimum, is predicted qualitatively by the exp-6 potential, though difficulties are encountered when a quantitative agreement[61] is sought. Thus, here again we find the theory to be inadequate in accounting for this unique effect observed in a few systems.

On the basis of the various characteristics of α_T variation discussed above, it is evident that, at best, the existing theory is able to explain the broad features on a qualitative basis only. For a good quantitative agreement of the various aspects of α_T variation one will have to await the development of a non-spherical molecular kinetic theory. The authors hope that the knowledge of the broad qualitative features of α_T variation discussed here will help in choosing the proper molecular model and the aspects of non-spherical molecular dynamics which should be emphasized in a formal study of such systems.

Summary

Some less known properties and characteristics of the thermal diffusion factor (α_T) of binary gas mixtures, e.g. the pressure dependence of α_T, reversal in the sign of α_T under different conditions and the abnormal variations observed in the temperature dependence of α_T, are critically discussed making use of the literature data on the dependence of α_T on temperature and composition of the components of many binary gas mixtures which have been thoroughly investigated both experimentally and theoretically. All the available experimental evidences are cited in the case of each effect and an attempt is made to examine the plausibility of the existence of the different effects, and to provide an explanation wherever possible. The major deficiencies of the existing theory are brought out and the various types of experiments which should be planned for throwing further light on the subject have been indicated. It is pointed out that only a formal theory of non-spherical molecules will be able to assimilate and explain the various observed α_T characteristics.

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Evaluation of Drug Toxicity*

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AS an outcome of crash programmes of developmental research financed liberally by pharmaceutical houses and by their competitive investment in 'admass' psychology beamed towards the gullible consumer, the past few years have witnessed an unprecedented spiralling upward trend in the manufacture, marketing and consumption of a bewildering variety of chemotherapeutic agents. This 'explosion in drugs' compelled the editor of the learned American journal *Clinical Pharmacology and Therapeutics* to warn the public against the imperceptible, none the same imminent, danger ensuing from the unbridled growth in the rate of drug intake [Modell, W., *Clin. Pharmacol. Therap.*, **2** (1961), 1]. Free access to countless stimulants, depressants, tranquillizers and the like has led to an alarming increase in self-medication, admittedly to survive the stress of modern living conditions. Consequently, several new forms of untoward reactions to drugs have been discovered in recent years and it is almost a mathematical inevitability that incidence of drug toxicity may increase faster than even the output of drugs. The problem of drug toxicity has assumed a new dimension of danger with the unravelling of the tragic story of thalidomide. Since 1962 when the teratogenic effects of thalidomide were first reported in the human embryos, over 2600 studies of the action of diverse drugs inducing malformative changes in mammalian embryos have appeared to date in medical literature [Cahen, R. L., *Clin. Pharmacol. Therap.*, **5** (1964), 480]. Other toxic symptoms of pathological importance are drug induced blood disorders such as dyscarias, aplastic anaemias, primaquine induced haemolysis prevalent particularly in some members of the Negroid species, penicillin allergy, hepato toxicity caused by salicylates, peripheral neurosis due to INH intoxication, and innumerable defective detoxication mechanisms.

Drug Toxicity: Clinical and Experimental Problems

Several high level conferences have been held in recent years to sort out the clinical and experimental problems arising out of drug toxicity. The Second International Congress of Pharmacology held at Praha in September 1963 devoted an entire session jointly with WHO to discuss mechanism of drug toxicity. The series of papers included in the

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volume under review were presented at a conference on drug toxicity held in New York in March 1964 sponsored by the Division of General Medical Sciences, National Institute of Health, Washington DC. Scientists drawn from the disciplines of pharmacology, biochemistry, toxicology, immunology, clinical investigation and embryology representing leading pharmaceutical and government research institutes participated in the conference. The first session was devoted to basic concepts concerning selective toxicity; the second session highlighted the importance of metabolic studies in the evaluation of drug toxicity. Genetic aspects of drug toxicity were discussed in the third session. The fourth session was devoted to a discussion of possibilities of evolving newer experimental methods of assessing drug toxicity and the last session surveyed clinical aspects of toxicological evaluation. The papers brought to light a mass of new data relevant to toxicity and the discussion was, as with other symposia of the New York Academy of Sciences, frank and useful. Anyone interested in reviewing the current problems of drug toxicity can greatly profit by critically studying the material presented in this volume.

Comparative Biochemistry of Tissues and Elaboration of Drugs with Selective Toxicity

The field of comparative biochemistry of different tissues of the same species and of the same tissue of different species with respect to drug accumulating mechanisms and subtle differences in cytological structure and enzyme activity can be profitably exploited for elaborating agents possessing selective toxicity. A rational approach like this as pointed out by Adrian Albert could presumably lead to chemotherapeutic agents specific for the infectious agent and non-toxic to the host. The selective action of penicillin on bacterial cell wall formation has made available a number of related reagents useful in the study of the enzymology of mucopolysaccharide biosynthesis. It should not be difficult from this experience to visualize possible means of synthesizing drugs active in collagenous diseases. The sequential inhibition of a chain of events in a biosynthetic pathway is an effective functional modus of a toxic agent. Thus the need for a better insight into the mechanism of toxication at the molecular level is all the more urgent as rightly stressed by Welch in his report on the action of nucleotide analogues. The adverse effect of drugs on the homeostatic equilibrium maintained by hormones as a prelude to their toxicity is illustrated by Gillette in his studies on the effect of drugs on the mobilization of steroids. In a discussion on drug allergy, Parker envisages possibilities of drugs or their metabolites combining with proteins in order to stimulate antibody production.

Liver Microsomal Enzymes and Drug Toxicity

One of the striking effects elicited by certain drugs is the induction of liver microsomal enzymes such as hydroxylases, O-methyltransferases and demethylases. Such enzyme stimulation is accompanied by increase in the net mass of smooth membranes of the endoplasmic reticulum. This observation has opened up an elegant enzyme system for studies that can meaningfully supplement conventional histopathological investigations on drug toxicity. Induced enzymes studied by Renner and Merker include the ones metabolizing drugs like phenobarbital and the ones hydroxylating steroids. Conney reports that phenobarbital, phenylbutazone and chlorocyclizine alter the *in vivo* metabolism of steroids, essential cofactors and nutrients. Conjugation mechanisms mediated by liver play a vital role in drug elimination from the system as well as in carcinogenesis. By specific alkylation reactions in liver cell constituents, carcinogenic drugs can initiate a sequence of reactions culminating in active interference with the body's basic regulatory mechanism. The comparative toxicity of organophosphorus compounds appear to be related to differences in the specific activities of degrading and activating enzymes in the host tissues. The papers of this session thus focused attention on the potential area of biotransformations at tissue and enzyme levels as an important feature of the mechanism of drug toxicity.

In view of the demonstrated ability of a variety of drugs to modify microsomic enzyme levels, this effect could perhaps be profitably used as a parameter in drug toxicity evaluation studies. One can go a step further and say that a general screening of all chemotherapeutic agents through a battery of representative tissue enzymes for obtaining information on inhibition or activation of specific activity may prove to be a rational prelude to conventional short-term and long-term acute and chronic toxicity tests on a large number of animals. If the enzyme data thus obtained can be interpreted critically, this procedure could lead to economization of the experimental animals at present sacrificed for toxicity studies [Krishna Murti, C. R., *Proceedings of symposium on chemotherapy of bacterial and virus infection* (Central Drug Research Institute, Lucknow), 1958, 15].

Genetic Factors in Drug Toxicity

The five papers by Motulsky, Evans, Granick, Marks and Banks and Siddell and Lehmann on genetic factors in drug toxicity emphasize the need for assessing the role of mutant genes, genetically determined polymorphism in drug metabolism, and the deficiency of the erythrocyte enzyme glucose-6-phosphate dehydrogenase as a predisposing factor in drug induced haemolytic anaemia. One cannot overemphasize the importance of faithfully recording side reactions in individual cases in clinical trials to provide objective basis for determining individual biochemical variation in drug toxicity.

Drugs and Mammalian Embryo

The five papers in the session on drugs and the mammalian embryo by Wilson, Leuz, Villee, Fonts and Hart and Kerbelle *et al.* present striking experimental evidence of embryological changes brought about by drugs such as actinomycin D, the epidemiology of congenital malformations, placental transfer of drugs and hepatic drug metabolism in the prenatal period. Remarkable results can be achieved in experimental teratogenic studies by the use of

electron microscopy and isotope labelling as revealed by the work of Keberle, Loustalot, Maller, Faigle and Schmid on thalidomide using pregnant rabbits.

Toxicity Tests in Animals and Clinical Drug Toxicity

In the last two sessions on the applications of new knowledge to toxicity tests in animals and clinical aspects of drug toxicity, several interesting problems have been posed with reference to the predictability of conventional animal toxicity tests, drug metabolism and excretion studies. Three assumptions as pointed out by Lasagna appear to be axiomatic in planning clinical evaluation of drugs: " (a) No clinically useful drug is devoid of toxicity; (b) no human being should be exposed to needless risk; and (c) the harm caused to present and future generations by lack of adequate remedies in many areas of medicine renders it imperative that new drugs, with their attendant hazards, continue to be introduced into research and practice". Gilman in his opening remarks of the session asks the extremely relevant question: 'Is it possible to produce a useful drug the administration of which entails no risk'? and expresses his own doubt about the possibility of such an event in our present state of biochemical knowledge of tissue reactions.

Toxic Manifestations of Common Drugs

The last session also includes a very valuable inventory of blood disorders, hepatic disturbances and other toxic manifestations produced by drugs used commonly in medical practice. Chloropromazine, amidopyrine, sulphonamides, thiouracil, pyribenzamine, tolbutamide, diamox, chloramphenicol, mesantoin, quinacrine, primaquine, nitrofurantoin and aminopyridine are all suspects from this point of view. The need for further documentation of toxicity data scattered widely in medical literature stands out prominently in this connection. The Federal Food & Drug Agency of USA now requires a declaration on the label of drugs indicating their potential side effects. Since toxic effects and teratogeny are not confined to man-made drugs, there is need for a careful assessment of the toxicity of vegetable drugs, particularly in their crude form. Such studies would indeed be of special significance to conditions prevalent in this country.

Conclusion

Perusal of the present volume has been a very profitable and stimulating experience for the reviewer. The data presented and the discussions of results should convince all, if conviction was needed, of the imperative necessity to understand the mechanism of drug action in unequivocal physico-chemical terms. This understanding can come only by the coordinated efforts of research workers drawn from a multiplicity of disciplines. In the evolution of a drug, the chemist who produced it and the pharmacologist who tested it on animals were considered all along adequate to constitute an effective research team. Studies on the effects of drugs on enzymes and other cellular constituents have hitherto been treated as of only academic interest and of no practical consequence. Teratogenic effects, blood disorders and hepatotoxic symptoms observed in clinical practice by the indiscriminate use of drugs have invalidated this view. They have also brought to light the vital role that the auxiliary biological scientists can play in a concerted programme of drug research.

Symposium on Neutron Monitoring for Radiological Protection

A Symposium on Neutron Monitoring for Radiological Protection will be held in Vienna from 29 August to 2 September 1966 under the auspices of the International Atomic Energy Agency. The symposium will deal exclusively with the instruments and methods used for neutron monitoring in the field of radiological protection. Both personnel monitoring and area surveillance will be discussed; only the most recent developments in the techniques of personnel dosimetry for radiation accidents will be considered.

Requests to present papers or participate in the symposium should be submitted through the appropriate national authorities responsible for atomic energy matters, from whom detailed information and application forms may be obtained. The Scientific Secretary of the Symposium is Shri S. Somasundaram, Division of Health, Safety and Waste Disposal, of the International Atomic Energy Agency, Vienna. Abstracts of papers for consideration by the Scientific Secretary must be received on or before 28 March 1966.

Toxins & Plant Diseases*

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THE germ theory of disease was known to the science of plant pathology[1] earlier than to human pathology, yet, the pathogenic role of toxins in infectious diseases was first established in human medicine. The term toxin in plant pathology has actually been borrowed from the conceptual sphere of human medicine.

The idea that harmful effects of microbial infections of man and animals might be caused by their poisons was entertained even before the germ theory of disease was established by Pasteur in 1865. Both the endo- and exotoxins were foreseen by Vallisnieri[2] in 1713. Loeffler who discovered the diphtheria bacillus in 1884 obtained evidence to show that the bacterium produced a toxin in the host tissue[2]. The credit for the discovery of toxin is generally given to Roux and Yersin[3].

Thus, a toxin can be defined as a substance of microbial origin involved in host pathogenesis. Toxins are normally proteins which are antigenic and have antitoxins. In addition, by introducing small doses of a toxin it is possible to develop immunity in the host against the toxin and the organism producing it. Nevertheless, we should remember that all these attributes were little known when Roux first visualized the presence of a toxin in the disease caused by *Corynebacterium diphtheriae* (Flügge) L. *et* N. In the diseased animals he observed symptoms in regions far away from the seat of infection. This, he thought, might not be due directly to the microbe itself but rather to its metabolic products that have been translocated.

Is there a toxin known to plant pathology? The answer has to be given with a certain amount of reservation. However, if we were to approach the problem in the same spirit as Roux, then we do have toxins in infectious diseases of plants. In plants symptoms are known to be produced in regions far away from the seat of infection and the most outstanding examples are the vascular wilts caused by *Fusarium* spp. and *Verticillium* spp. Here the seat of infection is the root and the pathogen invades at best the stem, while the pathogenic manifestations are seen in the leaves, hence the term 'wilt toxins' and the concept of toxins in plant diseases. In the leaf spot diseases there is also strong evidence to suggest that toxic metabolites of the pathogens are involved in producing the characteristic symptoms.

In fact, the situation is not far different in human medicine. Although the concept of toxin was first introduced in infectious diseases of man and animals, after the first flush of success in demonstrating toxins in three or four diseases (diphtheria, tetanus and botulism), their demonstration became increasingly difficult. Direct evidence to suggest that toxic metabolites of microorganisms produced *in vitro* (suspected toxins) are responsible for damage under natural conditions of human infections has not been forthcoming in other infectious diseases. Even if forthcoming, the toxic metabolites do not appear to be the only substances responsible for pathogenesis. It is perhaps better to follow the French legal practice of assuming guilt until innocence is proved and suggest that it is reasonable to assume, in the absence of evidence to the contrary, that all poisonous substances of microbial origin play a pathogenic role, but it is unwise to assume that they always play an exclusive or even an important part.

While the majority of infectious diseases of human beings are of bacterial origin, those of the plants are of fungal origin. Nevertheless, plant pathogens might produce toxins which conform to the original definition of the term. But up to now all substances that have been described as toxins, be it a vivotoxin or host-specific toxin, do not satisfy the above-mentioned definition.

We have certain practical problems in that if a plant pathogen like the vascular wilt organism were to produce a protein or a polysaccharide as its toxin, it cannot be freely translocated in the living system of the plant. The plant has to necessarily break down the macromolecule into smaller units prior to systemic translocation. Breakdown of the protein or polysaccharide might mean the breakdown of the toxin. Hence the role of macromolecular toxins in vascular wilts appears improbable. This does not, however, rule out the role of certain proteins — specific enzymes — in pathogenesis. But are these enzymes toxins? For example, it is known that at least one particular lethal toxin of *Clostridium welchii* Holland is the enzyme lecithinase. It is difficult to decide where to draw the line between an enzyme and a toxin.

In the case of leaf spot diseases where the symptoms are circumscribed to a short distance around the focus of infection, the problem of systemic translocation does not arise and it could be that macromolecular toxins are involved in these diseases. The reason why we have not been able to detect true toxins even in these cases appears to be lack of intensive investigation. Moreover, it is quite obvious, a toxin will not only be fixed to specific sites in the host tissue but will also be capable of killing the host even in low concentrations. Thus we will not be able to demonstrate the presence of the original molecule in the host but only the metabolized toxin or the degradation product of host-toxin interaction. Hence it will be very difficult to demonstrate a true toxin *in vivo*. It has been postulated[4] that the detection of a toxin *in vivo* will demonstrate its role in

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pathogenesis. It would appear that the term vivotoxin has helped us to understand the involvement of certain type of microbial poisons in certain plant diseases. But it has not and it cannot help us to identify a true toxin. In fact, in the case of human diseases the involvement of specific toxins in specific diseases has been demonstrated not by *in vivo* detection but by applying the following criteria[2]: (i) the organism is known to produce the toxin; (ii) virulent variants produce the toxin and the avirulents do not; (iii) infection of the toxin separately from the germ produces symptoms that mimic the disease; (iv) the infecting organisms produce the disease without spreading extensively and organs far removed from the seat of infection are affected; and (v) the disease can be prevented by immunization against the toxin.

It is also possible that a toxin may play a pathogenic role without fulfilling all these criteria.

It has been contended that since the known vivotoxins are not host-specific, one should look for host-specific toxins in plant pathology. In this connection the recent work on victorin, a toxin produced by *Helminthosporium victoriae* Meehan & Murphy in the Victoria blight disease of oats, is of interest. This substance is claimed to be highly potent and toxic only to the pathogen-susceptible host[5-7]. Perhaps we are dealing with another class of microbial poison. Nevertheless, even this host-specific toxin is not a true toxin. For this we take the analogy from human medicine. For example, it would be hard to believe that certain human pathogenic bacteria produce toxins which are specific only to human beings and not to other species of animals. Because of their non-specificity they cannot be removed from the class of toxins. By the same token it would be erroneous to think that only the host-specific toxins are to be reckoned with in plant pathology. In recent years many new words have been coined, such as phytotoxin, vivotoxin, pathotoxin and host-specific toxin, and in our opinion they are terms coined to support the importance of particular toxins rather than as absolute and final definitions. What should be borne in mind is the fact that in infectious diseases of plants, microbial poisons or toxins play a role. In an attempt to demonstrate this, quite a few substances have been isolated and shown to be involved in the diseases to varying degrees. Their chemical structure and biological activity is of considerable interest to warrant a study. However, if in this attempt we come across substances that conform strictly to the original definition of a toxin it would have proved beyond doubt the concept that microorganisms are pathogenic only if they are toxigenic[8].

In this review are enumerated some of the toxins produced by plant pathogens, which have been shown to be involved in pathogenesis, the emphasis being on their role, mode of action and their significance in the disease. Before we take up the individual toxins for our discussion it is of interest to know something about their range of action and selectivity.

The parasites producing the toxins might be discussed under two groups, namely those with long-range action and those with short-range action. In the parasites of the first group, the site of infection and the regions showing the symptoms are far removed from each other. In medicine, tetanus is the standard example. The pathogenic bacterium develops anaerobically in a peripheral wound and its toxin penetrates into the host tissue, diffusing along the nerve tracts to the brain and there producing the characteristic paralysis of the motor system. In the plant world, this group is represented by a series of economically important wilt diseases. In all these cases the parasite attacks the roots and transmits its toxin through the vascular system into the shoots. In the parasites of the second group the toxins operate directly on the tissues surrounding the focus of infection. Not only the parasite but also the toxin it produces and the damage done are localized; the site of disease coincides very largely with the site of infection. In human pathology this type of infection is very rare. *Clostridium histolyticum* (Weinberg & Seguin) Bergey *et al.*, a wound parasite, causes necrosis at its point of attack and dissolves the tissues down to the skeleton. In plants, on the other hand, numerous diseases are of this type, e.g. most of the leaf spot diseases.

Evidence so far obtained shows that the toxins have certain selective predilection for their host tissues. Some of these affect only the vascular bundles of the stem, petiole and leaves while others affect only the intercostal fields of the leaves. A few others produce only chlorotic halos in the leaves. This selective predilection of toxins for host tissues might not be so highly specific as our experience has shown with toxins affecting man and animals. This is due to the fact that in plants the tissue organization has not reached that level of differentiation as in man and animals, in addition to the fact that they lack a blood vascular system. It must also be borne in mind that in plants, unlike in man and animals, ageing is a dual process — age of the individual organs and age of the plant as a whole. It is known that tissues of organs of different ages show varying degrees of sensitivity to the same concentration of the toxin.

TOXINS — THEIR CHEMISTRY AND MODE OF ACTION

Among the many toxins that are to be discussed here, fusaric acid will be dealt with in greater detail as its chemistry and mode of action have been thoroughly worked out, and it is today a model for workers on toxins in plant pathology.

Fusaric Acid

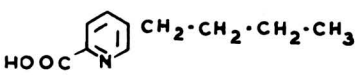
Fusaric acid was first isolated as the metabolic product of *Fusarium heterosporum* Nees, a non-specific parasite[9]. In 1952, it was recognized as an important toxin of *Fusarium lycopersici* Sacc., *Fusarium vasinfectum* Atk., and *Gibberella fujikuroi* (Saw) Wr.[10]. It is now known to play a pathogenic role in the wilt of tomato[11] and cotton[12] and the Bakanae disease of rice[13], the respective diseases caused by the above-mentioned pathogens. Subsequently, we have evidence to show that fusaric acid is involved in the Panama disease of banana caused by *Fusarium cubense* E. F. Smith[14].

Role in Pathogenesis

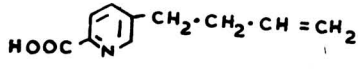
Fusaric acid is called a wilt toxin as it is now known to play a distinct role in the wilt of tomato and cotton plants, more than in any other disease. The pathogen after gaining entry into the root is seen to generalize in the vascular tissue of the host. Even when the pathogen is restricted to the roots, symptoms like vein clearing and epinasty are manifest on the leaves. This appears to be due to toxic metabolites translocated to the distant parts of the plant from the focus of infection. Some of the toxic symptoms on the diseased plants could be produced by the pure toxin fusaric acid. Moreover, it has been firmly established that a concentration of $10^{-4}M$ of fusaric acid is reached in the infected plants, a concentration lethal to the host tissues[12]. The symptoms of fusaric acid injury appear first in the stems and soon after in the leaves. In the stem it is mainly the cortical tissue over the vascular bundles that is destroyed and light gray-green sunken furrows appear on the stems and gradually spread to the petioles. Next the petioles start to bend downwards (epinasty) in the same way as the injury caused by ethylene. In contrast, the conducting strands in the stems and petioles are apparently uninjured and are not browned[15]. With higher concentration the whole stem tissue is weakened and necrosis of the stem sets in. In the leaves necrosis extends in the interveinal areas and subsequently along the whole length of the veins. About 24 hr after toxin intake the leaves curl and become flaccid.

Chemistry and Mode of Action

Fusaric acid, 5-*n* butylpyridine-2-carboxylic acid, has the empirical formula $C_{10}H_{13}O_2N$ and molecular weight 179 (Yabuta *et al.*[9]). It has the following structural formula:



Up to the present this constitutional formula, which made possible the synthesis of fusaric acid, has remained undisputed[16-18]. Pure fusaric acid has a melting point of 98-100°C.[18,19]. But biogenic preparations often contain traces of dehydrofusaric acid and possess, therefore, a higher melting point[17,20] (up to 109°C.). The existence of dehydrofusaric acid in preparations of fusaric acid was demonstrated by Stoll[20]. It has the empirical formula $C_{10}H_{11}O_2N$ and molecular weight 177 with melting point 118-120°C. It has the following structural formula:



Hydrogenation of dehydrofusaric acid with platinum catalyst and glacial acetic acid converts it into fusaric acid. Surprisingly the unsaturated dehydrofusaric acid appears after the appearance of saturated fusaric acid in culture filtrates of *F. lycopersici*[19] as well as in those of *G. fujikuroi*[21].

Mode of action in higher plants — One phase of the research on fusaric acid has been with reference to its effect on the water permeability of the plasma membrane[22,23] and on the water economy of tomato plants[24]. The test objects used in the permeability studies were the epidermal cells of *Rhoeo discolor* (L'Her.) Hance and the green alga *Spirogyra nitida* (Dillwijn) Link. The host plant, tomato, was found unsuitable on technical grounds for these experiments and only a few experiments were conducted with its medullary cells. The measure of injury to the water permeability of plant protoplasts is expressed as a quotient (Q) of the time taken by cells for deplasmolysation after treatment with water divided by the time taken after treatment with the toxin concentrations.

Fusaric acid begins to measurably impair the water permeability of protoplasts of the epidermal cells of *Rhoeo* at a low concentration of $10^{-9}M$ and that of *Spirogyra* at $10^{-8}M$. With increasing concentrations of the toxin, i.e. between 10^{-7} and $10^{-6}M$, the water permeability is temporarily increased and the Q value is double that of the control in *Rhoeo* and one and half times in *Spirogyra*. The medullary cells of tomato show initial response to fusaric acid only at a concentration of $10^{-7}M$ and hence are less sensitive than the other two model objects. But for this, the injury pattern is similar to that in the other two plant species. Thus the initial increase in water permeability in all the three test objects follows a similar course. This shows that the physiological injury suffered by these protoplasts is not determined by the plant species.

With further increase in toxin concentration the water permeability of the injured protoplasts returns to the normal value or, the protoplasts behave like the controls in water. The Q value is 1 at the toxin concentration of about 10^{-5}-$10^{-4}M$.

At still higher concentrations of the toxin a third decisive phase of injury sets in. Perhaps this phase of the injury plays a vital role in pathogenesis. It is now known that this concentration of fusaric acid may be attained in cotton plants under the diseased conditions[12]. In contrast to the earlier common behaviour of all the three test objects, this phase of the injury is determined by the type of plant protoplasts and hence the plant species. In *Spirogyra*, toxin concentrations above $10^{-5}M$ induce an initial decrease in water permeability which is quickly reversed and results in permanent increase in permeability of the protoplasts. *Rhoeo* and medullary cells of tomato respond in a manner opposite to that of *Spirogyra*. The decrease in water permeability which sets in at toxin concentrations above $10^{-5}M$ continues with increasing toxin dosage and a definitive 'waterproofing' sets in. It looks as if the plasma membranes have coagulated.

Dehydrofusaric acid (5-*n* butylenepyridine-2-carboxylic acid) injures the water permeability of plant protoplasts, i.e. *Spirogyra* and *Rhoeo* in a manner similar to that of fusaric acid except that the slight 'waterproofing' stage in *Spirogyra*, seen in the second phase of the injury, is absent here.

In this alternating injury to the water permeability of plant protoplasts by increasing concentrations of fusaric acid, two constituents of its

molecule are involved; the pyridine ring and the *n*-butyl group in the β-position. The first phase of the injury caused by fusaric acid in all the three test objects studied, namely the transient increase in the water permeability, can be induced, in addition to the above toxin, by pyridine alone and 20 other derivatives of fusaric acid, whose sole common constituent is the pyridine ring. These are differentiated from one another only by their side chains. These substances at higher concentrations are not capable of producing that phase of the injury caused by fusaric acid concentrations above $10^{-5}M$, i.e. the tight 'waterproofing'. Thus according to Bachmann[22] the pyridine ring of the fusaric acid molecule controls the first phase of the injury, viz. the transient increase in the water permeability of the plasma membrane. The carboxyl group in the α-position of the pyridine ring (α-picolinic acid) or the methyl group in the β-position (β-picoline) or their combination giving rise to 5-*n* methylpyridine-2-carboxylic acid are not toxic at concentrations above $10^{-5}M$. The specific injury to the plant protoplasts at higher concentrations must, therefore, be determined by the *n*-butyl group in the β-position. Thus, 3-*n*-butylpyridine which differs from fusaric acid molecule only in its lack of carboxyl group in the α-position produces the same effect as fusaric acid. In fact, a similar effect could be demonstrated with pyridine derivatives possessing the alkyl side chain. Homologous compounds lacking the side chain have no effect at higher concentrations.

Not only the effect on the permeability of plant protoplasts at higher concentrations of fusaric acid is brought about by the aliphatic side chain, but the quantitative nature of the damage is also determined by the length of the chain. The pathogenic action of the side chain at first increases with the length of the chain; starting from 3-methylpyridine the peak of toxicity is attained with 3-*n* butylpyridine, 3-*n*-amylpyridine and 3-*n*-hexylpyridine producing no appreciable increase in toxicity. The significance of the carboxyl group in the fusaric acid molecule with reference to its toxicity is not yet clear.

Two different processes are concerned in the intake of water by protoplasts — the familiar physical osmotic component which obeys Fick's law of diffusion, and an active non-osmotic component that is supported by the energy metabolism of the living cells. Unlike the former, the non-osmotic water intake acts independently of the concentration gradient. The transport mechansim in this case is not by diffusion but through a system of transport molecules. The molecule to be taken is closely bound to a protein and carried along with it[25]. This non-osmotic water intake acts at the expense of the energy released by cell respiration. Conversely, impairment of cell respiration leads to a dysfunction of non-osmotic water intake. Hence impairment in water permeability brought about in toxigenic wilting could be due to the injury to an essential energy-releasing process which controls the water intake. It is possible to demonstrate this with simple plasmolysis experiments by suitably blocking the appropriate enzymes with specific poisons.

A few experiments done by Bachmann[22] are worth mentioning. When epidermal cells of *Rhoeo*, in which the enzyme cytochrome oxidase is blocked previously by sodium azide, are subjected to increasing concentrations of fusaric acid, the water intake at lower toxin concentrations shows the marked rise characteristic of the pyridine ring. On the other hand, the curve showing the decrease in water permeability at toxin concentrations above $10^{-5}M$ is completely absent. Bachmann concludes that the *n*-butyl group in the β-position, which is responsible for the action of fusaric acid at higher concentrations, is rendered inactive by the previous blocking of the enzyme cytochrome oxidase.

On the other hand, if one previously arrests oxidative phosphorylation in *Rhoeo* protoplasts with 2,4-dinitrophenol and subjects them to increasing concentrations of fusaric acid, the curve showing increased permeability conditioned by the pyridine ring is absent at lower fusaric acid concentrations. At concentrations above $10^{-4}M$ the characteristic decrease in water permeability brought about by the alkyl side chain sets in. By these experiments Bachmann[22] demonstrated that the pyridine ring of the fusaric acid molecule was inactivated by the previous blocking of oxidative phosphorylation. Thus the pyridine ring in some way disturbs the oxidative phosphorylation.

Fusaric acid thus impairs the energy metabolism and in consequence the non-osmotic water intake of the plant protoplasts by two different mechanisms at higher and lower concentrations.

Fusaric acid ethyl ester causes injury to the water permeability of plant protoplasts in a manner similar to that of fusaric acid, although it cannot form metal chelates. Hence, it is unlikely that this injury caused by fusaric acid is due to chelation with heavy metals.

Mode of action in microorganisms — How fusaric acid has actually been interfering with the different enzyme systems was elucidated by studying its mode of action in microorganisms. In the classical sense of the term, fusaric acid is a weak antibiotic. It inhibits the growth of bacteria, yeasts and yeast-like fungus *Candida vulgaris* Auct. There are many examples of competitive antagonism to an essential metabolite in the field of antibiotics and antivitamins. However, very few cases have been demonstrated in the field of phytopathologically interesting toxins. That such possibilities exist is shown in the wildfire disease of tobacco which shall be dealt with later.

Although the action of fusaric acid is manifold, we may reasonably expect that some of the injuries caused are basic and more fundamental in nature, and, therefore, common to all organs and organisms susceptible to fusaric acid. If this is so, the other host-specific and tissue-specific injuries play an additive role in the clinical picture, or, they superimpose on the ground effect. Is the basic injury caused by fusaric acid attributable to its antagonism of some essential metabolite of the susceptible organisms or tissues? If so, is it possible to remove its toxicity to susceptible organisms or tissues by administering the hypothetical metabolite?

Pyridine derivatives and certain of the vitamins of the B group, which are analogous in structure

to fusaric acid, were screened for their capacity to nullify the toxicity of fusaric acid to microorganisms without success[26]. However, it is now clear that the toxicity of fusaric acid to bacterial species and certain fungi stems from the fact that it interferes with a metabolite essential for the growth of these organisms. This demonstration became possible because it was observed that the sensitivity of *Bacillus subtilis* Cohn, and *Escherichia coli* (Mig.) Cast., to this antibiotic is increased greatly in a synthetic medium than in a medium with beef extract or yeast extract. Subsequent work led to the demonstration of the presence of a substance in yeast extract that could reverse the toxicity of fusaric acid to these organisms, and this reversal appears to be competitive[26], as shown by the cross strip technique[27,28]. Thus we have evidence that fusaric acid inhibits the growth of *Candida vulgaris* by competitively inhibiting the action of a substance essential for its metabolism. A similar picture was obtained in tests conducted with *Bacillus subtilis* and the fungus *Ustilago sphaerogena* Burr. ex Ellis *et* Everh. However, with *Escherichia coli* and *Saccharomyces cerevisiae* Hans., the yeast factor and fusaric acid showed non-competitive antagonism[26].

Studies made on the effect of fusaric acid and pyridine derivatives on the water permeability of plant protoplasts show that the specific injury caused by this toxin at concentrations above $10^{-4}M$ is, to a large extent, due to the length of the aliphatic side chain of the pyridine ring. It was, therefore, of interest to study the activity of the yeast factor against structural analogues of fusaric acid with differing lengths of the aliphatic side chain.

Experiments were conducted to study the toxicity of the following substances with a decreasing length of the aliphatic side chain on the test organism *Candida vulgaris*: fusaric acid (5-*n* butylpyridine-2-carboxylic acid), dehydrofusaric acid (5-*n* butylenepyridine-2-carboxylic acid), 5-ethylpicolinic acid (5-ethylpyridine-2-carboxylic acid), 5-methylpicolinic acid (5-methylpyridine-2-carboxylic acid) and picolinic acid (pyridine-2-carboxylic acid). In addition, one pyridine derivative was used which did not contain the carboxyl group, namely 3-*n* butylpyridine. It was observed that picolinic acid and 3-*n* butylpyridine showed no toxicity. But with all the other compounds tested, greater toxicity was observed with an increase in the length of the aliphatic side chain. Thus, given the basic structure of α-picolinic acid, the length of the alkyl side chain of the analogues determines the magnitude of their toxicity[26].

The toxicity of the pyridine compounds was competitively reversed by the factor from yeast extract. However, with decreasing toxicity of the ethyl and methyl analogues the activity of the factor increased. In fact, the toxicity of fusaric acid to cut shoots of tomato plants could be partly removed by the anti-fusaric acid factor isolated from yeast extract[26].

We have already seen that the specific injury to the water permeability of plant protoplasts at higher concentrations of fusaric acid is determined by the *n*-butyl group in the β-position of the fusaric acid molecule which somehow interferes with the enzyme cytochrome oxidase. In the toxicity studies with microorganisms we are dealing with concentrations of fusaric acid above $10^{-5}M$ and the magnitude of the toxicity is determined by the *n*-butyl group. On the basis of the existing definition of competitive antagonism we might conjecture that fusaric acid interferes with the functioning of an essential metabolite (yeast extract factor) which is very closely associated with the enzyme cytochrome oxidase.

During the course of, and immediately after these experiments[26], Braun[29], working on the conversion products of fusaric acid by certain microorganisms, got certain interesting results. The fact that the metabolite which reverses the toxicity of fusaric acid was also found in the cultures of *Fusarium lycopersici*[26] led us to believe that a substance closely related to fusaric acid and capable of antagonizing the toxin is formed by the fungus during the biosynthesis of fusaric acid, or, conversely during the metabolization of the toxin by the fungus[29]. Many of the substances formed during the conversion of fusaric acid by fungi and a series of synthetic substances closely related to fusaric acid were investigated without much success. As opposed to these, it was found that ferioxamine, an organic substance of microbial origin with a moiety of iron in it, could remove the toxicity of fusaric acid to the test organism *Candida vulgaris*, with an inhibition index of 970. It was found that a gram atom of iron reverses the action of even 1000 molecules of fusaric acid. In such a relationship there is no possibility of direct chelation because a single atom of iron could not possibly bind 1000 molecules of fusaric acid. This finding was explained by the fact that fusaric acid specifically attacks the iron metabolism of the test organisms[29].

That the toxicity of fusaric acid to microorganisms could be reversed by a factor from yeast extract, probably a structural analogue of fusaric acid, as well as by iron, brings in certain new points of importance. Such instances of antagonism by a structural analogue and an inorganic ion are known. The inhibitory effect of pyridine-β-sulphamide on *Streptobacterium plantarum* Orla-Jensen was reversed by nicotinic acid, nicotinamide as well as by iron[30]. Thus the mode of action of this antibiotic could not only be explained on the basis of its blocking the action of the vitamin but also due to a blockage of the needed iron.

Detoxication

Tomato plants rapidly metabolize fusaric acid when it is administered to them. If tomato cuttings are allowed to take up a definite amount of fusaric acid whose carboxyl group has labelled carbon (^{14}C), then it is possible to account for 85 per cent of the original radioactivity in the extracts of these shoots made after 48 hr[31,32].

One of the conversion products of fusaric acid detected in the tomato shoots, which varies quantitatively depending on the plant variety, is now definitely known to be a non-toxic substance. It has been identified as N-methyl fusaric acid amide ion which appears to be saturated with organic acids in the plant[32]. N-methyl fusaric acid amide

neither induces toxic symptoms in tomato plants, nor inhibits the growth of microorganisms. The methylation of the toxin at the N-atom of the pyridine ring leads to a detoxication and, therefore, constitutes an antitoxic defence reaction. Such instances are known in the case of animals reacting to toxins. For example, the pyridine ring is known to undergo methylation and is thereby rendered harmless in the animal body. Again, nicotinic acid when introduced in the animal body undergoes transformation to trigonelline (N-methyl nicotinic acid)[33]. N-methylation as a detoxication mechanism has been demonstrated for the first time in plants with the toxin fusaric acid[32].

Of the three varieties of tomato studied, the wilt resistant variety was seen to inactivate 20-24 per cent of the administered fusaric acid by N-methylation, while the two wilt susceptible varieties, Bonny best and Tuckswood, inactivated only about 8 per cent of the fusaric acid by this method. Thus this defence reaction finds expression in the degree of susceptibility of tomato varieties to the toxin and thereby perhaps to the disease[32].

Antitoxic reaction — The toxic injury of fusaric acid to cut shoots of tomato plants could be partly removed by the factor isolated from yeast extract, the anti-fusaric acid factor[26]. It was concluded that a part of the damage to tomato shoots caused by fusaric acid is attributable to its antimetabolite character. This is most conspicuous in the leaves, where damage caused by fusaric acid is little affected by *p*H changes. Moreover, the leaves being the active centres of metabolism offer more reactive groups than the stem. The discrepancy between the stem and leaf injury is also due to the problem of non-uniform translocation of the toxin and antitoxin in the plant.

Within the wide range of phanerogamic hosts, the sensitivity of the various species to fusaric acid differs both quantitatively and qualitatively. Rye, maize and peas react only mildly to fusaric acid; their *dosis minima* is about 10 times that of beans, rice or tomato. Cotton plants react more sensitively, their *dosis minima* being about 1/100 that of rye, maize or peas[15]. On the basis of the antimetabolite nature of fusaric acid it would appear that the quantity of toxin needed to cause the injury to any organism or organ would depend on the proportional distribution of the antagonist (metabolite). Thus the quantity of antitoxin (metabolite) naturally present in the tissues of the plant species might determine their sensitivity to the toxin.

Significance of Fusaric Acid in Disease

It has been contended by certain workers[34] that since in the diseased cotton plants only 17·2 mg. of fusaric acid per kg. fresh weight has been detected[12] and as the toxic level of fusaric acid is 150 mg. per kg. of fresh weight, this toxin may not play an important role in the disease. This statement is erroneous for the following reasons: (i) The *dosis minima* for cotton is around 10-20 mg./kg. while it is 150 mg./kg. for tomato plants[15]; and (ii) since 50-70 per cent of the fusaric acid gets metabolized in the plant, the quantity detected in the diseased plant is the free fusaric acid or the unmetabolized fusaric acid which is only a portion of the total quantity of the toxin the pathogen synthesizes in the host plant. Thus a concentration lethal to the host plant is definitely attained in the cotton plants infected by *Fusarium vasinfectum*. It would thus appear that fusaric acid is a decisive agent in the causation of disease in cotton and perhaps in the actual disease other factors also play a synergistic role.

Lycomarasmine

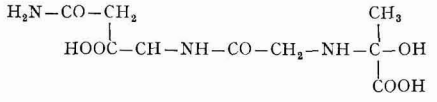
Lycomarasmine is a wilt toxin of the tomato wilt pathogen *Fusarium lycopersici* Sacc., isolated and described earlier to fusaric acid. Described by Clauson-Kass *et al.*[35] in 1944, this toxin unlike fusaric acid appears to be specific to *F. lycopersici*.

Role in Pathogenesis

Lycomarasmine when administered to tomato plants produces injury in the intercostal fields of the leaves, and rolling of the leaf tips. No appreciable injury is produced on the stems and petioles. Thus the symptoms of tomato wilt simulate the toxic injury manifest by the synergistic action of lycomarasmine and fusaric acid. While fusaric acid has been demonstrated in the infected tomato plants no such direct evidence exists for lycomarasmine. Its very chemical nature did not lend its detection, *in vivo*, by chemical or bioassay methods, in small amounts. The minimum dose of lycomarasmine needed to cause appreciable injury to tomato shoots is 150 mg. per kg. fresh weight.

Chemistry and Mode of Action

Lycomarasmine is a polypeptide with molecular weight 277·3 and empirical formula $C_9H_{15}O_7N_3$. The following structural formula has been tentatively assigned for this toxin[36]:



The toxicity of this substance seems to arise from the fact that it can form metal chelates and this is most obvious with iron. Thus the injury caused by lycomarasmine-iron complex is infinitely greater than that caused by lycomarasmine alone. Lycomarasmine when administered to tomato plants causes a shock phase within the first few hours, when there is a depressed rate of water intake as well as transpiration. Soon this is reversed and the rate of transpiration far exceeds the normal one bringing about the wilt. The pathogenic effect of this toxin is partly due to the fact that its molecules on their way through the tomato shoots chelate iron ions of the host, which themselves would not be able to migrate, and transport them into the leaves in the form of lycomarasmine-iron complex. Here part of the complex is decomposed, e.g. on account of photochemical effects or due to exchange by more potent chelating agents in the cell itself. Thus, on the one hand, part of lycomarasmine molecules are regenerated as chelating agents and cause appropriate local deficiencies, on the other hand, iron ions set free in the 'wrong' place lead to local

iron plethora. Gäumann and Naef-Roth[37] assume that the excessive transpiration occurring in consequence of lycomarasmine poisoning is mainly due to such iron injuries.

If cut tomato shoots are allowed to take in the lycomarasmine-iron complex saturated with iron, 1:1, they show the same characteristic curve of disturbed transpiration as they do with pure lycomarasmine, although the saturated lycomarasmine-iron complex cannot chelate iron ions of the host on the way[38]. The opposite occurs if lycomarasmine is shielded from iron so that, on the one hand, it carries no iron and, on the other hand, it cannot chelate on its way through the shoot iron ions of the host. In this case the transpiration of the tomato shoots does not show the characteristic curve of lycomarasmine injuries[39]. Hence, lycomarasmine with iron is without effect in the field of excessive transpiration.

This assumption, that the iron ions of the host transported by lycomarasmine to the 'wrong' place play an important part in the disturbing of the water economy of tomato shoots, cannot be verified in tomato tissues themselves, owing to methodological difficulties. For this reason analogous experiments were carried out with protoplasts of *Rhoeo discolor*[40] as a model. Pure lycomarasmine does not impair the water permeability of *Rhoeo* protoplasts, as was assumed in a working hypothesis for the tomato shoots. An increase in the number of iron ions, which are carried into the interior of the *Rhoeo* protoplasts with lycomarasmine as a vehicle, produces in the biologically sensible concentration range an excess of the water permeability of the *Rhoeo* protoplasts. The working hypothesis mentioned at the beginning is, therefore, borne out by the measurements with *Rhoeo* protoplasts.

By this confirmation of the working hypothesis the desensitization of tomato shoots after repeated application of lycomarasmine can be partly explained. If a tomato shoot is allowed to take in a certain amount of lycomarasmine on two successive days, the additional second dose produces a smaller effect than the first. This may be partly due to the fact that the first dose of lycomarasmine removed away a great percentage of the chelatable iron ions so that the second dose met with only a few iron ions.

Detoxication

Another aspect of the study of this toxin has been its antagonism to the yeast growth factor strepogenin[41], which is an oligopeptide containing glutamic acid. As against glutamic acid present in strepogenin, lycomarasmine has aspartic acid and to this has been ascribed its antagonistic action. This growth factor when supplied externally removes some of the toxic symptoms of lycomarasmine injury in tomato, leading to the conclusion, that it may interfere with the strepogenin metabolism of the host.

Piricularin

Pyricularia oryzae Cav., a facultative saprophyte belonging to the group of Hyphomycetes, is the causal organism of the 'blast' disease of rice and has a wide distribution in all rice growing tracts of the world. This being essentially a leaf spot disease, spindle-shaped necrotic spots develop on the leaves. Sometimes black spots or rings are observed on the inflorescence rachis and the nodes of the culms. In severe attacks the heads emerge prematurely, completely blasted and appearing whitened, long before the normal time of ripening[42]. The symptoms of the disease indicate the effective role of some toxic substances.

It appears that two of the metabolic products of the fungus are responsible for the characteristic symptoms of this disease. One of them was identified as α-picolinic acid and the other as piricularin[43]. Both the toxic substances have been isolated from the diseased plants and they appear to play a definite role in the disease. It is proposed to deal here with the chemistry and mode of action of piricularin[44].

Role in Pathogenesis

Piricularin is now known to be definitely involved in the pathogenesis of *P. oryzae*. When punched on to the leaf blades of rice plants, piricularin produces a characteristic spot in the leaves and administration of piricularin also brings about a stunting of the plants. Immersion of the aquatic green alga *Nitella* in a solution of 1 p.p.m. of piricularin for 24 hr produces coagulation of protoplasm. In addition, this toxin has inhibitory action on the growth of fungi and gram-positive bacteria.

Chemistry and Mode of Action

Piricularin has been assigned a tentative empirical formula of $C_{18}H_{14}N_2O_3$, melting point of 73·5°C. and a molecular weight of 306. It is highly stable in water, especially at acid *p*H.

The toxic action of piricularin appears to be due to its inhibitory effect on the activity of the enzymes, peroxidase, catalase, cytochrome oxidase and ascorbic acid oxidase, even at low dilutions (1/100,000-1/200,000). At still lower concentrations (1/1,600,000) the toxin stimulates respiration and growth of rice plants. This stimulative effect of piricularin causes increase in all fractions of organic phosphorus, especially nucleic acid phosphorus and protein phosphorus. Data on the RNA content clearly show a definite increase, especially in the ribosomal RNA by this stimulative effect. Rice plants showing stimulative effects with piricularin are resistant to blast infection, while piricularin at higher concentrations, when it is inhibitory to the rice plants, makes the plants susceptible to the disease.

Detoxication

The deleterious effect of piricularin is removed by chlorogenic acid or ferulic acid, each of them being the natural phenolic constituents of the rice plants. It is presumed that the combination of piricularin with these substances is a natural detoxifying mechanism.

Since piricularin is inhibitory to the germinating spores of the blast fungus, *P. oryzae*, which produces this toxin and such inhibitory effect is normally not observed in the cultures of *P. oryzae*,

it is of interest to know the non-toxic nature of piricularin in culture solutions. It is now known that piricularin combines with a peculiar protein, the piricularin-binding protein, and in this combined state it is non-toxic to the organism that produces piricularin. However, in the bound form it is toxic to plants. In contrast to this, piricularin in combination with chlorogenic acid, although non-toxic to the rice plant, is toxic to the blast fungus. The piricularin-binding protein with a molecular weight of 69,000 and containing 2 copper atoms in the molecule, has been demonstrated to be a new copper oxidase. This protein oxidizes various phenolic compounds, ascorbic acid and indole acetic acid.

The other enzymological properties of piricularin-binding protein are: (i) Michaelis constant: $4{\cdot}54 \times 10^{-4}$ (substrate pyrogallol), $2{\cdot}45 \times 10^{-3}$ (orcinol), $1{\cdot}56 \times 10^{-3}$ (phloroglucinol); (ii) oxygen consumption for oxidation of substrates: one mole oxygen for the oxidation of one mole of *para-* or *ortho*-diphenol and $\frac{1}{2}$ mole oxygen for the oxidation of *meta*-diphenol; (iii) inhibitors: KCN, NaN_3 (both exhibit 100 per cent inhibition at $10^{-4}M$); Na diethyldithiocarbamate (68 per cent inhibition at $10^{-3}M$); CO (20 per cent inhibition at CO : O_2 ratio of 95 : 5), 8-hydroxy quinolin (15 per cent inhibition at $10^{-2}M$) and piricularin; (iv) piricularin-binding protein oxidizes cytochrome *c* smoothly; and (v) piricularin-binding protein is smoothly reduced, coupling with the system of lactic acid and lactate dehydrogenase from baker's yeast.

Victorin

Helminthosporium victoriae Meehan & Murphy causes blight of oats only in the variety Victoria or its derivatives. It is a soil- and seed-borne pathogen, which causes severe leaf blight as well as stem and root necrosis of susceptible oats. Infection usually occurs near the soil line and the first symptoms are yellow to orange-red stripes in the leaves. Litzenberger[45] demonstrated that the culture filtrates of the fungus contained a toxin that can reproduce accurately the visible symptoms of the disease. The name victorin was given to this toxin by Wheeler and Luke[46]. The toxin is highly specific in its activity and is claimed to produce the symptoms only on the pathogen-susceptible variety of oats.

Role in Pathogenesis

Victorin is able to produce accurately the symptoms of the disease on susceptible host plants. In addition, this substance inhibits the growth of roots of susceptible plants. There is no direct evidence to show that the toxin is involved in the disease, as it has not been detected in the diseased plant. The role of this toxin in the disease is based on the following criteria: (i) the toxin is active only on the susceptible hosts; (ii) the symptoms of the toxic injury caused by the toxin and the pathogen are similar; and (iii) virulent strains produce toxin and the avirulent strains do not.

A bioassay based on the inhibition of root growth has been developed by Luke and Wheeler[47] for evaluating this toxin, in which the titre is expressed as the dilution required to cause a 50 per cent inhibition of root elongation. Pringle and Braun[5] have modified this by using, as end point, the lowest concentration of the toxin in a serial dilution that would completely inhibit root growth of susceptible seedlings.

All mention of the potency of victorin is based on the effect of this toxin on root growth. In our opinion it would be advisable to develop a method by which it should be possible to correlate the potency of the toxin directly by the intensity of the injury or necrosis of the stem, leaves and roots. We have the following reasons for advancing this argument: (i) The minimum concentration of toxin needed for inhibition of root growth may not be the same as that needed to produce the necrotic symptoms on the plant; this is all the more valid as the toxin has not been completely purified; and (ii) there is no evidence to show that the inhibition of root growth and the necrotic symptoms on the host plants are brought about by identical physiological disturbances in the host. Victorin is claimed to be the most potent toxin known in plant pathology but unfortunately on the basis of its effect on root growth.

Chemistry and Mode of Action

The chemistry of this toxin is not yet completely worked out and the present indications are that it is a polypeptide linked to a tricyclic secondary amine with a molecular weight around 2000 (ref. 5, 47 and 48). When the pure toxin is treated with saturated sodium bicarbonate for 24 hr at room temperature, it cleaves into two substances. One of the products is a peptide containing aspartic acid, glutamic acid, glycine, valine and one of the leucines. The second product of sodium bicarbonate cleavage was crystallized and has been found to be a new base called 'victoxinine'[48]. This has an empirical formula of $C_{17}H_{29}NO$. Evidence indicates that it is a tricyclic secondary amine[48]. Victoxinine is less toxic than the parent molecule.

The effect of victorin on certain functional systems of the susceptible oats is known, but how exactly this is brought about remains to be investigated. Victorin affects the permeability of the plasma membrane, as well as the respiration of the susceptible oats[6]. In our opinion these are effects rather than causes. Since we are dealing with a specific and a potent toxin, it would be of interest to know the exact mode of action of this toxin.

Detoxication

Unlike the previously mentioned toxins, victorin is able to incite toxic symptoms only in the blight-susceptible variety of oats. Perhaps this could be explained due to the presence of a specific enzyme on the surface of the cells of the susceptible and not of the resistant variety[49]. This enzyme has to necessarily transport the toxin molecule into the cell before the toxin acts. Such a phenomenon is known in the case of susceptibility of certain micro-organisms to drugs. The strain of *Mycobacterium tuberculosis* susceptible to isonicotinic acid hydrazide has the enzyme peroxidase active on the surface layers, while it is absent in the drug-resistant

strain[50]. Thus the resistance of this bacterium to this drug could be explained on the basis of the inability of the drug to get into the bacterial cell.

There are indications that the non-toxic nature of victorin to the resistant oats is due to its inability to enter the host tissue. If this is true, it may not be a true case of toxin resistance, in the sense that there is no inactivation of the toxin within the cells.

The Wildfire Toxin

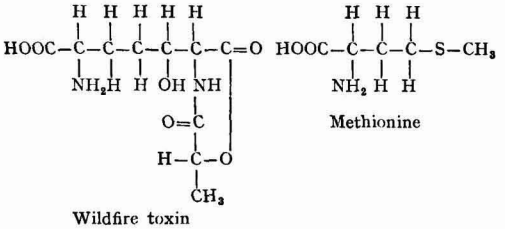
As opposed to the toxins described above which are of fungal origin, the toxin of wildfire disease of tobacco is produced by the bacterium *Pseudomonas tabaci* (Wolf & Foster) Stev. The disease was first described by Wolf and Foster in 1917 in America and is now found to occur in most of the tobacco growing regions. Because of the suddenness of appearance, the rapidity of spread and the severe nature of the disease in the field, the term wildfire has been applied to this disease.

Role in Pathogenesis

Johnson and Murwin[51] first demonstrated that the culture filtrate of the pathogen could produce the lesions characteristic of the disease on the host plants, and that the toxin is not host-specific although the pathogen is. This is the only decisive toxin formed by this pathogen and it, therefore, acts by itself within the host. The toxin produces chlorotic halo in the leaves when punched on, similar to the disease symptom.

Chemistry and Mode of Action

The wildfire toxin has been found to be a derivative of a new amino acid[52]. Complete hydrolysis of the pure toxin with strong HCl yields lactic acid and large amounts of this new amino acid which has been found to be α,ε-diamino-β-hydroxypimelic acid. The name tabtoxinine has been applied to this substance. The amino acid is the second member of a new type of α-amino acids (sulphur-free diamino dicarboxylic acid) found to occur naturally. Tabtoxinine has been found to be biologically inactive in so far as its ability either to elicit the formation of or to reverse the chlorotic effect of the wildfire halos in a tobacco leaf is concerned.



Wildfire toxin

Methionine

The wildfire toxin is most probably the lactone of α-lactylamino-β-hydroxy-ε-amino pimelic acid[53]. The toxin is biologically a very active substance and 0·05 μg. gives rise to a typical chlorotic lesion when introduced into the tobacco leaf.

In studying the mode of action of this toxin, Braun[54] has used the unicellular alga *Chlorella vulgaris* Beijerinck. He demonstrated that the toxin inhibited the growth of this organism. However, when yeast extract or liver extract is added to the toxin there is a reversal of its deleterious effect. This suggested that the toxin interfered with the functioning of some essential metabolite necessary for the growth of this alga. Yeast or liver extract provided this metabolite when added to the culture externally. A further investigation showed that L-methionine, one of the constituents of the above extracts, reversed the action of this toxin on this alga.

One of the probable pathways of methionine synthesis in bacteria and fungi is as follows: cysteine → cystathione → homocysteine → methionine. The three precursors when tested separately were found to be incapable of overcoming the effect of this toxin. The immediate precursor, homocysteine, forms methionine by transmethylation. If the synthesis of methionine in *Chlorella* follows a pattern similar to that described for other organisms, then the toxin might exert its biological effect either by blocking the methylation of homocysteine or by interfering with the utilization of methionine that is normally synthesized by *Chlorella*. To test the first probability, homocysteine was administered with choline or betaine, two good methylating agents, in the presence or absence of *p*-aminobenzoic acid, to test the organism subjected to the wildfire toxin. The toxicity was, however, not reversed. Since transmethylation is a complex phenomenon we are not in a position to conclude that the toxin does not interfere with the methylation of homocysteine. However, it was proved by Braun[55] that the wildfire toxin competes with methionine for active centres on the enzyme that normally combines with or acts on methionine. This could be demonstrated by the competitive nature of the antagonism between methionine and the wildfire toxin with *Chlorella vulgaris*.

A similar phenomenon could not be demonstrated with tobacco leaves treated with the toxin and methionine. These results suggested either that the mechanism of action of the toxin is different in the two plant species, or that the action was the same, but it was difficult to demonstrate in the higher plants, due to certain practical difficulties. Nevertheless, one could conclude that the wildfire toxin is a structural analogue of methionine and its biological activity is due to its behaviour as an antimetabolite.

The relationship of methionine and the toxin can be seen clearly by comparing the structure of these compounds. In the toxin the sulphur atom of methionine has been replaced by two carbon atoms. A change such as this commonly converts the metabolite into antimetabolite. One of the carbon atoms of the toxin bears the oxygen atom and the other bears the nitrogen atom. In addition, in the toxin, the oxygen and the nitrogen bonds have been reduced, and the methyl group has been oxidized to a lactone grouping. From previous studies it appears that the chemically high reactive group, lactone, present in the toxin, may make it a potent antimetabolite. This lactone most probably binds the toxin by a covalent bond to the site in the plant cell that is normally occupied by methionine[55].

Other Toxins of Phytopathological Interest

In addition to those described above there is mention in literature of toxins playing a role in certain other diseases. However, in these instances, the toxins have not yet been characterized or when characterized the mechanism by which they cause injury to the host has not been fully established.

Alternaric Acid

Alternaric acid produced by *Alternaria solani* (Ell. & Mart.) Jones & Grout has antifungal activity but not antibacterial. In addition it is highly phytotoxic. This substance is an optically inactive, unsaturated dibasic acid with an empirical formula of $C_{21}H_{30}O_8$. If it is introduced into the healthy shoots of tomato or potato, lesions similar to those observed in natural conditions of disease are produced in the stems and leaves. The effect of alternaric acid on the water economy of host plants is similar to that observed with the toxin lycomarasmine, but it is highly potent. This toxin or a substance closely related to this has been detected in the naturally infected plants and there is considerable evidence to show that it is involved in the disease caused by *A. solani*[56].

The black-spot disease of Japanese pears is caused by *Alternaria kikuchiana* Tanaka. This fungus produces what is claimed to be a host-specific toxin that damages only the susceptible variety of pears. More than one toxin appear to be involved in this disease. One of them is named phyto-alternarin[57,58] but its chemistry and mode of action are very little understood.

Diaporthin

Diaporthin is a specific toxin produced by *Endothia parasitica* (Murr.) And., the causal agent of blight of chestnut, of unknown chemical structure but with the empirical formula $C_{13}H_{14}O_5$. This toxin has a wide host spectrum of action ranging from bacteria to higher plants. The main toxic injury in higher plants is the necrosis of the conducting vessels resulting in the collapse of the stem tissues into longitudinal grooves. The conducting strands of the leaves are also affected[59].

Toxin of Periconia circinata (Mangin) Sacc.

Periconia circinata (Mangin) Sacc., the causal agent of the blight of certain cultivars of grain sorghum [*Sorghum vulgare* var. *subglabrescens* (Steud.) A. F. Hill], produces a powerful toxin which appears to be specific to the pathogen-susceptible plants. Preliminary investigations indicate that this is likely to be a low molecular weight polypeptide derivative, as that of *Helminthosporium victoriae*. Unlike victorin, *Periconia* toxin appears to be a stronger acid and more stable[60]. Like victorin, a distinct correlation between the pathogenicity of the strains and their capacity to produce toxins has been claimed[61]. Complete chemistry and mode of action remain to be investigated.

Conclusion

In conclusion we propose discussing a few pertinent points that confront the workers in this field.

One comes across quite often evidence to show that toxins produced by widely different fungi and vastly different in their chemical nature bring about certain common changes in the host. For example, it is known that the permeability of the plasma membrane can be affected by fusaric acid as well as victorin. In our opinion, the changes in permeability reported are the ' end effects ' and not the cause. One has to go deeper into the problem and find out the target in the host cells that is hit by the toxin. In the case of fusaric acid it is now beyond any doubt that it interferes with the iron metabolism[26,29,62] and as such the enzyme cytochrome oxidase at concentrations above $10^{-5}M$. A block in the above-mentioned enzyme affects the non-osmotic water intake and as a consequence the permeability of the plasma membrane. In the case of victorin, we have no experimental evidence to show the cause that brings about the changes in the permeability of the plasma membrane.

It is likely that the plant tissues, being not so specialized as animal tissues are, do not have multitudinous ways of expressing their reaction to toxins and hence similar effects that are produced by the action of different toxins do not in anyway prove their non-specificity.

Toxins from parasites with short-range action (leaf spot diseases) when administered to cut shoots act systemically. In natural conditions of disease, these toxins are not systemic but localized and as such it has been contended that these toxins may not play a role in the diseases. However, this variation can be explained on the basis of the following facts. The quantity and the route through which the toxin is administered is very important. In the experiments with the cut shoots the toxin gets translocated along the vascular bundles of the stem and leaves and on reaching the specific tissue produces specific symptoms. However, in the natural conditions of the disease the limited growth of the pathogen and the small amount of the toxin are localized to specific areas by virtue of host defence reactions. In addition the toxin seldom reaches the conducting strands, and even if they do so, the concentration may not be enough for effective systemic translocation. For example, if the toxin of a bacterium causing a boil or wound in man were to be introduced from an external source into the blood stream it would have a systemic effect. However, in the natural conditions of the disease the pathogen and the toxins are localized around the focus of infection due to host defence reaction. A similar situation is obtained in plants as well.

Up to now we have dealt with only instances of facultative parasites that could grow and produce toxins in synthetic media. Whether we are dealing with host-specific toxins or not, it has been possible to isolate and purify them from cultures and study their pathogenic role. It has been mentioned earlier that one might run into difficulties if one starts postulating criteria for the role of toxins in plant diseases, i.e. specificity, vivotoxic nature, etc., setting aside the original meaning of the word toxin.

Even with a facultative parasite like *Helminthosporium victoriae* it has not been possible to detect

the toxin, victorin, *in vivo*. This is as it should be. When we are to deal with obligate parasites, as they cannot be cultured, the problem will become more complicated. That the rust fungi, like the many species of *Puccinia* or the white rust fungus *Albugo*, might also damage their hosts by toxins is beyond doubt. But it will be very difficult to prove this, as we will not be in a position to detect the toxins in the diseased tissues. Nevertheless, it should be possible to demonstrate the role of toxins in these diseases just as it has been shown in bacterial infections of human beings by methods other than of *in vivo* detection.

It must be understood, as it has been emphasized in the beginning of this review, that all and every symptoms in any and every infectious disease cannot be accounted for as being due to toxins only.

Summary

The genesis of the term toxin in infectious diseases of man and animals has been traced and the possibility of toxins playing a role in plant infections is discussed. The chemistry, role and mode of action of some of the important toxins known to be involved in specific plant diseases are presented. A critical evaluation of conflicting terminologies now current in plant pathology, i.e. phytotoxins, vivotoxins and host-specific toxins, has been made. The possibility of toxins playing a significant role in certain plant diseases caused by obligate parasites is also discussed.

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Edema of Protein-Calorie Malnutrition

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THE body of an average man contains water roughly equivalent to two-thirds of his body weight and solids comprise only one-third. Nearly two-thirds of the total water lies within the cells, associated with cellular solids and is in dynamic equilibrium with the extracellular water. The pituitary gland, the adrenal cortex, liver and kidneys and physico-chemical forces like osmotic pressure, Donnan membrane equilibrium are involved in the maintenance of the normal water balance in man. However, in certain pathological conditions, there is a large increase in the degree of hydration which leads to an abnormal accumulation of fluid in the extracellular compartment. This condition is referred to as 'Edema' and may be due to various nutritional and other factors. In the present article the work carried out at the Nutrition Research Laboratories, Hyderabad (formerly at Coonoor), on edema due to protein-calorie malnutrition is reviewed under the following heads: (i) important techniques employed in the study; (ii) investigations carried out in clinical conditions of edema and experimentally induced protein deficiency in animals; and (iii) the hypothesis put forward by the investigators.

Techniques

Total body water and extracellular water — A water-soluble compound which can negotiate the cell membrane barrier freely and is capable of attaining a uniform concentration throughout the intracellular and extracellular water is employed in the determination of total body water, while a compound which does not cross the cellular membrane is employed in determining the extracellular water. These compounds should not be metabolized during the period of observation and must not be toxic. Urea has been the compound of choice for the determination of total water in normal subjects and has been widely employed in the study of edema of protein-calorie malnutrition. It was suggested that urea may be metabolized in malnourished subjects and total water as determined by urea would be an overestimate. It, therefore, became essential that the validity of the urea method had to be established. Srikantia and Gopalan[1] carried out comparative studies using urea and antipyrine to estimate total body water in malnutrition and showed that the urea method was suitable for the estimation of total body water even in malnourished patients. Thiocyanate is widely employed for the determination of extracellular water. It is recognized that thiocyanate gives a slight overestimate of the absolute amount of extracellular water but is satisfactory as a practical tool for the clinical investigations of edema.

In the thiocyanate method, an initial sample of blood is collected from the subjects under basal conditions and the bladder is completely emptied. An accurately known amount of urea is given orally and thiocyanate is administered intravenously. Urine is collected over the following 6 hr instead of the usual 2 hr, since the subjects are edematous. A second sample of blood is collected at the end of 6 hr. The amounts of these compounds excreted in the urine and the concentrations of urea and thiocyanate in the initial and final samples of blood are determined. These experiments enable the calculation of the amount of total body water and extracellular water (ECW).

Cell solids, fat and mineral contents in the edematous stage and after rehabilitation — After the edema clears, it is assumed that hydration of cellular solids in these subjects would be normal and the intracellular water determined would constitute about 67 per cent of cell mass. The other constituents are calculated as shown below by the method of McCance and Widdowson[2].

Bone minerals = 7·5 per cent (cell mass + extracellular water)

Fat = body weight − (extracellular water + cell mass + bone minerals)

An estimate of these constituents in the initial edematous stage is done as follows.

(i) Bone minerals = bone minerals − (Ca + P)

(Initial) (Final)

where (Ca+P) represents the calcium and phosphorus intake during the rehabilitation period

(ii) Total solids = body weight − total body water

(iii) Cell solids = total solids − bone minerals

(iv) Fat is taken as nil in the extreme stage of chronic starvation, an assumption supported by a few post-mortem examinations of some subjects

The assumption that the minerals are completely retained would lead to an underestimate of the bone minerals at the initial stage, but the magnitude of this error is negligible.

Bioassay of antidiuretic hormone — In the presence of antidiuretic hormone (ADH) of pituitary origin in circulation, the excretion of a dose of water fed to animals is significantly inhibited. Young male rats fasted for 12 hr are employed in these assays. They are orally fed with water to the extent of 5 per cent of their body weight and the material to be assayed for the hormone is administered intraperitoneally. The percentage of water excreted over the following 240 min. is taken as a measure of the antidiuretic activity of the extract; the smaller the volume of urine excreted, the greater the ADH content of the material.

Antidiuretic potency of liver and inactivation of pitressin by liver in vitro — Liver has been shown to be an important site of inactivation of ADH and the inactivating system has been shown to be

enzymatic in nature[3]. In this assay, liver homogenates are injected intraperitoneally to hydrated rats prepared as detailed under bioassay of antidiuretic hormones and the amount of water excreted is taken as a measure of the antidiuretic potency of liver. The ability of liver to inactivate pitressin *in vitro* is measured by incubating liver homogenates with 2 units of pitressin at 37°C. and the enzyme inactivated at intervals. The active pitressin in the incubated samples is assayed with hydrated rats.

Bioassay of ferritin — Physiologically active ferritin depresses or inhibits the constrictor response of capillaries to epinephrine. The capillary bed of the mesoappendix of fasted rats is used for the bioassay. The time taken for the capillaries to respond to epinephrine is determined and the serum or any biological fluid that is to be assayed for ferritin is intravenously administered to the rat and the response to epinephrine determined again. The duration of time over which the constrictor response remains depressed is a measure of the ferritin content of the material. In these studies specificity for ferritin is achieved by the use of antiferritin serum.

Clinical Edema and Experimental Edema

Edema in Children and Adults

Edema due to protein malnutrition is common in children soon after weaning, all over Asia, Africa, South and Central America. A comprehensive description of this condition is given by Gopalan and Patwardhan[4] and Venkatachalam *et al.*[5]. The condition in adults is not encountered as often as in children, and is described by Gopalan *et al.*[6]. The edema present in such cases may be of various grades affecting only the extremities, to edema involving extremities, trunk and face. The condition in children is commonly referred to as 'Kwashiorkor' or 'Nutritional edema syndrome' and that in adults as 'Nutritional edema' or 'Hunger edema'. Though a wide range of constituents have been studied in different tissues in subjects with edema, only those that are relevant to the problem of edema are discussed here.

Blood chemistry — Serum total proteins and albumin are low in kwashiorkor and nutritional edema[4,6]; Ramanathan[7] found low serum total proteins, albumin, urea, non-protein nitrogen, total cholesterol and cholesterol esters and a high concentration of globulins in children suffering from kwashiorkor. The electrophoretic pattern of serum proteins in nutritional edema of adults reveals a decrease in albumin, a slight increase in α_1 globulin and a considerable increase in γ globulin concentrations. In some subjects a band corresponding to β_2 globulin appears during therapy but disappears with continued high protein treatment[8].

The serum sodium and chloride levels are low or normal in adult nutritional edema[6] while serum potassium level is in the range of high normal levels[9]. In kwashiorkor, serum potassium and magnesium levels are found to be normal[10]. Srikantia[11] demonstrated active ferritin in plasma obtained from children suffering from kwashiorkor and adults with nutritional edema, but none in children suffering from protein malnutrition without edema. All these changes are reversed following on successful therapy with high protein diets.

Urinary excretion of antidiuretic hormone, 17-ketosteroids and electrolytes — Nutritional edema in adults is associated with excretion of low volumes of urine and in some patients the oliguria is profound and the urine volume is as low as 200-400 ml./24 hr. Gopalan[12] demonstrated that urine from patients suffering from nutritional edema contains an antidiuretic factor. Patients excreted lower amounts of potassium in urine after the disappearance of edema as compared to the levels of excretion at the initial stages on a constant dietary intake of potassium[9]. In patients suffering from edema, excessive intake of sodium chloride causes greater retention of water while restriction of salt intake brought about no marked beneficial effects.

Urinary excretion of 17-ketosteroids is low in nutritional edema and kwashiorkor. These values increase on treatment with high protein diets[13].

Body composition and basal metabolism — Gopalan *et al.*[14] determined the total body water and extracellular fluid in adult patients suffering from edema and found them to be high. Evidence has been obtained to show that cellular solids are low and there is excessive intracellular hydration as well. After treatment, and when edema completely disappears, total body water — both intracellular and extracellular — shows a fall while plasma volume shows an increase. Venkatachalam *et al.*[15] demonstrated that in edematous patients basal metabolism is low at the time of admission and there is an appreciable rise after treatment. The reduction in basal metabolism is due to a reduction in the quantity of metabolizing tissue and not due to a reduction in oxygen consumption per unit of tissue.

Response to water load and renal function — Gopalan and Venkatachalam[16] studied the response to a water load both in the erect and recumbent postures in normal and edema cases. While the water excreted in both the postures is low in patients with edema, the excretion in erect posture shows a greater degree of impairment. Water excreted in the erect posture is about 55-60 per cent of that excreted in recumbent posture in normals, while in edema cases it was only 7-40 per cent of that obtained in the recumbent posture.

Srikantia and Gopalan[17] observed that the renal plasma flow and glomerular filtration rates are normal in subjects suffering from nutritional edema. However, the urine flow per minute is considerably reduced.

Experimental Edema in Animals

Response to water load and inactivation of pitressin in protein deficiency — Gopalan and Ramanathan[18] induced protein deficiency in monkeys by feeding low protein-low calorie and low protein-high calorie diets. They observed that the response to a water load is more defective in animals receiving high calorie-low protein diets than in animals receiving low protein-low calorie diets.

The antidiuretic potency of liver homogenates and their ability to inactivate pitressin *in vitro* are affected by starvation. Liver homogenates obtained from rats fasted for 48 hr cause a lower excretion of

water when injected to hydrated animals and are less efficient in inactivating pitressin *in vitro* as compared to liver homogenates of fed rats[19].

Low protein diet brings about a similar reduction in the ability of liver homogenates to inactivate pitressin *in vitro*. Restriction of calorie intake by rats fed low protein diets has a beneficial effect[20].

Sequence of changes preceding edema formation and clearance — Srikantia and Gopalan[21] followed, at regular intervals, the changes in serum protein, response to water load, and the content of extracellular water in monkeys maintained on a low protein diet. Active ferritin has been detected only in the plasma of children and adult edema patients and not in the plasma of children suffering from protein deficiency but not associated with edema. Ferritin has, therefore, been assayed in plasma collected from the monkeys to study its relation with edema formation. Some of these monkeys received a supplement of chlortetracycline (aureomycin) from the beginning of the low protein regimen since it has been shown that the *in vitro* release of ferritin under unaerobic conditions, from liver slices of rats could be inhibited by treating the rats with aureomycin, prior to killing[22].

A large increase in extracellular space (36 per cent body weight from an initial value of 20 per cent) occurs in all the monkeys around the 13th to 16th week of experimental diet. A consistent finding in each of the animals which developed edema is the appearance of ferritin in circulation a week or two before the thiocyanate space increased. It is also interesting that the increase in extracellular water is not gradual but abrupt during this period. The response to water load shows a gradual impairment and by the time the edema appears, it decreases to about one-third of the control value. There is a sudden worsening of the response at the time when ferritin is demonstrated in circulation. The decrease in serum albumin is also gradual till about the 10th week and thereafter no further decrease is observed. On re-feeding a high protein diet, the first change that occurs is the disappearance of ferritin from circulation. The extracellular water significantly decreases and the response to water load improves by about three weeks though these values do not return to the level of the control values. The serum albumin levels show only a slight increase by this time.

In the animals receiving aureomycin along with low protein diet, only one out of three monkeys developed edema. The changes in extracellular water and appearance of ferritin in circulation in this animal are similar to those which occur in monkeys that do not receive aureomycin. There is no increase in extracellular water and no ferritin in the circulation of animals which received aureomycin and do not develop edema. On the other hand, the decrease in body weight and serum albumin is comparable, in all animals which received a low protein diet, irrespective of aureomycin supplementation. The impairment in response to the water load test is much less than that in animals which develop edema.

Effect of low protein diets on the metabolism of ferritin — Ferritin is stored mainly in the liver and spleen of normal animals and is not released into circulation. However, in a number of conditions characterized by edema and oliguria[23] ferritin is detected in blood. Srikantia[24] studied the metabolism of ferritin in rats and monkeys. Rats were maintained on low protein-low fat and low protein-high fat diets for varying periods and blood and liver wash were assayed for ferritin. *In vitro* destruction of ferritin by liver slices of these rats was also determined. Ferritin appeared in the plasma of only those rats whose liver wash contained ferritin. At the end of 16 weeks all the animals fed on low protein-high fat diet show ferritin in plasma and liver wash and the ability of liver to destroy added ferritin is lost. Some animals on this diet develop ascites by this time. These changes do not occur in rats maintained on low protein-low fat diets.

Monkeys develop edema on low protein diets. Biopsy samples from liver and blood samples were collected at the end of 16 weeks, by which time the animals developed edema. Both blood and liver wash contained ferritin at this time.

Hypothesis of Edema Formation

A consideration of the body composition and the mechanism of water excretion in normal subjects becomes pertinent to an understanding of edema development and the hypothesis put forward to explain this phenomenon. The normal body composition and water balance are presented in Charts 1 and 2.

In healthy conditions the composition of the various compartments is well maintained. Fluid exchanges across the cell membrane are determined

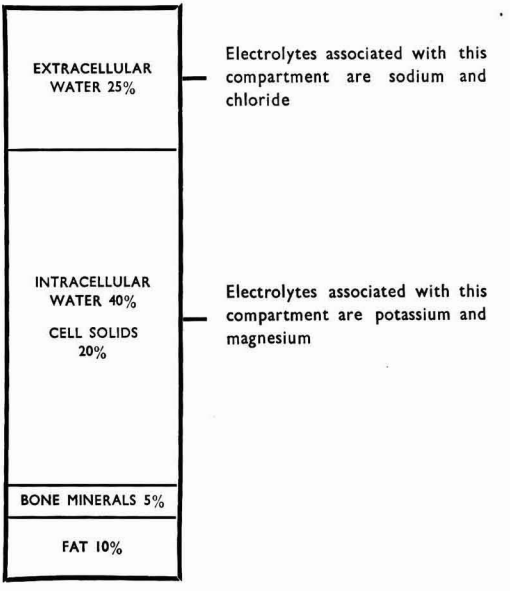

Chart 1 — Normal body composition

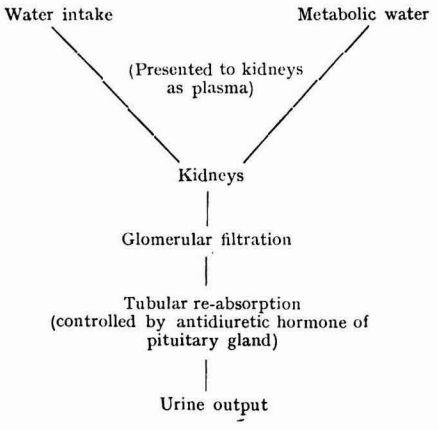

Chart 2 — Normal water balance

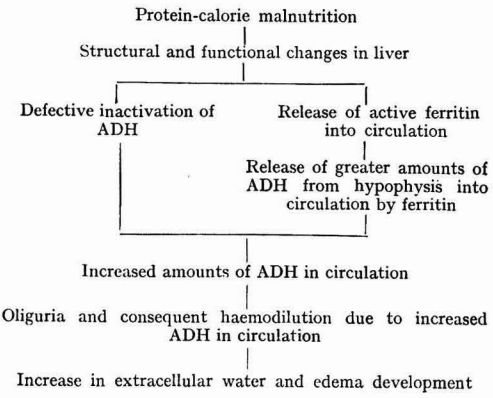

Chart 3 — Edema development in protein-calorie malnutrition

by a number of factors, among which are the colloid osmotic pressure of proteins, the intra- and extracellular distribution of potassium and sodium. These adjustments are mainly accomplished by the kidney through intrarenal mechanisms. These may be influenced by extrarenal factors, of which the role of antidiuretic hormone and the adrenal cortical hormones is well established. Normally, the antidiuretic hormone is inactivated in the liver. The antidiuretic action of ferritin is mediated via the hypophysis which elaborates the ADH in response to the action of ferritin[25].

All these factors have been investigated during the production of experimental edema and in clinical edema and have been discussed in the previous section. Based on these observations, the latest hypothesis put forward by the Nutrition Research Laboratories to explain the development of edema is presented below.

The liver shows both structural and functional changes in kwashiorkor and nutritional edema[6,26,27]. It is significant that marasmus, another clinical condition in children due to protein malnutrition, is not associated with edema and the liver is normal in this condition[28]. The normal renal function, the normal or lowered levels of sodium in serum, and urinary excretion pattern of sodium and potassium exclude the possibility of any of these factors playing a role in the development of edema due to protein-calorie undernutrition. The low serum albumin concentration implicated earlier to explain edema formation is not adequate. There is no correlation between the degree of edema and the extent of decrease in serum albumin concentration in clinical conditions of edema. At the time of disappearance of edema there is no striking increase in the albumin level in serum. In studies on experimental production of edema, the fall in serum albumin has been observed to occur much earlier than any increase in extracellular water and there is no immediate rise in albumin level following edema clearance. On the other hand, ferritin seems to herald the increase in extracellular water observed in experimental production of edema. In monkeys, the response to water load becomes worse and the extracellular water shows an abrupt rise within two weeks following the appearance of ferritin in circulation. The disappearance of ferritin from circulation is the first change to be observed on refeeding the animals with a high protein diet. This is followed by a gradual increase in the response to water load and reduction in the extracellular water content. This would indicate that release of ferritin from the damaged liver played a primary role in the production of edema in protein-calorie malnutrition. This hypothesis of edema development is schematically presented in Chart 3.

Summary

The work carried out in the Nutrition Research Laboratories, Hyderabad, on the edema of protein-calorie malnutrition has been reviewed. The techniques employed in the studies are briefly described, and the results of investigations carried out in clinical conditions of edema and experimentally induced protein deficiency in animals are presented. The latest hypothesis put forward by the investigators at the Nutrition Research Laboratories to explain the development of edema in protein-calorie malnutrition is given. The main steps in the hypothesis are: Structural and functional changes are induced in liver as a result of protein-calorie malnutrition leading to defective inactivation of antidiuretic hormone (ADH) and release of active ferritin into circulation, which is responsible for the release of greater amounts of ADH from hypophysis into circulation. The increased amounts of ADH in circulation induce oliguria and consequent haemodilution, resulting finally in excess amounts of extracellular water and edema development.

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Fifteenth General Assembly of the International Scientific Radio Union

The Fifteenth General Assembly of the International Scientific Radio Union will be held at Munich during 5-15 September 1966. The main topics suggested for the scientific sessions under the various Commissions are as follows:

Commission I — Atomic standards of time and frequency; standard frequency and time signal transmission; form of signals; radio standards and measurements up to and above 1 Gc/s.; and basic standards and physical measurements.

Commission II — Experimental analysis of the atmosphere; models of atmosphere; theoretical and experimental investigation of propagation in non-ionized media; effects of propagation on the measurements of distance, angle of arrival and Doppler effect; propagation below the earth's surface; and propagation and radiometry for millimetre and sub-millimetre wavelengths.

Commission III — The D-region; electron distribution, collision frequency and radio wave absorption; relations between the stratosphere and the ionosphere; F-region morphology; electron distribution in the topside F-region; dynamics of the ionosphere; formation of Es; travelling disturbances — tides; and VLF and ELF natural emission.

Commission IV — Ionization density in the magnetosphere; whistlers; VLF emissions; and micropulsations.

Sub-Commission IVa — Atmospheric noise (review of IQSY results): characteristics of atmospherics at the source, propagation of atmospherics and VLF radio waves and statistical characteristics of atmospherics; whistlers (review of IQSY results); VLF and ELF noise phenomena; and abnormal propagation phenomena due to nuclear explosion.

Commission V — Radio telescopes; high sensitivity devices and receiving methods; extragalactic radioastronomical observations, planetary radio and radar astronomical observations; and radio spectroscopy.

Commission VI — Diffraction and scattering; coding and modulating systems; antennas; linear and non-linear circuits; satellite communication systems; microminiaturization; source free solution in ionized regions; and radiation and scattering in ionized regions.

Commission VII — High sensitivity receiving methods; progress in building blocks of high frequency receivers; practical progress; microminiaturization, optoelectronics and cryogenic coils for high magnetic fields; and new domains: lasers and helicons.

Besides the meetings of the above Commissions, there will be a number of joint sessions on the following subjects (the number of the Commission with which the joint session is to be held is given in parentheses): velocity of light (Comm. I); laser radar measurements (Comm. II); microminiaturization (Comm. VI); Space Radio Research Committee, report on COSPAR assemblies with particular reference to URSI participation, and synchronization of clocks by means of satellites (Comm. I); report of highlights of the Belgrade Symposium on Solar-terrestrial Physics (Comm. III, IV and V); and radiometry in planetary atmosphere (Comm. II).

REVIEWS

and Academic Press Inc. New York)

THIN LAYER CHROMATOGRAPHY: A LABORATORY HANDBOOK edited by Egon Stahl (Springer-Verlag, Berlin), 1965. Pp. xvi+553. Price DM 68

It is remarkable that within the short span of ten years since the description of the method of thin layer chromatography (TLC), this method has received wide recognition and has been extensively used as a tool for rapid separation of micro- and macro-quantities of a variety of compounds. The underlying principle of separation is the same as in column chromatography and, therefore, the thin layer chromatograms are considered as 'open columns'. The book under review is the English edition of the original German monograph. It records the vast knowledge on this technique and the articles have been written by an international group of scientists who have themselves contributed towards the progress of this technique.

The book is divided into two parts — (1) General section and (2) Special section. The former deals with the instrumentation and working procedure for TLC. The historical aspects of the development of TLC are given in the first chapter. Every minute detail of the procedure employed, including information on the commercially available apparatus and adsorbents is given. Special techniques used in TLC like continuous flow and multiple development methods, two-dimensional separations and the documentation of thin layer chromatograms have been described very clearly. Various methods used in the quantitative evaluation of thin layer chromatograms have been objectively reviewed. A chapter solely devoted to isotope techniques demonstrates the importance of TLC in the chemical and biochemical investigations with radioisotopes. A mathematical description of the processes occurring during chromatography is included in the chapter on the theoretical aspects of thin layer chromatography. The theoretical plate concept and the relationship between chromatographic behaviour and chemical structure are also discussed in this chapter. The special section deals with the separation of various classes of compounds and an attempt is made for the analytical classification of material. Thus, the special section begins with the lipids and ends with the hydrophilic compounds. Methods for the separation and estimation of neutral lipids, phospholipids, fatty acids, terpenes, steroids, water-soluble and fat-soluble vitamins and related compounds, synthetic organic materials like dyestuffs, insecticides, antioxidants and other food additives, active constituents of the medicinal plant extracts, amino acids and peptides, nucleic acids and their hydrolysis products, sugars and their derivatives and inorganic ions are described in detail and thus the investigators can directly use these methods. A chapter each on the use of TLC in pharmaceutical analysis and in clinical diagnosis and pharmacology is included. A useful chapter on 157 generally used spray reagents has been included. The book has extensive reference lists to original publications. Research workers in all the laboratories where thin layer chromatography is routinely used for separations and analysis will do well to possess this valuable handbook.

V.C.J., T.R. & P.S.S.

CHEMISTRY AND BIOCHEMISTRY OF PLANT PIGMENTS edited by T. W. Goodwin (Academic Press Inc., New York), 1965. Pp. xiii+583. Price 117*s.* 6*d.*

The book is based on a colloquium on the chemistry of plant pigments held by the Biochemical Society in 1962, which demonstrated the need for publishing the proceedings and for supplementing it by chapters on the chemistry of plant pigments and their analysis. The editor states in the preface that, owing to unforeseen circumstances, it was not possible to include the projected chapter on plant cytochromes. The book consists of four parts: (I) Nature, distribution and biosynthesis; (II) Function; (III) Metabolism in senescent and stored tissue; and (IV) Analytical methods. The parts are understandably unequal in length.

Part I on the chemistry and biosynthesis constitutes the major part of the book because of the wealth of information now available, and similarly because so little is known about pigment changes in senescent and stored tissue. Part III (C. O. Chichester and T. O. M. Nakayama) consists of only 15 pages. In Part I excellent accounts are given of the chlorophylls (A. S. Holt; L. Bogorad), the carotenoids (B. C. L. Weedon; T. W. Goodwin), phycobilins (C. Ó hEocha), phytochrome (W. L. Butler, S. B. Hendricks and H. W. Siegelman), flavonoids (T. Swain; J. B. Harborne; H. Grisebach), and quinones (R. H. Thomson). Weedon's treatment of the chemistry of the carotenoids is especially well written and provides the best short review of their isolation, structure and stereochemistry which has appeared so far. Swain's chapter on the nature and properties of flavonoids, probably because of the pressure to which he is submitted in writing reviews and editing books, does not do justice to the subject. It contains several erroneous formulae: morin (p. 222); peltogynol and distemonanthin (p. 229); artocarpin (p. 231); the erroneous structure XXVI for cyanomaclurin was not suggested by Dean, and the correct structure for cyanomaclurin appeared in *Tetrahedron Letters*, 317 (1963). Betacyanins and betaxanthins find room in this chapter, because they were long assumed to have a flavonoid character!

The three chapters in Part II, function in photosynthesis (C. P. Whittingham), functions of carotenoids other than in photosynthesis (J. H. Burnett), and the physiological functions of phytochrome (S. B. Hendricks and H. A. Borthwick), are particularly valuable because other books on natural colouring matters leave these aspects untouched. The two chapters on phytochromes by Hendricks and his colleagues are in themselves an authoritative monograph on this important group

of plant pigments which control photoperiodism, etiolation and other phenomena familiar to plant physiologists. They are also typical of the entire volume which will be of equal interest to organic chemists, biochemists and botanists.

Part IV deals with only three groups of pigments: chlorophylls (M. Holden), carotenoids (B. H. Davies), and flavonoids (T. Swain), but it is a very useful introduction to methods of isolation, identification, and quantitative estimation.

A few minor errors have been noticed, such as the biflavonyl linkage in Fig. XIII, page 294, and ' glucoses ' for ' glucosidases ' in line 2, page 453.

K.V.

ENGINEERING PHYSICAL METALLURGY by Y. Lakhtin; translated from the Russian (Gordon & Breach Science Publishers, New York), 1965. Pp. 471. Price $ 14.50

This publication, a translation from the Russian text by Nicholas Weinstein, is an addition to many other publications on the same subject, but with a notable difference. Before defining this difference, it should be acknowledged that the publication covers a very wide scope based on the theoretical principles of physical metallurgy and its practical implications in relation to cast iron and plain carbon steels; alloy, tool, special and stainless steels; non-ferrous metals and alloys and light alloys and also the rare metals and their alloys which many other standard texts on physical metallurgy have hitherto mostly omitted. Considered in the background of such a vast scope of subject coverage, the notable difference in this publication and other texts on the subject lie in the former's inclusion of the almost up-to-date and latest references to the current researches on physical metallurgy. It is almost a stupendous task which, it must be conceded, has been accomplished in this publication remarkably well.

The publication would be of significant value to the mechanical engineers engaged in research, design and mechanical engineering industry; to the metallurgists in understanding the theoretical basis of practical and often empirical hypotheses encompassing the physical metallurgy of ferrous and non-ferrous metals and alloys; to the students of metallurgy in having before them a readily assimilable text and, lastly, to the teacher in making his daily task more interesting and indeed lighter. The references to the latest researches in diverse fields of physical metallurgy are refreshing inasmuch as these cover the results of workers in and outside the Soviet Union.

There are 22 chapters contained in the text, followed at the end by the subject index and technical references.

The illustrations and charts are exceedingly clear, the printing neat and invites reading and the general get-up highly attractive. Undoubtedly, the publication will be a highly favoured text for most research and teaching institutions for many years to come and an indispensable addition to the technical libraries the world over, apart from lying on the desk of the teacher and the taught alike.

The author is to be complimented on a very fine exposition of the fundamentals of physical metallurgy and heat treatment of metals and alloys covering ferrous, non-ferrous and light alloys and rare earth group of metals which have come to the forefront in recent years.

The translator has equally done a worthwhile job with his ability to interpret and translate in lucid technical English, what indeed would have been an equally formidable task if he were writing the book itself.

B. R. NIJHAWAN

JET AIRCRAFT POWER SYSTEMS: III, edited by Jack V. Casamassa & Ralph D. Bent (McGraw-Hill Book Co. Inc., New York), 1965. Pp. vii+408

The preface to the third edition states that the book is expected to provide the basic information required by a technician or his instructor to obtain a clear understanding of the construction and operation of jet propulsion engines. In the spectrum of jet propulsion engines in use today, the book deals largely with air-breathing engines, more particularly the turbine engines. The chapters on the ramjet engine and the rocket engine give no more than a few pictures and descriptive information.

The information on the turbine engines is up to date and presented systematically. There are a variety of diagrams and pictures in addition to the description, but it is doubtful if the diagrams are self-explanatory; there is not a close enough coordination between the pictures and the description.

There is hardly any analysis of any aspect of the performance of the units, even though the definitive terms are clearly explained. A chapter on performance under the design and off-design conditions appears to be a real lacuna.

The book has a place in an institutional library, but it is difficult to see even a trade-technician to whom it can be recommended as adequate or necessary.

S. N. B. MURTHI

ULOTRICHALES by K. R. Ramanathan (Indian Council of Agricultural Research, New Delhi), 1964. Pp. ix+188. Price Rs 21.50

This compendium on Ulotrichales, one of the inclusive yet compiled, incorporates all the significant advances in this remarkably interesting but confusing group of algae. It fills the particular need of the Indian phycologists, coming as it does 50 years after Heering's work in 1914.

The topical divisions are well conceived and the facts are marshalled with clarity. Introduction deals with the classification and delimitation of the order and is essentially based on the nature of the thallus; it is a modification of the systems proposed by Fritsch (1935) and Smith (1955). The heterotrichous forms have been relegated to Chaetophorales and the parenchymatous forms to Ulvales. Hence, both these groups are excluded from this volume. The Prasiolaceae has been assigned to Ulvales, because of its multiseriate and parenchymatous thallus and dimorphic diplo-haploidic life history.

The Ulotrichales, as dealt with in this volume, includes two sub-orders, Ulotrichineae and Sphaeropleineae, the former including three families, viz.

Ulotrichaceae, Microsporaceae and Cylindrocapsaceae and the latter including one family, Sphaeropleaceae. Twenty-three species have been described under *Ulothrix*, 5 under *Uronema* and *Cylindrocapsa*, 3 under *Binuclearia* and *Radiofilum*, 13 under *Geminella*, 12 under *Hormidium*, 8 under *Stichococcus*, 11 under *Raphidonema*, 21 under *Microspora* including two doubtful ones and 6 under *Sphaeroplea*. The remaining genera, *Hormidiella*, *Catena*, *Gloeotilopsis*, *Heterotrichopsis*, *Ulotrichopsis*, *Psephonema*, *Microsporopsis* and *Cylindrocapsopsis*, are monotypic.

There are a few omissions which, however, do not in anyway minimize the value of the book. For example, on page 141, the author indicates the paucity of information on the cytology and development of *Cylindrocapsa* species which has, however, been worked out recently by Sarma (1962) in *Cylindrocapsa involuta* and by Chowdhary (1963) in *Cylindrocapsa sytonemoides*. A special chapter on the ecology of this group would have been highly useful.

Genus *Cylindrocapsopsis* has been given a separate identity from *Cylindrocapsa*, on the basis of the stellate chloroplast and the peculiar mode of sexual reproduction in former. The author rightly cautions about the exclusiveness of these characters to *Cylindrocapsopsis*, since very little information is available on the nature of the reproductive cycle in species of *Cylindrocapsa*. Bourrelly's (1961) recent observations on the axial stellate chloroplast in *Cylindrocapsa geminella* var. *minor* supports author's skepticism.

The keys given to distinguish various genera and species are simple and convenient. The illustrations, though grouped in plates, are so inserted as not to cause much inconvenience in referring to them along with the text. The bibliography is quite comprehensive and indication to the Indian works is thoughtful. The printing and get-up of the book are good.

Throughout this book, one is impressed by the clarity of expression and urbanity of style. This book deserves a place on the bookshelves of all scientists and teachers engaged in this field.

G. S. VENKATARAMAN

THE CHEMICAL FOUNDATIONS OF MOLECULAR BIOLOGY by Robert F. Steiner (D. Van Nostrand Co. Inc., New York), 1965. Pp. xii+468. Price $ 12.00

The phrase 'molecular biology' has been in use for nearly a decade and finds somewhat general acceptance. One can choose to wonder about the need for, and the meaning of, the term as the old chemist does in the dialogue in Chargaff's *Essays on nucleic acids*. All the more so, since biochemistry and biophysics scarcely leave any area of biology untouched. Dr Steiner has his own difficulties with the new terminology and has chosen to define molecular biology literally as aspects of biology which can be described at the molecular level. A most liberal definition would be that molecular biology deals with the structural and functional foundations underlying all biological expression.

In *The chemical foundations of molecular biology* the amino acids and proteins, and the nucleotides and nucleic acids receive major attention, and quite properly so. Six chapters (221 pages) cover the amino acids, the structure, size, shape, charge and spatial organization of proteins, the catalytic properties of enzymes, and finally specific structural details relating to eight selected proteins. The use of optical rotatory dispersion, UV absorption in the deep ultraviolet, and infrared spectra in the study of protein structure have not received the mention they merit. More extensive treatment of the chemical structure of proteins in Chapter 3 would have been in order, considering the diversity of methods for structure elucidation, involving degradation and sequence analysis, and the interesting homologies in structure of related proteins, from different species, which yield some clues to evolution in protein structure and function. Appendix A summarizes the methods by which the structure of the B-chain of insulin was elucidated.

The chemistry of nucleotides, the physical, chemical and biological aspects of the structure and function of nucleic acids, viruses and protein biosynthesis are covered in four chapters (136 pages). The treatment brings out modern developments. The RNA code in relation to mutational alteration of bases and observed amino acid exchanges in proteins could have been dealt with in more detail than is given in Chapters 3 and 10. The structure elucidation of DNA and RNA molecules remains even today an inviting but none the less formidable problem. In this context, Chapter 10 could have included a record of the advances made in determining the primary structure of s-RNA (alanine) in 1964, or even as the knowledge was at the end of 1963.

Other parts of Dr Steiner's book cover the biosynthesis of polysaccharides, energy transformations in cells and basic genetic principles. Appendices B-D cover basic thermodynamic concepts, synthesis of polyamino acids, and biological oxidation and reduction.

There are some relatively minor errors in the book. The representation of the structure of hydroxylysine on page 29 (Fig. 2.1) is incorrect. The statements that diiodotyrosine and thyroxine are to be found in several thyroid proteins (p. 37), and that the possibility of peptide bond formation through the side-chain amino and carboxyl groups of proteins is not realized in naturally occurring polypeptides (p. 45) are open to question. Insulin A chain contains 21 amino acid residues, not 20 (pp. 64 and 443). On page 404 (Fig. 12.18) dihydroxyacetonephosphate is labelled triose phosphate. The representation of the primary amide group (pp. 209 and 210) as an amine group, $-C-NH_2$, and the statement (p. 210) "category 2 contains enzymes which catalyse the conversion of primary amino groups to hydroxyl groups" are misleading. Less than a dozen typographical errors were present and noticed.

Omissions and errors apart, the book is lucidly written and this reviewer enjoyed reading it. Students of biochemistry and related disciplines will find this book of value. The book deserves too a place in every library.

L. K. RAMACHANDRAN

THE CONTROL OF FERTILITY by Gregory Pincus (Academic Press Inc., New York), 1965. Pp. xvii +360. Price $ 9.00

It is rightly said that the pioneering researches of Gregory Pincus on steroidal contraceptives have ushered in a new era in the history of mankind, since for the first time the human female has a positive means for emancipating herself from the fetters of repeated childbirth. It is, therefore, in the fitness of things that he should record his deep and sustained experience of research in fertility regulation in the compass of a monograph. This is indeed what he has done primarily, but most judiciously he has done so over a scaffolding of the present-day knowledge on hormonal, biochemical and nervous factors controlling oogenesis, ovulation, ova development and transport implantation, spermatogenesis and sex hormone elaboration in mammals and in human subjects. The chemical agents affecting these specific events in the reproductive processes *vis-à-vis* fertility and their *modus operandi* have been reviewed *in extenso*, with particular reference to steroids. Sections have been also devoted to the immunologic regulation of fertility and intrauterine contraception.

Meticulous attention has been given by the author to indicate the hiatuses in our knowledge on reproductive mechanisms, the questions demanding answers and the areas where further research is urgently needed. The implications of the extensive animal experiments carried out over the past decade to the present-day methods of fertility control have been discussed in detail.

To all those interested in the biological, biochemical and physiological aspects of family planning and population control, this excellent monograph will be invaluable. Dr Pincus deserves our gratitude for this timely presentation.

A.B.K.

RAPID MIXING AND SAMPLING TECHNIQUES IN BIOCHEMISTRY edited by Britton Chance, Rudolf H. Eisenhardt, Quentin H. Gibson & K. Karl Lonberg-Holm (Academic Press Inc., New York), 1964. Pp. xii+400. Price $ 9.00

This book consists of the papers presented at the first international colloquium held in Philadelphia in 1964 under the auspices of the International Union of Biochemistry. The techniques and equipment for rapid mixing and sampling are discussed under two major sections: (i) rapid physical methods for generating non-equilibrium states in a biochemical system and physical measurements of the chemical change, and (ii) rapid methods for stopping a reaction or for withdrawing samples for measuring the extent of the reaction. Though photolysis and sudden temperature change techniques are briefly considered, the first section deals mainly with techniques such as rapid mixing and fluid flow, injection of a sample into a fixed volume, stop flow methods and continuous flow methods. The physical methods used for following the reactions include spectrophotometric and fluorimetric methods and EPR. Among the techniques for rapid stopping and sampling discussed in the second section are liquid-liquid quenching, rapid freezing and rapid removal of samples from a reaction mixture. The study of reactions which take place in very short periods of the order of less than 10 sec., which was initiated by Hartridge and Roughton, has made very rapid progress in recent years and is being extended to periods of the order of milliseconds and even nanoseconds. The application of laser techniques is likely to give a further impetus to the development of this field. These techniques find application in the study of intermediary metabolism, the kinetics and mechanism of action of enzymes and of photosynthesis. The list of participants includes nearly all the leading workers in this field and the standard of the articles and discussions is very high. This volume will be found to be indispensable to research workers and specialists interested in the development or use of rapid reaction technique especially for the study of biochemical problems.

V. JAGANNATHAN

PUBLICATIONS RECEIVED

THE RATIONAL USE OF DYES IN BIOLOGY by Edward Gurr (Leonard Hill Books Ltd, London), 1965. Pp. xii+422. Price 105*s*.

PROCEEDINGS OF THE SUMMER SEMINAR IN MAGNETOHYDRODYNAMICS edited by P. L. Bhatnagar (Department of Applied Mathematics, Indian Institute of Science, Bangalore), 1965. Pp. ix +376. Price Rs 12.50

ENERGETICS IN METALLURGICAL PHENOMENA: Vol. I, edited by William M. Mueller (Gordon & Breach Science Publishers, New York), 1965. Pp. xiv +425. Price $ 19.50 (ref. edition); $ 9.50 (paper back edition)

NON-LINEAR PARTIAL DIFFERENTIAL EQUATIONS IN ENGINEERING: Vol. 18 of Mathematics in Science and Engineering — A Series of Monograph and Text-books, by William F. Ames (Academic Press Inc., New York), 1965. Pp. xii+510. Price $ 16.00

THE USE OF SURFACTANTS IN THE PETROLEUM INDUSTRY; authorized translation from the Russian; edited by P. A. Rebinder (Consultants Bureau Enterprises Inc., New York), 1965. Pp. xviii+346. Price $ 45.00

COSMIC RAYS by D. V. Skobel'tsyn (Consultants Bureau Enterprises Inc., New York), 1965. Pp. 254. Price $ 27.50

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

NOTES & NEWS

A new theory of stimulated Brillouin and Raman scattering

A new theory of the stimulated Raman and Brillouin effect based on the coupled wave concept introduced by J. A. Armstrong *et al.* [Armstrong, J. A., Bloembergen, N., Ducuing, J. & Pershan, P., *Phys. Rev.*, **127** (1962), 1918] has been developed by Y. R. Shen and N. Bloembergen of the Gordon McKay Laboratory, Harvard University, Cambridge, Massachusetts. Several attempts have been made earlier to explain the observed characteristics of the Raman and Brillouin scattered radiations both from the classical and from the quantum mechanical points of view. Although a qualitative explanation of many features of the observed characteristics of the radiations has been given by the earlier theories, there are still a number of important experimental observations which have not received detailed explanation. The deductions from the new theory are found to be in qualitative and in some cases quantitative agreement with most of the experimental results including the directional properties of the Raman radiation. However, it should be emphasized that a number of simplifying assumptions made in the analysis have not been met under actual experimental conditions. According to the coupled wave concept on which the present theory is based, stimulated Raman and Brillouin scattering can be described as due to the interaction of light waves with optical and acoustic-phonon waves respectively. In the formulation of the theory the coupling parameters have been derived both classically and quantum mechanically and the wave equations for the coupled wave problem solved taking into account the appropriate boundary conditions. Saturation effects, generation of higher order Raman radiation and the effect of mode structure in the laser beam have been treated in a more approximate and qualitative fashion. It is pointed out that all the experiments made so far in this field are not sufficiently well defined to provide a test of the theory or to unravel the various physical mechanisms involved in the stimulated Raman effect. A new experimental arrangement which could possibly provide better information about the mechanism involved has also been suggested [*Phys. Rev.*, **137** (1965), A 1787].

A new phenomenon in semimetals and semiconductors

A new phenomenon in the electrical conduction of extremely small ohmic contacts (area $\approx 10^{-10}$ cm.^{2}) between a normal metal and a single crystal of Bi, Sb or semiconducting Bi-Sb alloys, at low temperatures (2-4°K.), has been observed at the IBM Watson Research Centre, New York. While observing the *E-I* characteristic of the ohmic contact, it was found that as the current is increased, at a critical value of the current I_C, the resistance changes abruptly from a lower value R_L to a higher value R_H. It was also observed that this effect had no polarity with respect to bias voltage and that the critical value of the current where the jump in the resistance value takes place as the current is decreased ($I_{C'}$) was less than I_C. Thus a hysteresis loop is formed by a complete cycle of current whose area is dependent on the sweeping speed.

The small contact areas were derived by evaporating a metal through a pinhole in an oxide layer on the cleared surface of a single crystal. The substrate was kept near room temperature to avoid any alloy formation during evaporation and Al, In and Ag were used as the counter electrode. The effect was dependent on the metal used, the temperature and the magnetic field. The plot of dV/dI versus I for different magnetic fields showed multiple peaks at currents near I_C, thus indicating that the current flows through more than one pinhole. It was also found that I_C is linearly dependent on magnetic field and falls to zero at a value H_C and that H_C and I_C will probably fall to zero at some temperature T_C above 4·5°K.

These observations are similar to those observed in the case of superconductors. Since the conditions of observations eliminate the possibility of any other material associated with the contact becoming superconducting and since the calculated ratio of R_H/R_L ($\approx 1{\cdot}5$) agrees closely with the ratio of the spreading resistance for a flat contact to that of a hemispherical contact ($=\pi/2$), it is believed that a small portion of the non-metal under the contact point becomes superconducting. It is proposed that the superconductivity of the semimetal may be due to production of excess carriers at the region of contact [*Phys. Rev. Lett.*, **15** (1965), 152].

Observation of Jahn-Teller tunnelling by acoustic loss

The observation for the first time of certain acoustics characteristics which could be attributed as due to quantum mechanical tunnelling through a potential barrier connected with the Jahn-Teller effect has been made for the first time at the Bell Telephone Laboratories, Murray Hill, New Jersey. The characteristics which could be attributed to the Jahn-Teller tunnelling were observed during investigations of low temperature acoustic loss and velocity change which arise from incorporation of small quantities of Ni^{3+} and Mn^{3+} into single crystals of yttrium aluminium garnet (YALG) and corundum, and of Mn^{3+} into yttrium iron garnet and lithium gallium spirel. The presence of either of these two ions leads to a large acoustic loss and to a reduction in sound velocity which increases rapidly with decreasing temperature. Both ions enter octahedral sites and have unpaired *e* electrons in the ground state, Mn^{3+} being in the weak field ^5E state and Ni^{3+} in the strong field ^2E state. These states are expected to show a strong

Jahn-Teller effect which has been observed by spin resonance for the case of nickel in corundum and YALG. The observed effects have been attributed to relaxation under stress between the possible directions of the Jahn-Teller distortion. At high temperatures, the relaxation tends to the exponential temperature dependence characteristic of thermal activation over a potential barrier. However, at low temperatures, the relaxation time becomes independent of temperature. The only possible explanation for this observation is that there is tunnelling through the potential barrier [*Phys. Rev. Lett.*, **15** (1965), 19].

High resolution images by the 'photocharge process'

A new light sensitive plastic film that produces high resolution images has been developed at General Electric's advanced technology laboratories. This film contains a photosensitive compound — an organic chemical dissolved in a polymer. The new technique is called 'photocharge process' and uses a photoelectric potential generated in the film when exposed to light to produce images. Unlike the conventional electrophotographic process, photocharge requires no external electrical charges or fields. It is expected that the process may find its early applications in microimaging memories for graphic and pictorial information and in high resolution inexpensive reproduction.

When thermoplastic photovoltaic materials are cast as a thin film on a rigid or flexible substrate, they can be selectively illuminated by a light image. When the films are heated to their flow point and cooled quickly, deformations result which correspond to the illuminated areas of the film. The other advantage in using the new film is that it needs no chemical developer, only light and heat producing a completely developed picture. The images produced by the film can be seen with the naked eye by reflected light. They can also be projected as bright continuous-tone black and white images through refractive or diffractive optics.

It seems that the mechanism by which these films operate entails the photogeneration of either positive ions and electrons, or negative ions and holes. As both the charge carriers begin to diffuse, driven by concentration-gradient, mobility differences between bulky ions and the much lighter electrons or holes result in charge separation, analogous to the bulk photovoltaic effect. Since the film material is a good insulator, the flow of electrons or holes gives rise to space charge which impedes further penetration. Thus, these charges act as a uniform layer below the film's surface producing an internal field in the medium which is primarily a function of the average separation distance and the number of charge carriers. When the film is temporarily molten, electrostatic pressure due to the film deforms the film. On extensive heating, the charge carriers recombine because of coulombic attraction and the enhanced mobility of the liquid state, and the film can be used again.

The resolving power obtained by using photocharge process is such that two lines less than 1/10,000 in. apart are readily distinguished. Twenty-five re-uses have been obtained with a single film with no apparent degradation of image quality or film sensitivity [*Chem. Engng News*, **43** (24) (1965), 48].

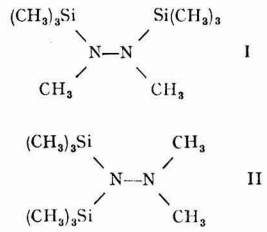
Regeneration of X-ray damaged cells by DNA

Dr Mathilde Krim and coworkers at Sloan-Kettering Institute for Cancer Research (New York City) have demonstrated that the radiation damage caused to cells by X-rays can be repaired by subsequent administration of DNA to the cells. Loose monolayers of Chinese hamster cells grown on cover slides were irradiated with X-ray doses of 10-800 roentgens and divided into two groups, one treated with human leucocyte DNA at a concentration of *c.* 200 mg. per ml. and the other untreated group was for comparison. Cytological examination of these samples revealed that the extraneous DNA, when administered for as little as 30 min., significantly assisted in the spontaneous repair of radiation damage to chromosomes. Also, DNA treatment reduced the number of cells with chromosome abnormalities. Dr Krim explains that the breaks visible in chromosomes at the metaphase of mitosis undergo spontaneous repair by joining of the broken ends and if the broken ends are joined correctly the damage is no longer demonstrable cytologically. It was also found that half the non-treated cells were killed with 200 to 300 roentgens depending on the handling of cells before and after irradiation. But when DNA was added to the cells after irradiation the dose needed was as high as 400 roentgens to kill 50 per cent of the cells. The regenerative effect of DNA is different from the preventive action exerted by non-cellular agents like cysteine and cysteamine. DNA is non-toxic and heterologous preparations can be used.

The importance of the above work is that it is the first demonstration of the beneficial effect of DNA on the regeneration of chromosomes in cells on irradiation with X-rays [*Chem. Engng News*, **43** (17) (1965), 40].

Unusual rearrangement in organosilylhydrazines

The facile migration of silicon from one hydrazine nitrogen atom to another in organosilylhydrazines, observed accidentally for the first time by workers at the University of Wisconsin, Madison, has led to the conclusion that structures of some organosilylhydrazines will have to be reconsidered, since structures in the past have been assigned without considering the possibility of rearrangement. In an attempt to synthesize 1,2-bis-(trimethylsilyl)-dimethylhydrazine from 1,2-bis-(trimethylsilyl)-hydrazine it was found that on adding two equivalents of *n*-butyl lithium, followed by two equivalents of methyl iodide, a 60 per cent yield of pure, colourless 1,2-bis-(trimethylsilyl)-dimethylhydrazine formed. However, the NMR spectrum of this product showed four peaks instead of the expected two. Two peaks appeared in the Si-C-H region (9·98 and 9·89 τ) and two peaks in the methyl region (7·43 and 7·57 τ) and the relative peak areas were 3 : 3 : 1 : 1 respectively. Thus, it was concluded that the product contains



about equal amounts of two isomers (I) and (II) which were separated by gas chromatography. Presence of rearranged isomer was confirmed by first solvolysing the isomers with 1-propanol and then identifying the two respective dimethylhydrazines. Similarly, rearrangement in 1,2-bis-(trimethylsilyl)-methylhydrazine has also been studied.

Studies carried out using various amounts of base shed some light on the mechanism of the rearrangement. The first step is a rapid proton transfer to the base generating the hydrazine anion which then rearranges rapidly if one equivalent of alkyl lithium is used, rearrangement is immediate. With smaller amounts rearrangement is immediate up to the amount of base added and slow after that, suggesting that the first step is rapid proton transfer to the base generating hydrazine anion which then rearranges rapidly.

Other bases which catalyse the rearrangement of 1,2-bis-(trimethylsilyl)-dimethylhydrazine include phenyl lithium, Grignard reagents, triphenylmethyl sodium and the sodium radical anions of naphthalene and anthracene [*Chem. Engng News*, **43** (15) (1965), 46].

Cobaloximes

A number of cobalt complexes, cobaloximes, have been prepared and found to have chemical properties remarkably similar to those of vitamin B_{12}. The compounds are prepared by reacting bis (dimethylglyoxime) complexes of cobalt (III) with Grignard reagents such as phenylmagnesium bromide, methylmagnesium bromide, etc. The cobaloximes can be reduced to substances analogous to vitamin B_{12r} and vitamin B_{12s}. These reduction products can be used to synthesize many new organometallic compounds of cobalt in aqueous solution. The essential species present in solution of both vitamin B_{12s} and cobaloximes are anions in which the spin paired cobalt (I) ion is probably five coordinated. This causes an increase of the antibonding character of the highest filled orbital which is responsible for the strong nucleophilic character of cobalt. The characteristic colour of the reduced species stems largely from the low energy *d-d* transitions. The reactions of both B_{12s} and cobaloximes with alkylating agents are nucleophilic displacement reactions.

Most organo cobaloximes are remarkably insensitive to oxygen but decompose easily in light, a behaviour also exhibited by B_{12} derivatives. Photolysis of cobalt-carbon bonds producing alkyl radicals has been used to alkylate thiol groups. This reaction is analogous to the stoichiometric formation of methionine from homocysteine in which methylcobalamine is the source of methyl group [*Chem. Engng News*, **43** (37) (1965), 61].

N-Acetylpyrrole

Acetylation of pyrrole in the 1-position has been a difficult job because of the formation of other products, viz. 2- and 3-acetylpyrroles. Also the methods employed are tedious and the yields low. A relatively simple method with yields as much as 90 per cent has been developed. In the present method, equimolar amounts of freshly distilled pyrrole and freshly distilled N-acetylimidazole are heated under reflux at atmospheric pressure. The acetyl group transfers from imidazole to pyrrole, of course without the use of any catalyst. N-Acetylimidazole is comparatively easier substance to get into the pure state. The progress of the reaction is followed by NMR spectroscopy. It is found that after heating under reflux for 90 min., the N-acetylpyrrole content of the mixture is 90 per cent and does not change appreciably on continued heating [*Chem. & Ind.*, (1965), 1426].

Chemistry of trimethylsilyl radical

Studies on the chemistry of trimethylsilyl radical did not make any headway earlier due to the non-availability of the source. It has been found that bis (trimethylsilyl) mercury, when heated or exposed to light, decomposes to form trimethylsilyl radicals. Bis (trimethylsilyl) mercury, formed by treating mercury amalgam with trimethylsilyl chloride, is a yellow solid melting at 102-4°, without decomposition, to an orange liquid. The compound decomposes thermally in cyclohexane with a half-life of about 3 days at 190°C. In the presence of light it decomposes rapidly in cyclohexane, but slowly in aromatic solvents.

When bis (trimethylsilyl) mercury decomposes in cyclohexane it produces mercury and trimethylsilyl radicals which combine to give hexamethyldisilane with traces of trimethylsilane. Heating the compound in toluene, benzene or chlorobenzene gives a number of products for the formation of which the presence of trimethylsilyl radical is essential.

One of the most interesting reactions of trimethylsilyl radical is the displacement of trichlorosilyl radicals from the aromatic ring.

$$PhSiCl_3 + Me_3Si. \longrightarrow PhSiMe_3 + Cl_3Si.$$

The above reaction also occurs in the gas phase at 500°C. [*Chem. Engng News*, **43** (37) (1965), 60].

A new method for the reduction of aromatic nitro compound

The only methods hitherto available for the reduction of nitrobenzene and its alkyl derivatives in alkaline medium are that of Bechamp and the catalytic hydrogenation. A new method based on the reducing power of a mixture of sulphur and sodium hydroxide in acetone-methanol mixture has been developed. In a typical case sodium hydroxide (3 g.), sulphur (1·5 g), acetone (10 ml.), methanol (10 ml.) and nitro compound (0·0248 mole) are heated for 1·5 hr on a water-bath. The amine formed is removed by either of the following methods: (a) distilling the solvent followed by removal of the amine by steam distillation; (b) extraction of the amine with benzene; and (c) extraction with benzene followed by further extraction with dilute acid. The yield of the amine is 75 per cent if the

quantity of sodium hydroxide taken is double that of sulphur. Heating beyond 1·5 hr does not improve the yield [*Chem. & Ind.*, (1965), 1496].

New microchemical techniques

The problems which confront a microchemist are accurate weighing, achieving complete combustion and making qualitative and quantitative analyses of reaction products. Recently, some new approaches in simplifying the above problems have been developed.

Among the new approaches is a technique of weighing hygroscopic materials in the submicro scale. A dry sample atmosphere is provided by a small membrane-type pump to drive air through a 4 m. long section of polyvinyl chloride tubing. The air stream passes through an activated silica gel packing and then through a grid plate supporting a platinum weighing boat. The hygroscopic sample is introduced into the boat in a counter current flow of dry air and the boat is then transferred to the balance. A silica gel insert around the balance pan provides a dry atmosphere for weighing.

In the case of polymer analyses a new combustion furnace has been designed for the pyrolysis of polymers in the sample injector of a gas chromatograph. A moving wire, a hydrogen flame and a flame detector are the main components of the system. Sample drops (μl.) fall and attach themselves to the moving wire which carries the sample into a hydrogen flame where the solvent burns. A flame detector connected with programmed-temperature chromatographic columns can then be used to determine both type and amount of polymers in solution. A modified Wickbold combustion furnace has been devised for sulphur analysis in p.p.m. Instead of aspirating the sample the modified furnace eliminates the vacuum pump by using a sample reservoir and pressurizing the sample into the burner with carbon dioxide. Higher temperature employed favours the thermodynamic stability of SO_2 and minimizes formation of SO_3.

A microchemical technique, injection enthalpimetry, has been developed for titration reactions that require an excess of titrant. It involves rapid injection of 20 μl. of the appropriate concentrated reagent and measurement of the temperature change as a temperature pulse. The heat evolved depends on the number of moles reacted and is thus proportional to sample size [*Chem. Engng News*, **43** (37) (1965), 64].

Enzymic synthesis of the methyl group of methionine

The importance of N_5,N_{10}-methylenetetrahydrofolate reductase in the biosynthesis of N_5-methyltetrahydrofolate and consequently of the methyl group of methionine is well established [Friedkin, M., *Annu. Rev. Biochem.*, **32** (1963), 185]. Evidence also exists to indicate that this enzyme is a flavoprotein, catalysing the reduction of N_5,N_{10}-methylenetetrahydrofolate as shown below.

$$N_5,N_{10}\text{-Methylenetetrahydrofolate} + FADH_2 \rightarrow N_5\text{-methyltetrahydrofolate} + FAD$$

The methyl group of methionine is derived from N_5-methyltetrahydrofolate; however, the reaction by which reduced flavinadenine dinucleotide (FAD) is generated for methylenetetrahydrofolate reduction has not been clarified. It has been suggested that methylenetetrahydrofolate reduction directly catalyses the oxidation of NADH during the reduction of methylenetetrahydrofolate [Cathou, R. E. & Buchanan, J. M., *J. biol. Chem.*, **238** (1963), 1746].

Recent studies of H. M. Katazen and J. M. Buchanan [*J. biol. Chem.*, **240** (1965), 836] are in extension of the earlier work. The enzyme N_5,N_{10}-methylenetetrahydrofolate reductase has been purified 100-fold and the purified enzyme did not utilize NADH to reduce N_5,N_{10}-methylenetetrahydrofolate. They have also shown that the utilization of NADH in this reaction required FAD and also the participation of another enzyme, flavin reductase. This second enzyme is for generating $FADH_2$ necessary for the reduction of methylenetetrahydrofolate.

$$NADH + H^+ + FAD \rightarrow FADH_2 + NAD$$

They have further demonstrated that the synthesis of the enzyme methylenetetrahydrofolate reductase in bacteria is controlled by methionine, an end product type of repression mechanism. Enzyme synthesis is repressed at high concentrations of either methionine or vitamin B_{12}. Evidence has been presented to indicate that B_{12} functions by increasing the production of methionine.

In the second paper the authors report the transfer of methyl group from the methyl donors to homocysteine to form methionine. In view of the earlier report of J. Bremer and D. M. Greenberg [*Biochim. biophys. Acta*, **46** (1961), 217] that a number of sulphydryl compounds can be methylated enzymatically by *S*-adenosylmethionine, these authors have studied the role of the B_{12}-containing enzyme in the transmethylation of 2-mercaptoethanol from *S*-adenosylmethionine and N_5-methyltetrahydrofolate. They isolated *S*-methylmercaptoethanol as the product of transmethylation from either donor. However, homocysteine was a better substrate when both homocysteine and mercaptoethanol were present in equivalent concentrations. Moreover, with this transmethylase system large variations in the concentrations of one methyl donor does not lower the contribution of the other methyl donor in the formation of thiol methyl products. From these experiments the authors suggest that the methyl groups derived from *S*-methyltetrahydrofolate and *S*-adenosylmethionine do not converge metabolically, i.e. they donate their methyl groups independently. These observations, therefore, exclude the possible involvement of adenosylhomocysteine in transferring methyl group of 5-methyltetrahydrofolate to other acceptors as suggested by M. A. Foster, M. J. Dilworth and D. D. Woods [*Nature, Lond.*, **201** (1964), 39].—K. SUBBARAO

Tryptophan synthetase A protein

The A protein subunit of *Esch. coli* tryptophan synthetase catalyses the reversible reaction of indole glyceraldehyde phosphate to indole and 3-phosphoglyceraldehyde. This enzyme consisting of a single polypeptide chain has been

purified. Recently, extensive genetic and biochemical studies have been carried out with this protein to determine the specific relationship between mutation in the A gene, the structural gene for the A protein and the subsequent structure and function of the A protein.

Mutation studies with the A gene-A protein system have led to the isolation and characterization of a large number of mutants incapable of forming a functional protein. Of the 60 mutants produced by ultraviolet light, the mutationally altered sites were found to be at restricted locations in the A gene. For example, there were 15 recurrences of A-23 mutations, 7 recurrences of A-46 mutations, 14 mutations at A-11 site, 16 mutations at A-34 site, 4 mutations at A-11 site and 1 mutation at A-169 site [Yanofsky, C., Carlton, B. C., Guest, J. R., Helniski, D. R. & Henning, V., *Proc. nat. Acad. Sci., Wash.*, **51** (1964), 266]. These findings support the idea that either these sites are preferred sites of mutation by ultraviolet light or changes at only certain positions will permit the recovery of an altered, detectable A protein.

To obtain evidence in support of the concept of collinearity between gene structure and amino acid replacements, J. R. Guest and C. Yanofsky [*J. biol. Chem.*, **240** (1965), 679] have studied the mutationally induced amino acid substitutions in the A protein produced by 5 mutant strains A-58, A-78, A-90, A-94 and A-169. The altered proteins were immunologically similar. But altered proteins differed from normal A protein in heat stability, solubility at low *p*H and electrophoretic mobility [Henning, U. & Yanofsky, C., *J. mol. Biol.*, **6** (1963), 16]. Some of the altered A proteins also exhibited characteristic peptide differences in trypsin, chymotrypsin and trypsin+chymotrypsin peptide patterns when compared to the normal protein [Helniski, D. R. & Yanofsky, C., *Proc. nat. Acad. Sci., Wash.*, **48** (1962), 173, 183]. However, with the altered A proteins produced by mutants A-58, A-78, A-90, A-94 and A-169 no peptide differences could be detected except in the case of trypsin+chymotrypsin pattern of strain A-169. Based on the amino acid changes associated with mutations in the tryptophan synthetase A gene, the above 5 mutants were divided into 3 categories. These changes were traced to the adjacent residues 13 and 14 of octadecapeptide. Sequence analysis established that two of the substitutions affected the glycine residue at position 13 and the third involved the serine at position 14. The conversion of glycine to cysteine in strain A-78 was not observed in earlier studies on the mutation alterations of protein structure. Three strains, A-58, A-90 and A-94, possessed the alteration, glycine to aspartic acid. The third amino acid substitution was characteristic of A-169 and involved the replacement of serine by leucine. This change in A-169 mutant generates a new chymotrypsin sensitive bond in the otherwise chymotryptic-resistant core. Also, a new chymotryptic-tryptic peptide overlapping TP 6 and TP 3 was found.

Genetic mapping studies with the above 5 strains showed that their sites of mutation were very closely linked with the A gene. The A-169 mutation clearly occurred in the coding unit adjacent and distal to the coding unit affected in the other 4 mutations. The recombination data suggested that the alterations in A-58, A-90 and A-94 were produced by identical mutational events and that A-78 strain arose by a mutation at another nucleotide in the same coding units. The linear relationship between the amino acid substitution and recombination distances between the corresponding loci on the genetic map unequivocally establish the collinearity of the gene structure and protein structure for the segment of the A protein represented by two tryptic peptides, TP 3 and TP 6. The results obtained also favour the 'degeneracy' of the genetic code.

Mutation studies have also been carried out with a revertant strain A-46, PR 9, in an effort to determine whether the amino acid difference characteristic of the revertant protein would influence the position of subsequent amino acid changes that would give inactive A proteins [Carlton, B. C. & Yanofsky, C., *J. biol. Chem.*, **240** (1965), 690]. In each of the mutants examined only a single amino acid difference was detected between the mutant protein and the parental protein. Although it is likely that each of the single amino acid changes is responsible for the inactivity of the A protein, sufficient comparisons were by no means performed to rule out other amino acid changes. The primary structure of the two of the revertant mutants and their genetic mapping again provided additional evidence for the collinearity of A gene and A protein.

Primary structure studies give some information on critical amino acid sequences required for maximum activity of the enzyme. But better information can be obtained from a detailed knowledge of proteins of the primary structure of the wild type enzyme which are required for substrate binding and turnover. Such information will eventually suggest explanations for the absence or presence of activity in the mutant and reverted A proteins. The evidence obtained from studies on chemical modification of wild type enzyme in the presence and absence of the substrate strongly indicated the requirement for one or more of the three cysteine residues as free sulphydryls in the catalytic activity of the A protein [Hardman, J. K. & Yanofsky, C., *J. biol. Chem.*, **240** (1965), 725]. This observation was based on results obtained with sulphydryl binding reagents and photo-oxidation experiments. However, it is difficult with the existing data to relate enzyme inactivation unequivocally to the oxidation of each amino acid residue. The relative rate of cysteine oxidation and enzyme inactivation was similar to enzyme inactivation by iodoacetate, suggesting photo-oxidation of two cysteine residues to be responsible for enzyme inactivation. However, the possibility that activity loss is a result of all the three types (cysteine, methionine and histidine) or any two types of amino acid residues, each contributing to a degree of activity cannot be ruled out completely. Corroborative evidence is, however, available for the possible role of histidine in substrate binding.

In view of the probable flexibility of the enzyme structure in

solution and the possibility that low molecular weight compounds such as an enzyme substrate alter the conformation of the molecule, it is rather difficult to define clearly an unambiguous role for a particular amino acid residue in which modification results in the loss of enzyme activity. Of the amino acid residues believed to be essential or involved in some manner in the catalytic activity or substrate binding properties of the A protein (i.e. cysteine, histidine and methionine) none has been found to be altered in enzymatically inactive mutationally altered A proteins. It is rather likely that the amino acid substitution found in defective proteins is important for a proper functioning of the normal proteins. However, a further substantiation is necessary to prove this.— T. S. ANANTHASAMY

Progress Reports

Radio Physics Research in Australia

Research activities and developments concerned with radioastronomy and cloud physics form the main content of the 1963-64 annual report of the Radio Physics Laboratory, CSIRO, Sydney.

The principal instrument in the study of radioastronomy is the 210 ft radio telescope commissioned in 1961 at the Australian National Radio Astronomy Observatory at Parkes, NSW, which is operated as a field station from the headquarters of the Radio Physics Laboratory. The chief features of the telescope are: (i) it can be directed with precision over a large part of the visible sky and (ii) it can receive radiation of any wavelength (from a few centimetres to several metres) and polarization.

To increase the resolving power of the 210 ft radio telescope, the laboratory's 60 ft telescope is combined with it to design an interferometer. This interferometer when completed will have a resolving power of 1′ at 20 cm. or 30″ at 10 cm. wavelengths as against the 1° of the 210 ft telescope used alone.

The 210 ft telescope is also used to find accurately the radio positions by means of occultation or eclipses of sources by the moon, by measuring the time of disappearance and reappearance from behind the moon. The nature of the magnetic fields in our own galaxy is also being studied by measuring the polarization and Faraday rotation of the emissions from the radio sources. Studies of different types of radio emissions from the Jupiter, finding the radio sources of our own galaxy, and survey of the galactic plane are the other investigations that are carried out with the telescope.

The observations on the hydrogen line profiles from the Small Magellanic Cloud when combined with the optical data obtained by employing the 210 ft radio telescope showed that part at least of Small Magellanic Cloud consists of two separate masses of gas and stars and that these are moving rapidly apart at a velocity (in the line of sight) of about 35 km./sec.

By means of the 210 ft telescope, information on the red shift, hydrogen content and dynamics of the irregular galaxies NGC 55 and 3019 and the spirals NGC 300 and 5236 has been obtained. These galaxies were found to be having masses of the order of 10^{10} solar masses, 5-10 per cent of the matter being made up of interstellar hydrogen, suggesting that they are in their early stages of evolution. Observations are being made of galaxies of other types, such as NGC 1313, 6744, 6822, 7793 and IC 1613.

Current research of the solar group of the laboratory is directed towards an understanding of the basic flare phenomenon and its geophysical and ionospheric accompaniments as well as the physical processes underlying the generation of solar emission. To aid this programme a new frequency (200-2000 Mc/s.) spectrograph has been designed and operated. By this instrument dynamic spectroscopy continuous in frequency from 5 Mc/s. has been achieved. Another new important instrument, the radioheliograph, is being designed to obtain extended and refined observational data at the metre wavelength, a region which cannot be studied by means of the available simple interferometers. The radioheliograph gives images of the sun in its radio emission at 80 Mc/s. The images will cover a field 2° in diam. and show the details of the sun's radio features with a resolution of about 3·5′.

In the field of cloud physics, detailed studies are being made of the nature and origin of the nuclei of condensation and ice formation whose concentration in the atmosphere plays an important role in the growth of cloud and the release of precipitation. With the aid of theoretical models as well as field investigations employing aircraft, the dynamics of convection have been studied. Conclusions from experiments conducted on rain-making, particularly by injecting silver iodide acting as artificial freezing nuclei, have been reported. A new series of area cloud seeding experiments with improved design are being initiated.

Nobel Prize Awards, 1965

The Royal Swedish Academy has awarded the 1965 Nobel Prizes for physics jointly to Prof. Richard Feynman (California Institute of Technology), Prof. Julian Schwinger (Harvard University) and Prof. Shino-ichiro Tomonaga (Tokyo Kyoiku University) for their distinguished contributions in the development of quantum electrodynamics. The three physicists, working independently, transformed the ' exchange play ' — a theory dealing with interactions between elementary particles — into mathematical form.

The Nobel Prize for chemistry has been awarded to Dr R. B. Woodward (Harvard University) for his meritorious contributions to the ' art ' of organic synthesis.

The Nobel Prize for medicine and physiology has been jointly awarded to French scientists André Lwoff, François Jacob and Jacques Monod, all working at the Pasteur Institute, for their researches into the mechanisms by which genes regulate vital biochemical processes.

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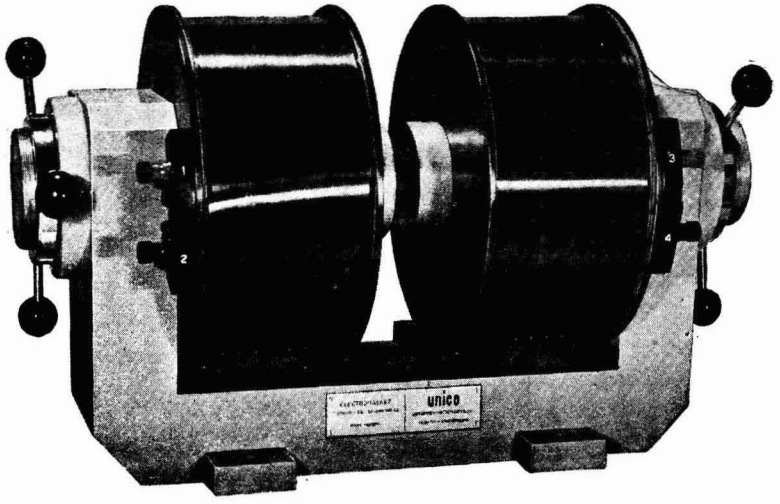

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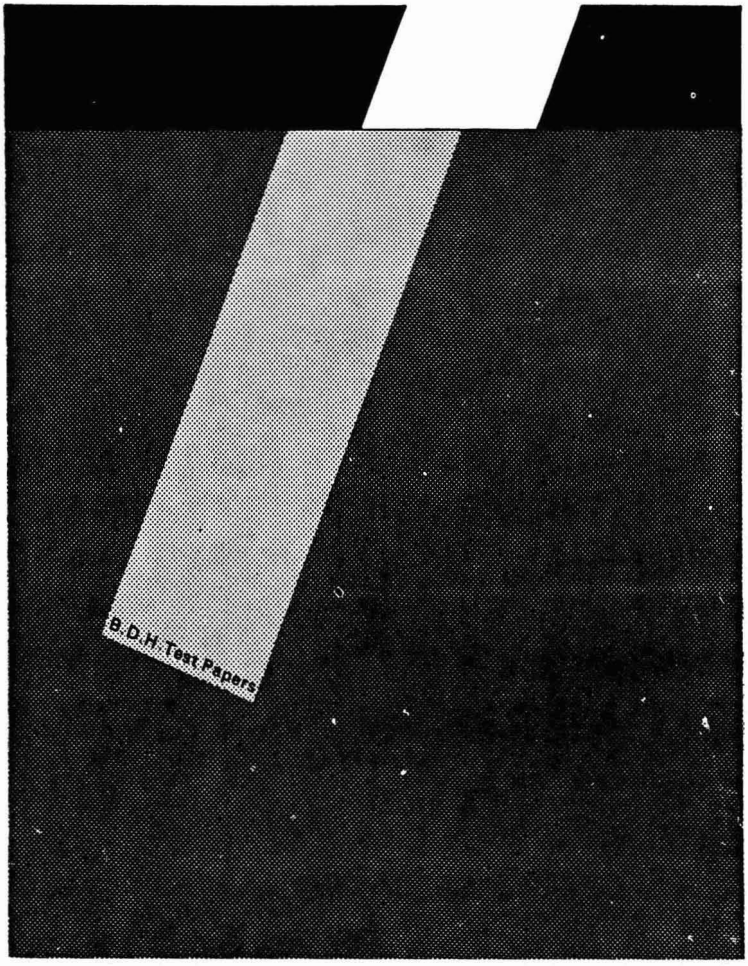

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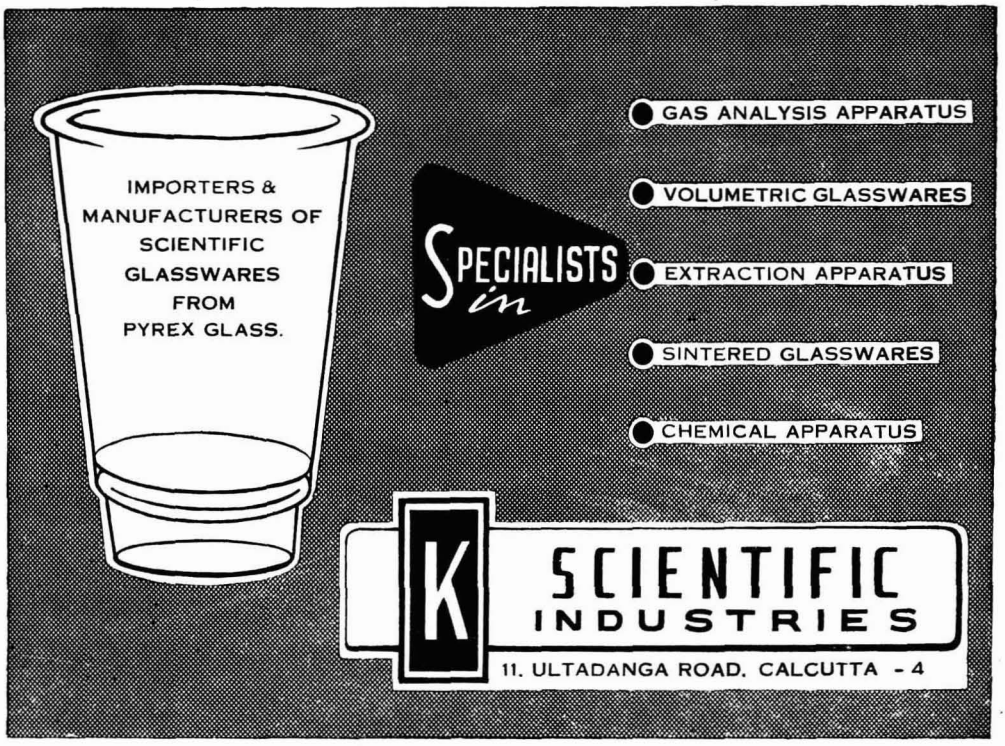

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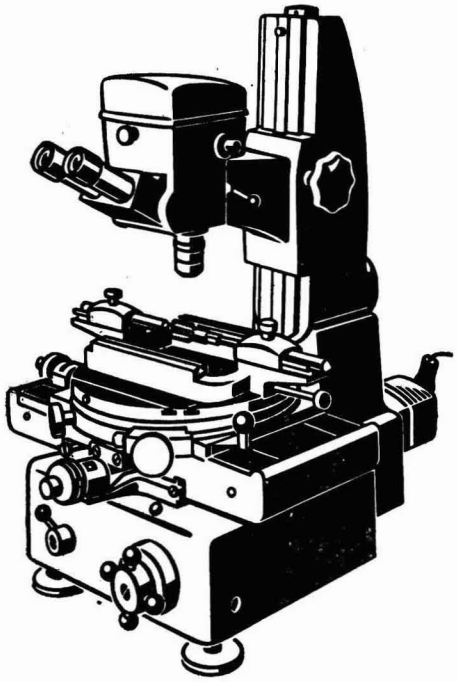

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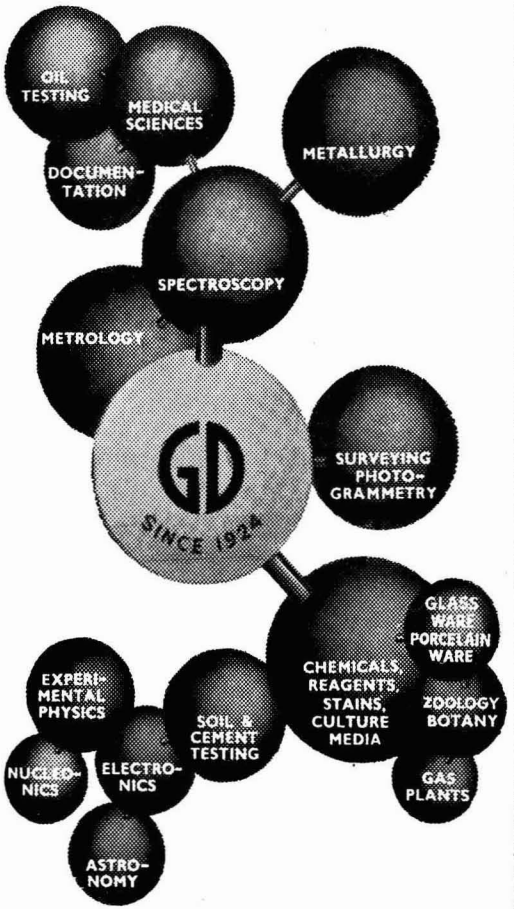

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