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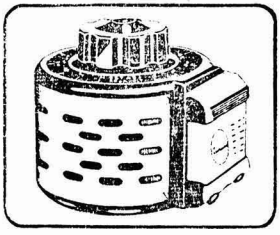
CONTENTS

For Index to Advertisers, see page A21

Current and Voltage Regulators



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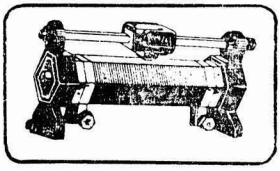

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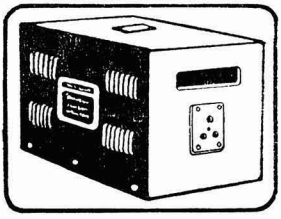

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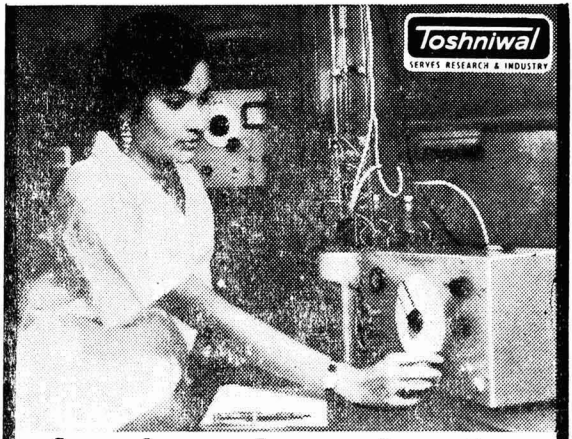

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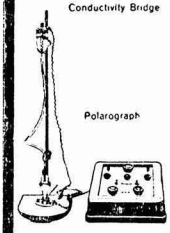

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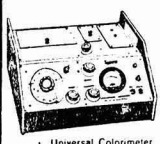

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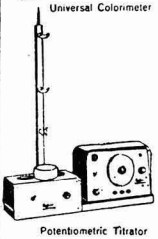

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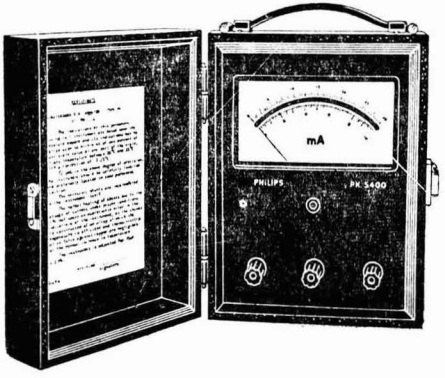

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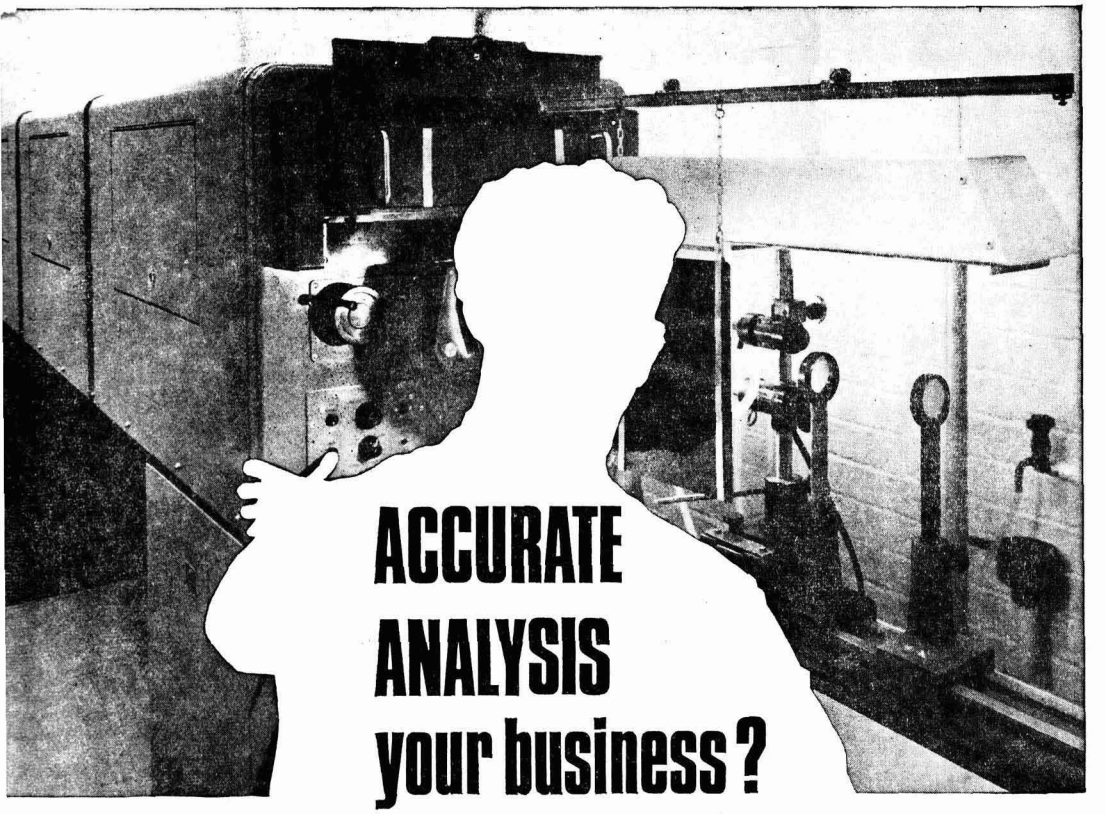

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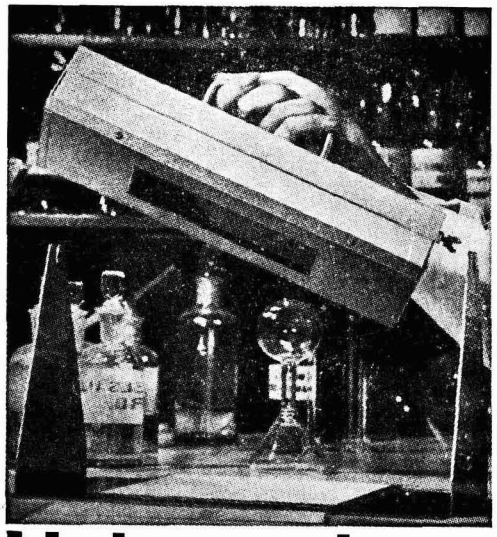

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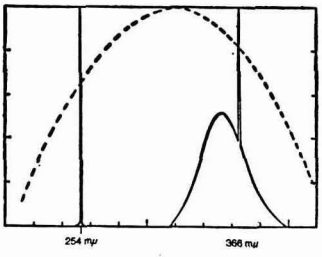

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Current Topics

International Cooperation in Running Abstracting Services

THE past few decades are marked by a steep rise in the rate of generation of new scientific and technical information which finds its way into and gets scattered over a large and increasing number of primary and other scientific periodicals. To keep himself abreast of the developments in his field of interest, the research worker, of necessity, depends largely on the abstracting periodicals as sources of organized information. Till such time as a more effective means of dissemination of scientific information is evolved, abstracting periodicals will continue to be the major avenues of disseminating nascent information. How efficiently and effectively the abstracting periodicals discharge their responsibilities is a matter of vital concern for the advance of science and technology, and calls for cooperation at international level. The problem has received the attention of the International Council of Scientific Unions (ICSU) and Unesco, who set up, some time ago, a joint committee to examine the feasibility of establishing a world system for scientific information. An important step taken recently by the ICSU Abstracting Board was to conduct a survey on the working of some major abstracting services in the fields of physics, chemistry and biology. The information, obtained through a comprehensive questionnaire, has been made available in the form of a report*.

The abstracting services covered under the survey were: Physics Abstracts, Physikalische Berichte, Bulletin Signaletique and Referativniy Zhurnal in the field of physics; Chemical Abstracts, Chemisches Zentralblatt, Bulletin Signaletique and Referativniy in the field of chemistry; and Biological Abstracts, Bulletin Signaletique and Referativniy Zhurnal in the field of biology. The aspects covered in the questionnaire were: general policy of abstracting; use of page proofs, use of authors' abstracts, indexes (classification used); general features (budget, staff, etc.); and use of computers.

The survey, while providing an insight into the working of the abstracting services covered, has served to highlight some of the important issues in the field of communication of scientific information. A significant conclusion arrived at from the trends in the coverage by the various abstracting services is the lack of precise and accurate information in the realm of primary literature. The major impediment for exhaustive coverage by abstracting periodicals in a particular discipline is the existence of inter-disciplinary and multi-disciplinary periodicals. The searching out of the last 5-10 per cent of material scattered over such periodicals is so time-consuming and expensive that all abstracting periodicals do not attempt it.

An important criterion determining the effectiveness of an abstracting service is the time lag between the date of issue of the original paper and the date of issue of the corresponding abstract. The time lag for the services covered in the survey varies from 3 to 8 months, with an average of 5 months. The findings of an independent survey conducted by the United States Atomic Energy Commission restricted to Physics Abstracts and Nuclear Science Abstracts [*Nature, Lond.*, **216** (1967), 737] show almost the same trend with regard to the lag period. The efforts of the abstracting services to bring down the gap to the theoretical minimum (3 months) can succeed only through the cooperation of primary periodicals. The positive steps indicated in this direction include: (i) obligatory provision of authors' abstracts for all original communications, including short communications, letters to the editor, etc. (at present only 60 per cent of the communications carry authors' abstracts); (ii) improvement of the quality of authors' abstracts (at present only 20 per cent of authors' abstracts are in a form directly usable by abstracting periodicals); (iii) provision of authors' abstracts in several languages (this is necessary to check the tendency of some abstracting services to scrutinize mainly the primary periodicals from their own country of origin); and (iv) supply of advance copies of abstracts and contents pages, and/or page proofs by primary periodicals to the major abstracting agencies.

The identification of the main problems coming into play in the speedy and effective dissemination of scientific information through the survey is indeed very timely. The report rightly stresses the need for an international policy under which it is made obligatory for the publishers of primary periodicals to provide page proofs, authors' abstracts and such other materials as are necessary for reducing the time lag and ensuring comprehensive coverage. It is a matter of satisfaction in this connection, that for over a decade, advance abstracts and contents pages of the primary periodicals brought out by the Council of Scientific & Industrial Research, India, are being regularly supplied by air mail to the main abstracting services in different countries and this service has contributed in no small measure in cutting down the gap between the publication of the periodicals and the noticing of the abstracts in the abstracting periodicals. A beginning in the supply of page proofs has been made recently. At present page proofs of the *Indian Journal of Biochemistry* and *Indian*

**Compared activities of the main abstracting and indexing services covering physics, chemistry and biology during the year* 1965 (Abstracting Board, International Council of Scientific Unions, Paris), 1967. Pp. 84.

Journal of Experimental Biology are air mailed to the Chemical Abstracts Service for their recently introduced computer-produced service, *Chemical-Biological Activities*.

The report under review makes a strong case for international cooperation in the field of scientific information. The ultimate objective should be single international abstracting services for various disciplines, fed by national abstracting services (on the lines of the Indian Science Abstracts). No effort should be spared towards the realization of this objective.

Summer School in Coordination Chemistry

K. S. NARAYAN
Department of Inorganic & Physical Chemistry, Indian Institute of Science, Bangalore 12

A SUMMER School in Coordination Chemistry was organized by the Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore, from 26 June to 7 July 1967 with financial support from the University Grants Commission. The object of the summer school was to provide an introduction to the research workers in the basic concepts and experimental techniques used in the study of coordination compounds. There were about 70 participants, including 40 from the Indian Institute of Science, Bangalore.

Prof. M. R. A. Rao (Indian Institute of Science) while welcoming explained the objectives of the summer school. In his inaugural address, Prof. S. Dhawan, Director, Indian Institute of Science, stressed the need for summer schools in view of the rapid advances being made in the frontiers of the various branches of science. The inauguration was followed by a talk by Prof. M. R. A. Rao on "Some recent trends in the study of coordination compounds". He spoke on the modern developments in the synthesis, structure and the techniques employed in the study of these compounds.

During the course of the summer school, fifty lectures were delivered by 16 speakers from various institutions. The lectures fall broadly under the following three categories: (1) fundamental concepts in the study of coordination compounds, (2) important techniques and their application to coordination chemistry, and (3) review of work in certain areas of coordination chemistry. Facilities for practical work were also provided. The practical work was aimed mainly at introducing the participants to special techniques, including the details of calculations and interpretations of data.

Topics covered in the first category were: (i) Crystal field theory, (ii) Symmetry in coordination chemistry, (iii) Normal coordinate analysis, and (iv) Molecular orbital calculations. Prof. P. T. Narasimhan (IIT, Kanpur), in the course of his lectures on crystal field theory, discussed the energy levels of a free atom, including atomic wave functions, angular momentum operators and matrix elements of the Hamiltonian for a 'many-electron ion'. He solved the energy level problem in the case of an octahedral field. He also dealt with fields of lower symmetry and ligand field theory. Dr C. C. Patel (Indian Institute of Science) gave a course of lectures on the applications of symmetry in the construction of hybrid orbitals for both σ and π bondings. Application of group theory in infrared spectroscopy, including qualitative correlation of symmetry and the number of bands, was also discussed by him. The details of normal coordinate analysis and its application in the characterization of group frequencies, infrared band intensities, and force constants were dealt with by Dr D. N. Sathyanarayana (Indian Institute of Science). J. Gopalakrishnan (Indian Institute of Science) gave a talk on the principles involved in the calculation of molecular orbital energies of metal complexes by the Mulliken, Wolfsberg, Helmholtz method.

Several speakers dealt with the experimental techniques employed in the study of coordination compounds. Dr A. Chakravorty (IIT, Kanpur) discussed the applications of NMR with special reference to solvation of cations, stereochemistry and reaction kinetics. He also illustrated the utility of contact shifts in solving a variety of problems. The lectures by Dr R. Srinivasan (Indian Institute of Science) dealt with the applications of ESR spectroscopy in coordination chemistry. He first outlined the basic principles underlying the technique, followed by the applications in the determination of the structure and the molecular orbital description of complexes and exchange interactions in binuclear complexes. Prof. C. N. R. Rao (IIT, Kanpur) gave the highlights of infrared spectroscopy, with particular reference to its limitations in the interpretation of spectra. He also touched on some aspects of far infrared spectroscopy and infrared dichroism. Dr C. C. Patel illustrated the use of energy level diagrams in the interpretation of electronic spectra of transition metal complexes. He also discussed the spectral characterization of distorted octahedral and lower symmetry fields and the importance of intensity and position of bands in structural assignments.

Dr H. B. Mathur (National Chemical Laboratory, Poona) discussed the applications of Mössbauer spectroscopy in the study of coordination compounds. After outlining the experimental technique, he explained the significance of isomer shift, quadrupole

splitting, and hyperfine magnetic splitting. This was followed by an illustration of the applications, with special reference to iron compounds. The determination of crystal structures by X-ray methods was the topic of the talks given by Dr H. Manohar (Indian Institute of Science). After giving a brief outline of the fundamentals of the methodology of solution of crystal structures, he discussed some recent interesting structures of coordination compounds. Metal peptide complexes and unusual coordinations of metal atoms were dealt with in detail during the course of his lectures. Dr S. Soundararajan (Indian Institute of Science) discussed the importance of dipole moments in the determination of chemical structures. Special emphasis was laid on metal carbonyls, chelates and charge transfer complexes.

The determination of the stability constants of complexes was the theme of the talks by two speakers. Dr A. D. Damodaran (Bhabha Atomic Research Centre, Bombay) spoke on the determination of stability constants by solvent extraction and ion-exchange methods. He pointed out the advantages as well as the limitations of the two methods. Dr R. S. Subrahmanya (Indian Institute of Science) discussed the polarographic and potentiometric methods in detail. After outlining the procedure for determining stability constants of systems containing mono- and poly-nuclear complexes, he discussed systems involving competition between two ligands. He also reviewed his recent work in this particular area.

Prof. D. Sen (IIT, Kharagpur) surveyed the work on complex compounds involving π-linkage. The stabilization of low oxidation states by ligands was also discussed. Subsequently, he covered the work on some typical non-transitional metal complexes, with special reference to beryllium, boron, silicon and rare gas compounds. The importance of complexes in the electrodeposition of metals and alloys was the subject of the lecture given by Prof. T. L. Rama Char (Indian Institute of Science). Dr D. Ramaswamy (Central Leather Research Institute, Madras) discussed the role of complexes in leather tanning and the part played by the complexes in coordination polymerization. He also outlined the work in progress at the Leather Research Institute. Prof. S. K. K. Jatkar (Poona University) spoke about the importance of spin isomers in coordination chemistry. During the course of his two talks, Dr Jagdish Shankar (Bhabha Atomic Research Centre, Bombay) reviewed the studies on the effects of nuclear recoil in cobalt chelates. He discussed the various mechanisms accounting for the annealing in the complexes.

A group discussion, presided over by Prof. S. K. K. Jatkar, was held on the modernization of the teaching of chemistry at the university level.

Balloon Performance from the Rate of Rotation of the Fan in F-type Radiosonde*

HAR SURENDRA SAHAI SINHA & S. P. VENKITESHWARAN
Meteorological Office, Amausi, Lucknow

THE rotation of fan in the F-type radiosonde shows many features in the atmosphere which are not available from the records of any other type of instrument; the rate of rotation of the fan gives valuable information on the existence of regions of turbulence in the atmosphere[1]. An estimate of the vertical component of the gusts in the turbulent regions can be made from the variation in the rate of rotation of the fan, based on the assumption that the rate of ascent of the balloon is uniform[2].

Variation in the Rate of Ascent of the Balloon with Height

It is well known that after a few seconds, a pilot balloon ascending freely attains a vertical speed v, when the air resistance D has become equal to the free lift L. D is a function of v, ρ (the density of air), and A (the horizontal cross-section of the balloon). From wind tunnel experiments on spheres it may be concluded that

$$D = b_1 \rho v^2 A \ (= L = \text{constant})$$

where b_1 is approximately constant.

It follows, therefore, that

$$(v/v_0)^2 = \rho_0 A_0 / \rho A$$

where v_0, ρ_0 and A_0 refer to conditions near the surface. If V is the volume of the balloon,

$$\frac{A_0}{A} = \left(\frac{V_0}{V}\right)^{2/3} = (\rho_0/\rho)^{-2/3}$$

and

$$(v/v_0) = (\rho_0/\rho)^{1/6}$$

Thus the rate of ascent is not constant, but varies with the air density and, therefore, with the height. The variations of $(\rho_0/\rho)^{1/6}$ with height for Poona and Agra are given in Table 1 for the summer (May and June) and winter (December and January) months.

*Paper presented at the convention organized by the Physical Research Committee of the Council of Scientific & Industrial Research at the Banaras Hindu University, Varanasi, in March 1967.

The mean rate of ascent of the radiosonde balloons at Minicoy was found from the time taken by the balloon to reach heights of 5, 10, 15 km. and above 15 km. up to the maximum height reached. Such data were also computed for the radiosonde ascents made at Lucknow, where the C-type (chronometric type) of instrument is in use. The mean rate of ascent in the height ranges 0-5, 5-10, 10-15 km. and above 15 km. are given in Table 2.

It is observed from Table 2 that even when layers 5 km. thick are considered, the rate of ascent, on an average, is higher in the region above 5 km. from ground level and tends to decrease only above 15 km.

Variation of Rate of Rotation of Fan with Height over Minicoy

The rate of rotation of the fan in the F-type radiosonde depends initially on the rate of ascent of the balloon. With a constant rate of ascent and in the absence of turbulence, the rate of rotation of the fan will gradually and steadily fall with height, due to decrease in density of the air. During an examination of the radiosonde data for Minicoy for different months and the variation of the rate of rotation of the fan with height, it was noticed that, on many days, the rate of rotation remained unchanged from ground up to 100 m bars. There were a few occasions in which it even showed a slight increase up to 100 m bars, above which it entered the tropopause and decreased rapidly (Fig. 1). The rates of ascent in the different layers on such occasions at Minicoy and Delhi are given in Table 3.

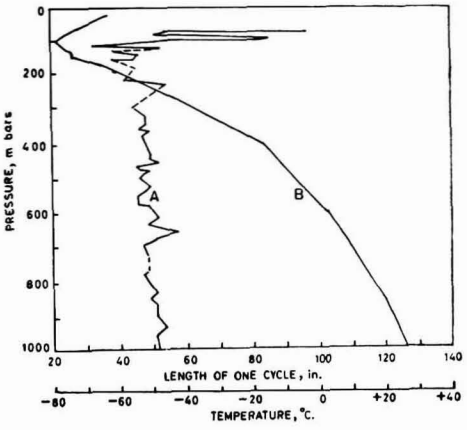

Fig. 1 — Variation of the rate of rotation of the fan and temperature with height over Minicoy (21 Feb. 1964) [A, rate of rotation of fan (data not available for dotted line); and B, temperature]

TABLE 1 — SEASONAL VARIATION OF $(\rho_0/\rho)^{1/6}$ WITH HEIGHT AT POONA, AGRA AND TEMPERATE LATITUDES

Height, km.:	0	2	4	6	8	10	12	14	16	18	20
					POONA						
May-June	1	1·02	1·05	1·09	1·13	1·17	1·22	1·28	1·33	1·42	1·52
Dec.-Jan.	1	1·02	1·05	1·09	1·14	1·18	1·23	1·28	1·35	1·43	1·53
					AGRA						
May-June	1	1·03	1·06	1·10	1·14	1·18	1·23	1·28	1·34	1·42	1·52
Dec.-Jan.	1	1·03	1·07	1·11	1·15	1·19	1·25	1·32	1·38	1·46	—
					TEMPERATE LATITUDES[3]						
Year	1	1·04	1·08	1·11	1·15	1·19	—	—	—	—	—

TABLE 2 — MEAN RATE OF ASCENT OF BALLOON

Height, km.	Mean rate of ascent (km./hr)		Average rate of ascent for whole flight		Average of maximum height reached	
	Minicoy (41 ascents)	Lucknow (47 ascents)	Minicoy	Lucknow	Minicoy	Lucknow
0-5	21·0	23·7	22·0	26·3	22·0	19·5
5-10	23·1	25·2				
10-15	22·9	28·3				
Above 15	21·9	26·7				

TABLE 3 — RATES OF ASCENT (KM./HR) IN DIFFERENT LAYERS WHEN RATE OF ROTATION OF FAN REMAINS UNCHANGED

	0-5 km.	5-10 km.	10-15 km.	Above 15 km.	Mean rate of ascent	Max. height reached (km.)
Minicoy (16 Feb. 1964)	25	27	31	27	27	22
Minicoy (21 Feb. 1964)	21	23	27	21	23	20
New Delhi (6 Jan. 1967)	20	25	27	21	24	19

Without going into the details about the occurrence of the regions of turbulence as indicated by the rapid fluctuations in the rate of rotation, it appears that the constant rate of rotation was maintained by the increasing rate of ascent of the balloon. The decrease in the rate of rotation at higher layers was due to the fact that the rate of ascent of the balloon in these regions ceased to increase with height. It was either constant or was decreasing due to the diffusion or leakage of gas through the balloon fabric which become very thin at these heights.

Calculated Rates of Ascents of Radiosonde Balloons Used in India (Type SR 875)

The empirical formula generally used to calculate the rate of ascent of a pilot balloon is

$$v = b \frac{L^{1/2}}{(L+W)^{1/3}}$$

where the rate of ascent v is in m./min., the free lift L and the weight of the balloon W are in g., and the constant b has the value 84. However, when larger balloons are used, the value of this constant has to be changed. For example, assuming the constant as 84, the rate of ascent of the balloon (wt 900 g.) used with the F-type radiosonde (wt 2020 g.), with a free lift of 1480 g., is 11·83 km./hr. But the actual observed rate of ascent was 21 km./hr. A value of 149, therefore, must be assigned to b to get the observed rate of ascent. In the case of the balloons used at Lucknow with C-type of instruments, the constant b was 149.

Thickness of Balloon at Bursting

The balloons at Minicoy generally reached 50 m bars where the temperature was of the order of —75°C. Since the balloon weighing about 900 g. was filled with hydrogen for a buoyancy of 4400 g., the hydrogen required at NTP was 3659 l. The approximate diameter at the 50 m bars level was 4·68 m. Assuming a density of 0·935 g./cm.^{3} for rubber, the thickness of the balloon at bursting was approximately 0·014 mm. Since, in the above calculation, the neck which is also included in the weight of the balloon does not function, the thickness of the balloon at bursting might have been a little less than 0·014 mm. This was, however, higher than Middleton's[3] value of 0·001 cm. which was probably based on the bursting diameter at ground level. Considering the effects of the low temperature at the 50 m bars level on the balloon fabric, the bursting thickness of 0·014 mm. is probably of the correct order.

Summary

If the rate of ascent of the balloon is constant, the rate of rotation of the fan in the F-type radiosonde should decrease with height, due to the decrease in the density of the air. It was observed from the radiosonde ascents over Minicoy that the rate of rotation of the fan remained almost unchanged up to even 100 m bars. It is shown that this is due to the increase in the rate of ascent of the balloon by about 2 per cent of the value near the ground per km. of ascent.

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Ultrasonic Studies in Liquids under High Pressure*

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THE propagation of acoustic waves in liquids at atmospheric pressure has been studied by many investigators as functions of frequency and temperature. The addition of hydrostatic pressure as a variable in the measurement of propagation constants will throw more light on the model of the liquid state. From the values obtained from pressure coefficient of velocity, several thermodynamic constants at different pressures may be determined. Excess absorption in liquids is interpreted in terms of structural and thermal relaxation. The addition of hydrostatic pressure determines exactly the type of relaxation mechanism applicable for a particular liquid.

Experimental Techniques

Different techniques have been used for measuring the propagation constant. The single crystal variable path interferometer was used by Swanson[1] up to 300 kg./cm.^{2}. Richardson and Eden[2] used the phase path pulse interference method with single crystal and reflector for the measurement velocity. Absorption was measured by placing another reflector of smaller dimension in the ultrasonic beam and displaying the two pulses reflected by the two reflectors on the oscilloscope. From the known distance between the two reflectors and heights of the two received pulses, the absorption coefficient was determined. Pressure was communicated to the main vessel by the liquid from the compression cylinder by screwing up a piston. They worked up to pressures of 10,000 lb./sq. in.

A single electric pulsed signal causes a quartz crystal to send out two acoustic pulsed signals simultaneously in opposite directions. The acoustic signals are reflected at the end plates and returned to the quartz plate. The crystal is always fixed and is closer to one end of the plates. The velocity and attenuation of the particular liquid are obtained from the difference in the measured arrival of times and amplitudes of pulsed signals reflected from the end plates. This method was used by Mifsud and Nolle[3].

Holton[4] used the method of multiple reflection. It consists of applying a pulsed r.f. voltage to a quartz crystal, placed inside a pressure-supporting vessel containing the test sample. The resulting ultrasonic pulses in the liquid are reflected back and forth over a definite path of length between the transducer and the reflector. After amplification, the complete echo pattern is displayed on the oscilloscope. The time difference between successive pulses furnishes data for velocity. Holton made measurements up to a pressure of 6000 atm.

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Results of Velocity Measurements

Measurement of sound velocity under pressure was made by Swanson[1] in non-associated liquids like benzene, chloroform, etc., up to a pressure of 300 kg./cm.^{2}. The velocity of sound in the liquids was found to increase with the increase of pressure. He studied the variation of the ratio of specific heats with pressure. The adiabatic compressibility and velocity computed from the data obtained by Swanson from static measurements were in reasonable agreement with the velocity determined experimentally. Biquard[5] measured the velocity of sound in benzene, water and chloroform up to a pressure of 600 atm. and his results are in agreement with those of Swanson.

Values of the velocity of sound in water as a function of temperature (at 30° and 50°C.) and pressure (0-6000 kg./cm.^{2}) were reported by Holton[4]. He particularly chose the liquid, water, to determine the effect of pressure and temperature on the association of water molecules. The graph plotted between $\frac{1}{v}\cdot\left(\frac{dv}{dt}\right)$ at 40°C. and pressure crossed the zero point at a pressure of 5300 kg./cm.$^{-1}$ from the positive side and had negative value for higher pressures. It may be recalled that all normal liquids have negative temperature coefficient of sound velocity. By this test, water too is a normal liquid beyond a critical pressure for the stated temperature. Holton also studied the variation of γ, the ratio of specific heats, with pressure, taking the P-V-T data for water from Bridgman's[6] results, and found it to decrease with increase of pressure.

Smith and Lawson[7] measured the velocity of sound in water at different pressures and temperatures. The temperature, at which maximum velocity occurs, shifts towards higher values with increase of pressure and the curves become flatter as pressure increases. These results are contrary to those obtained by Holton. The results of Smith and Lawson are supported by Litovitz and Carnevale[8] and Wilson[9] and seem to give support to Hall's model for structure of water.

Wilson[10] measured the velocity of sound in heavy water as a function of temperature and pressure in the ranges 0-100°C. and 14·71-14,000 lb./sq. in. respectively. The character of these curves is similar to those for water. The maximum sound speed shifts to higher temperatures with the increases of pressure at a greater rate in heavy water than in water and the slopes of the heavy water curves between 0°C. and the peaks are slightly greater than that for water. In the temperature range 0-20°C., the slopes are nearly equal.

The pressure dependence of sound velocity in primary alcohols was studied by Litovitz and

Carnevale up to a pressure of 6000 kg./cm.^{2}. It was found that the velocity increased with the increase of pressure. They found the molar sound velocity, R, to be constant. It was, however, observed by Wilson[11] that molar sound velocity does not remain constant in ethyl alcohol in the pressure range 147-14,000 lb./sq. in. Wilson and Bradley[12] measured the sound velocity in primary alcohols in the above pressure range and computed γ for ethyl alcohol.

Mifsud and Nolle[3] measured the speed of sound in several non-associated liquids under high pressures up to 1360 atm. It was shown that Tonks equation gives approximately correct values for the pressure coefficient of sound velocity in CS_2. Altenburg[13] developed an equation for the pressure coefficient of velocity which is also approximately valid for the liquids tested by him. From the study of the velocity variation with pressure, a model for the liquid state can be suggested or verified.

Effect of Pressure on Absorption

Mifsud and Nolle[3] measured absorption coefficients in the pressure range 1-1360 atm. in benzene, carbon tetrachloride and carbon disulphide. They applied the structural relaxation theory to CCl_4. The structural relaxation theory led to a reasonable prediction of the trend of pressure dependence of sound absorption in carbon tetrachloride but to an erroneous prediction of the magnitudes of absorption. Later investigators found that the thermal relaxation theory fits reasonably well. The thermal relaxational theory has been extended to carbon disulphide. According to this theory, the absorption data are consistent, approximately, with the theoretical prediction of the effect of pressure upon the relaxation frequency and upon the relaxation portion of the specific heat. Litovitz and Carnevale[8] measured the absorption of water up to a pressure of 2000 kg./cm.^{2}. The data showed that both the shear and compressional losses decrease as the pressure is raised. Comparison of these results with the values predicted by Hall's theory of sound absorption in water shows that the theory has got to be slightly modified to be in agreement with the experimental data. The modification consists of assuming that the open or ice type of packing is associated with higher free energy state than that of the closed type of packing. Measurements of sound absorption were made in primary alcohols up to a pressure of 2000 kg./cm.^{2} by Litovitz and Carnevale[14]. They found the two-state model for the structural relaxation process, which fitted well in the case of water, inadequate to describe the pressure dependence of sound absorption in alcohols, though alcohols and water belong to the same classification. They attributed the failure of this theory to the existence of more than two states. This is not unreasonable when considered in terms of the ultrasonic relaxation measurements made on n-propyl alcohol.

Absorption measurements in triethylamine, acetic acid, carbon disulphide and glycerol were made as a function of pressure at various frequencies[15,16]. In the case of glycerol, the relaxation frequency decreased with increase of pressure. Here the viscous relaxation effects are related to the motion of the molecule from one lattice side to another. As the pressure is raised and the density increased, it becomes more difficult for a molecule to jump from one site to another in the lattice and the time between the jumps increases. Thus, the relaxation frequency for intramolecular structural rearrangements which involve molecular motion should decrease with increasing pressure as observed experimentally. In the case of carbon disulphide, the relaxation frequency increased with the pressure. The relaxation effect here is due to the perturbation of equilibrium between vibrational and external degrees of freedom. As the relaxation time is determined by the efficiency of a collision in exciting or de-exciting an internal degree of freedom and the number of collisions per second, the effect of increasing the pressure is to increase the number of collisions per second without affecting the collision efficiency. It is for this reason that the relaxation frequency increases with increasing pressure in CS_2.

The pressure independence of the relaxation frequency in triethylamine and acetic acid indicates that neither vibrational nor translational relaxation for structural relaxation of the liquid lattice is involved in these liquids. This conclusion is in agreement with the assumption made by Heasall and Lamb[17] that the loss in triethylamine is due to rotational isomeric effect and the contribution to the loss is related to the difference in enthalpy between the two isomeric forms. In acetic acid, the loss is related to perturbation of the monomer-dimer equilibrium by the changes of temperature accompanying the sound wave. Theoretical work by Freedman[18] supports this view.

Summary

The various experimental techniques used for the determination of ultrasonic velocity and absorption with pressure in liquids have been discussed. From the measurement of the variation of velocity with pressure, the values of several thermodynamic constants with pressure can be determined and compared with the values obtained from static measurements. The molar sound velocity, R, in alcohols has been observed to be constant up to a pressure of 6000 kg./cm.^{2}. The pressure dependence of relaxation frequency is a useful study in distirguishing the type of relaxation present. Such a study has been found suitable in establishing the monomer-dimer relaxation in acetic acid, vibrational relaxation in carbon disulphide and isomeric relaxation in triethylamine.

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Metallic & Molecular Compounds of Antipyrine

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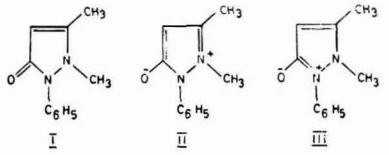
AN important keto derivative of pyrazoline, generally known as antipyrine (1-phenyl-2,3-dimethyl-5-pyrazolone), was first synthesized by Knorr[1,2]. Antipyrine receives its name from the antipyretic properties which are shared by several of its derivatives. These compounds have been widely used as analgesics and antipyretics, in the measurement of body water, topically as local anaesthetics, vasoconstricters and styptics in epistaxis.

Knorr[2] proposed structure (I) for this compound. But Michaelis[3] felt that the properties of antipyrine and its mode of formation could be most easily explained by a phenol betaine structure, the modern version of which is one with a formal positive charge on the nitrogen in the 2-position and a negative charge on the carbonyl oxygen (II). This structure satisfactorily explains the considerable solubility of antipyrine in water and its high dipole moment. However, infrared and Raman spectra of antipyrine and its derivatives, taken in different physical states, favour Knorr's formulation[4,5]. Experimental evidence for yet another form of antipyrine (III) has been furnished by Kitamura[6].



I II III

The absorption spectra in ultraviolet region, studied by Valyasko and Bliznyukov[7], tend to suggest that a state of resonance between many structures exists in the antipyrine molecule. They have also shown that the pharmacodynamic properties of antipyrine and the related substances have a definite relation to these canonical structures. They relate the increased toxicity and antipyretic action of 1-phenyl-2,3-dimethyl-5-pyrazolone (antipyrine) compared to those of 1-phenyl-3-methyl-5-pyrazolone to the increase in the basicity of the nitrogen atom, caused by the substitution of the methyl group in the 2-position, which in turn facilitates the development of the phenyl hydrazine structure. The dipole moments of antipyrine and some of its derivatives were studied by Brown *et al.*[8] and the results have been explained by considering antipyrine as a resonance structure, the two chief polar contributors being (I) and (II).

The large dipole moment of antipyrine (4·6-6·0D) favours its coordination to different metal ions via the oxygen atom in the carbonyl group. Further, the proton-accepting nature of this oxygen atom facilitates the formation of hydrogen bonded complexes with proton-donor molecules and groups. A large number of metallic and molecular compounds of antipyrine have been synthesized and an attempt to classify these is made in the following. However, it may be mentioned that very few chemical and physical studies on these have been carried out and in many cases structural problems like the nature of linkage to the metal ion and its coordination are not clearly understood. This fact necessarily restricts the scope of the discussion and the conclusions that can be derived from it.

Antipyrine Compounds of Rare Earths and Actinides

Well over 50 antipyrine compounds of rare earths and actinides have been reported in the literature. A number of these compounds are distinguishable by the presence of a single metal hexaantipyrine complex cation. The structure of these can be represented by the general formula $(MA_6)X_3$ where M is a trivalent metal ion, A, antipyrine and X, a monovalent anion. Dörfurt and Schliephake[9] have synthesized many compounds belonging to this category with M = La, Ce, Pr or Nd (all trivalent) and X = ClO_4^-, by mixing the solutions of appropriate amounts of the respective metal nitrate, antipyrine and either perchloric acid or ammonium perchlorate. The metal hexaantipyrine iodides were prepared by replacing the perchlorates by the corresponding iodides. Ryabchikov and Terenteva[10] have reported the preparation and solubilities of many such compounds with X = Cl^-, ClO_4^-, Br^-, I^- or IO_3^-. The

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corresponding thiocyanates were synthesized by Dutt and Mukherjee[11] by mixing the organic reagent and an aqueous solution of the rare earth nitrate and then adding a solution of ammonium thiocyanate. Recently, Krishnamurthy and Soundararajan[12] have studied the infrared and electronic spectra and have reported the conductivity and molecular weight data of the hexaantipyrine complexes of lanthanum and cerium perchlorates. They conclude that the coordination is through the carbonyl oxygen and the coordination number is six.

A second type of rare earth compounds have the general formula $M_2X_3.6A$. Dutt and his colleagues[11,13,14] have prepared metal hexaantipyrine selenites, dithionates and tetrathionates conforming to the above formula with M = La^{3+}, Ce^{3+} or Pr^{3+} and X = SeO_3^{2-}, $S_2O_6^{2-}$ or $S_4O_6^{2-}$. The preparation of these is similar to that of the metal hexaantipyrine thiocyanates. In the absence of further studies, definite conclusions about the nature of coordination in these compounds cannot be drawn. The anions may function as bidentate ligands to satisfy the coordination of one of the metal ions with the antipyrines coordinating to the other. It is also probable that both the cations have mixed coordination.

Mixed complexes of the type $M(NO_3)_3.3A$ where M = La^{3+}, Ce^{3+} or Sm^{3+} were prepared by Kolb[15] by adding antipyrine to a strong acid solution of the rare earth nitrates, evaporating and crystallizing the residue from ethyl alcohol. A similar gadolinium compound has been reported by Sarkar[16].

Rare earth antipyrine compounds such as $[MA_6]$-$[Cr(SCN)_6]$, $[MA_6]_2[Hg(SCN)_4]_3$ and $[MA_6][Cr(SCN)_4$-$(NH_3)_2]$ where M = La^{3+}, Ce^{3+}, Pr^{3+} or Nd^{3+}, which contain metal complex anions, have also been reported in the literature[10,14,17]. The only compounds of antipyrine with elements in the actinium series so far reported[15,18] are thorium hexaantipyrine perchlorate, $ThA_6(ClO_4)_4$, uranyl pentaantipyrine perchlorate, $UO_2A_5(ClO_4)_2$ and $2Th(NO_3)_4.5A$. The first two of these were prepared by mixing aqueous solutions of thorium nitrate or uranyl nitrate, ammonium perchlorate and antipyrine in molecular proportions and the third by adding antipyrine to an acid solution of thorium nitrate.

The principal use of rare earth antipyrine complexes is in the separation of rare earth elements by fractional crystallization[9,19]. Much physical study has not been reported on these compounds except in the case of terbium hexaantipyrine iodide. White *et al.*[20] have recorded the paramagnetic resonance spectrum of this compound and Van Uitert and Soden[21] have studied the effect of rare earth substitution upon the fluorescence of TbA_6I_3. Paramagnetic resonance studies on gadolinium hexaantipyrine iodide have also been reported[22].

Antipyrine Compounds of Transition Metals

The largest single group of antipyrine compounds of transition metals comprises those containing metal hexaantipyrine complex cations with general formula $(MA_6)X_n$ where n is determined by the ionization state of the metal ion. More than a score of such metal hexaantipyrine perchlorates, fluosulphonates, dichromates, permanganates, thiocyanates, chlorates, thiosulphates and picrates with M = Y^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cr^{2+}, Fe^{3+} and Cr^{3+} were prepared by Dörfurt and his colleagues[9,18,23] by mixing solutions of chlorides, nitrates or sulphides of the metals with aqueous solutions of antipyrine and a salt containing the respective anion. They have also prepared two chromic compounds with more than one complex ion, viz. chromic hexaantipyrine ferricyanide, $[CrA_6][Fe(CN)_6]$, and chromic hexaantipyrine ferrocyanide, $[CrA_6]_4[Fe(CN)_6]_6$. Divalent copper and monovalent silver form pentaantipyrine and triantipyrine complexes respectively. Preparation of zirconium(IV) hexaantipyrine nitrate and yttrium(III) tetraantipyrine nitrate by the addition of antipyrine in acid solutions of the respective metal nitrates also have been reported[15].

Patel and coworkers[24] have studied the physicochemical properties of the hexaantipyrine perchlorates of Mn^{2+}, Co^{2+}, Ni^{2+}, Cr^{3+} and Fe^{3+}. Spectral and magnetic data on these indicate them to be spin-free octahedral complexes. Infrared spectra of the solid complexes suggest that the carbonyl oxygen of antipyrine is the donor to the metal. Similar studies on copper(II) pentaantipyrine perchlorate also have been reported by Gopalakrishnan and Patel[25]. Paramagnetic resonance studies carried out by Srinivasan and Subramanian[26] indicate distorted octahedral and tetragonal site symmetries for Mn^{2+} and Cu^{2+} in the manganese and copper compounds respectively.

The next important type of compounds have the composition[27,28] $MX_2.A_2$ where M is a divalent metal ion (Cu^{2+}, Co^{2+} or Mn^{2+}) and X = Cl^-, Br^-, I^- or NCS. The halides were prepared by mixing the respective metal halide and antipyrine in solution and the thiocyanate (for Mn^{2+} only) by dissolving antipyrine in a metal nitrate solution and then adding ammonium thiocyanate. There are two possible structures for these compounds, one with two antipyrines and two anions coordinating the same metal ion and the other containing two complex ions $[MA_4]^{2+}$ and $[MX_4]^{2-}$. From transport studies in solution, Souchay[28] has assigned the latter formula to these compounds. However, spectral, magnetic and electrical conductance studies on some of these compounds by Gopalakrishnan and Patel[29] indicate a tetrahedral structure for them with the antipyrines and the halogens coordinating the metal ion.

Unlike in the above case, addition of ferric chloride or ferric thiocyanate to antipyrine in solution yields compounds[30,31] of formula A_3FeCl_3 and $A_3Fe(SCN)_3$. These compounds may be expected to be mixed complexes with the anions and antipyrines coordinating to the metal ion to satisfy the usual coordination number six for Fe^{3+}. The corresponding bromide and iodide are unstable. Antipyrine compounds of ferric halides with a different formula, viz. $2FeX_3.3A$, where X = Cl^- or Br^-, have been reported by Colzolary[32]. From the colour of the substance, he believes that a complex cation might have been formed.

Souchay[28] has reported the preparation of a series of compounds with the general formula $MA_n(H_2O)_mX_2$ where M = Co^{2+}, Ni^{2+}, Mn^{2+} or Cu^{2+}, X = NO_3^- or ClO_4^-, n = 2 to 4 and m = 0 to 5. They could be mixed complexes, antipyrine and H_2O being the ligands. Except in the case of

$CoA_3(H_2O)_5(ClO_4)_2$, it is found that $m+n = 4$ or 6. This probably shows that, in the present case, these metal ions can exist in two coordination states, one with coordination number four and the other with six. The same author has reported a number of compounds with composition $2CdX_2.MX_2.xA.yH_2O$ where $M = Co^{2+}$, Ni^{2+} or Cu^{2+}, $X = Cl^-$, Br^-, I^- or NCS^-, $x = 6$ to 9 and $y = 0$ to 3. Again for want of further data it is difficult to verify the formulae assigned by him or discuss structural problems. One can, however, expect these compounds to exist in mixed coordination state.

An appreciable number of ferrous and ferric antipyrine compounds of complex composition containing other groups such as H_2O, CN, NH_3, NO, NO_2, SCN, C_2O_4, HCl, pyramidone, pyridine and quinoline have been reported[31-35]. However, hydrogen bonding is believed to account for their stability to a large extent. The titanium compounds, $(AH^+)_2[TiO(o\text{-}C_6H_4O_2)_2]$ and $(AH^+)_4[TiO\{(O_2^-)HOC_6H_2CO_2^-\}_2]$, also can be considered to be stable by virtue of the presence of a system of hydrogen bonds[36,37]. On the other hand, copper salicylate antipyrine, $A(OH.C_6H_4.COO)_2.Cu$, is thought to be a mixed complex[38].

Three antipyrine compounds of gold, viz. $A_2.AuCl_3.HCl$, $A.AuCl_3$ and A_2Au, were prepared by Komada[39]. In the first two, stabilization is likely to have been achieved by hydrogen bonding. The third one is unusual in that the metal atom seems to have been directly bonded to antipyrine.

Antipyrine Compounds of Non-transition Metals

The most stable and commonly occurring coordination of antipyrine about metal ions seems to be sixfold. Dörfurt and his colleagues[18,23], who have done extensive work on such compounds, have prepared a large number of hexaantipyrine salts with the general formula $[MA_6]X_n$ where $M = Mg^{2+}$, Ca^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+} or Al^{3+}, $X = ClO_4^-$, BF_4^-, MnO_4^-, SO_3F^- or SCN^- and $n = 3$ for the aluminium compound and 2 for the rest. The preparation of these is similar to that reported for the analogous transition metal complexes.

Several zinc and cadmium salts of antipyrine with general formula $MX_2.A_2$ where $M = Zn^{2+}$ or Cd^{2+} and $X = Cl^-$, Br^-, I^-, NO_3^-, CN^- or SCN^- have been reported in the literature[28,40-42]. The general method of preparation of these compounds involves the addition of the components in molecular proportions in neutral solution. As in the case of the analogous transition metal compounds there are two possible structures for these represented by the formulae $MX_2.A_2$ and $[MX_4]^{2-}[MA_4]^{2+}$. The preparation of zinc and cadmium salicylates, $A_2.(OH.C_6H_4.COO)_2.M$ ($M = Zn^{2+}$ or Cd^{2+}), by mixing solutions of sodium salicylate, antipyrine and the respective metal chloride or metal sulphate has been reported by Schuyten[27]. Cadmium antipyrine compounds with formulae $(CdA)I_2$, $[A_2][CdBr_4].2A$ and $(CdA)[Hg(SCN)_4]$ have also been prepared[41,43,44].

Preparation and properties of a number of addition compounds of antipyrine with mercury salts with different compositions like $A.HgX_2$ ($X = Cl^-$, Br^- or CN^-), $A_2.HHgCl_3$, $A_2Hg.H_2O$, $A.HgI_2.H_2O$, $A.HgI$, $A.HgXX'$ ($X = Cl^-$ or I^- and $X' = NH_2^-$ or OH^-) are found in the literature[45-49]. Although much study on these compounds has not been done to facilitate a discussion of bonding in these, it is reasonable to expect hydrogen bonding to play the major part in their stabilization. A complex compound of divalent mercury with formula $HgI_4(HA)_2.6H_2O$ has also been reported[50].

Astre and Vidal[51] have prepared a stannic compound of composition $4A.SnCl_4.4HCl$ by adding solutions of stannous chloride and antipyrine in dilute sulphuric acid. Here, it may be noted that stannous chloride changes into stannic chloride in the presence of antipyrine. A compound with composition $3A.SnCl_4.3HCl$ is obtained when the stannous chloride is replaced by stannic chloride. HCl and antipyrine molecule may be hydrogen bonded in these compounds.

A solution of selenium monochloride in carbon tetrachloride reacts with a concentrated solution of antipyrine in chloroform at 0°C. to give diantipyryl diselenide, $Se:Se:A_2$, according to Konek and Schleifer[52]. This compound is of interest as the metal ion is directly bonded to antipyrine. Selenium tetrachloride also reacts vigorously with antipyrine to give diantipyryl selenium dichloride[52], A_2SeCl_2.

Kaufmann[53] has reported three addition compounds of antipyrine with strontium salts, viz. $4A.SrBr_2$, $6A.SrI_2$, $A.Sr(SCN)_2$. The presence of metal hexaantipyrine complex cation may be expected in $6A.SrI_2$. $4A.SrBr_2$ is probably a mixed complex with bromine also coordinating to the metal ion. The stabilization of $A.Sr(SCN)_2$ might have been achieved through hydrogen bonding.

Dick and Maurer[50] have prepared a bismuth compound of formula $BiI_4(HA).6H_2O$ by adding a solution of antipyrine to an acidified (with hydrochloric acid) solution of $K(BiI_4)$ at $pH = 1$. The absorption spectrum of this compound indicates the presence of $[BiI_4]^-$ ion. Antipyrine in this is probably hydrogen bonded to the complex cation. By a similar procedure at $pH = 5$-6 another bismuth compound $[BiA_3][BiI_6]$ was also prepared.

Compounds of antipyrine with calcium chloride and calcium iodide, $CaCl_2.A$ and $CaI_2.A$, have been prepared by Greenbaum[54]. No further study on these has been reported.

Molecular and Other Hydrogen Bonded Complexes of Antipyrine

The proton-accepting nature of antipyrine aids in the formation of molecular complexes with proton-donating organic molecules with ease. Several such complexes have been prepared and studied[55-61] and unlike in the case of its metal complexes, the mechanism of the formation of the molecular complexes is fairly well understood.

The largest number of such compounds are formed with polyvalent phenols and their derivatives. Most of them combine in more than one molecular proportion. Aromatic carboxylic acids, hydrobenzoic acids and aromatic acids with a side chain carboxyl also form molecular complexes with antipyrine. Complexes of antipyrine with α- and β-phenols also have been reported. Aliphatic or aromatic aldehydes and ketones do not combine with antipyrine but

some of their polyhalogen derivatives like chloral hydrate do. Aliphatic acids like oxalic acid, succinic acid, tartaric acid, adipic acid, fumaric acid and mesaconic acid combine with antipyrine in the ratio 1 : 2 whereas citric acid forms a 1 : 3 compound with it. Esters, as a rule, do not combine. The general trend seems to be that compounds with little chemical activity such as hydrocarbons, esters, aldehydes and ketones do not form molecular complexes with antipyrine whereas compounds of acidic or otherwise reactive nature do.

Extensive spectroscopic studies on these compounds have been carried out to determine the mechanism of complex formation. Taboury[62] has studied the Raman spectrum of a 1:1 complex of antipyrine and chloral hydrate and has found that the CO band of antipyrine at 1658 cm.$^{-1}$ is displaced and greatly weakened, showing thereby that association has taken place at the carbonyl group and not at the 2-position as was suspected by some workers. Further studies in Raman and infrared spectra by Taboury and his colleagues[4,5,63-65] confirm that these compounds are formed by H-bonding between the carbonyl oxygen of antipyrine and the ionizable hydrogen in the added molecule. Infrared studies on the compounds of antipyrine with phenol, thymol and α-naphthol by Oi *et al.*[66] also suggest that the stabilization of the systems is through hydrogen bond formation involving the carbonyl oxygen. The same conclusion has been drawn from a study of the spectrum of antipyrine in the near ultraviolet region in a three-dimensional system with ethyl alcohol in heptane as solvent[67].

A number of other non-metallic compounds of antipyrine such as A.HCl, A.HSCN, $A_3(HI,I_2)_2$, $A_2.HI.I_2$, $A_2.H_3PO_4$, $A_2.H_3AsO_4$, $ASOCH_3(OH)_2.2A.4H_2O$ and $ASO(CH_3)_2OH.2H_2O$ have been reported in the literature[30,68-71] and a system of hydrogen bonds is once again believed to be responsible for their stability. Normal salts of antipyrine with halogen acids were prepared and their infrared spectra were recorded by Cook[72]. The corresponding deuterated compounds also were studied in order to facilitate the identification of the bands due to the vibration of the protonating hydrogen atom. These studies suggest that protonation occurs at the carbonyl group. Recently, Ramamurthy[73] has reported the preparation and properties of an adduct of antipyrine and perchloric acid. From physico-chemical studies he has shown the structure of the adduct to $(A_2H)^+(ClO_4)^-$, the two antipyrines being linked by a symmetrical hydrogen bond.

Crystallographic Studies

Antipyrine crystallizes in the monoclinic system with space group $C2/c$ or Cc and with eight molecules in a unit cell of dimensions[74]: $a = 17{\cdot}83$, $b = 7{\cdot}43$ and $c = 16{\cdot}90$ A. and $\beta = 117{\cdot}2°$. Attempts to solve its structure by Romain[75] by means of Patterson diagrams were of no avail and the exact molecular geometry of antipyrine was not established.

Until recently the only structural information reported in the literature[21] concerning antipyrine compounds was about $Tb(C_{11}H_{12}ON_2)_6I_3$ which crystallizes in the rhombohedral space group $R\bar{3}$. No further details of this investigation are available.

Recently, Vijayan and Viswamitra[76-80] have carried out a systematic and detailed X-ray investigation on some metal hexaantipyrine perchlorates with general formula $M(C_{11}H_{12}ON_2)_6(ClO_4)_2$ where M = Mg^{2+}, Zn^{2+}, Pb^{2+} or Ca^{2+} to study the nature of the metal oxygen bonding in these and to elucidate the geometry of the antipyrine ring system. These compounds form an isomorphous series with space group $P\bar{3}$. This isomorphism is an interesting feature as the replaceable atoms have widely different ionic radii. For example, magnesium and lead in their doubly ionized states have ionic radii 0·65 and 1·21 A. respectively[81] and this is probably the only case where the replacement of Mg^{2+} by Pb^{2+} does not alter the isomorphism of the parent compounds.

In these compounds, the sixfold coordination of the divalent metal ion at the origin of the unit cell is satisfied by the carbonyl oxygen atoms in the $\bar{3}$-equivalent antipyrine groups disposed octahedrally about it. The $\bar{3}$-axis of the octahedron formed by these oxygen atoms coincides with that of the space group. Hence, the only distortion of the octahedron permitted by symmetry is an elongation or contraction along the $\bar{3}$-axis. This octahedron is quite regular in the magnesium compound, but gets elongated as the size of the metal ion increases presumably due to packing requirements. From the known electronegativity coefficients of the relevant atoms, the ionic character of the metal-oxygen bond in these compounds have been evaluated and it is found that the Mg-O and the Ca-O bonds are essentially ionic whereas the Pb-O bond is predominantly covalent. This deduction has been confirmed by the variation in the lengths of the metal-oxygen bonds.

In the antipyrine molecules in these compounds, both the phenyl ring and the pyrazolone ring are planar and are inclined with respect to each other by angles varying from 62·3° to 68·6°. The N-C bond that connects the two rings is formally single and hence this orientation is determined entirely by intramolecular and intermolecular steric effects. From bond length-bond order correlation curves[82-84], the contributions of the canonical forms (I), (II) and (III) have been fixed at 41, 37 and 22 per cent respectively. However, it might be mentioned that the association of the metal ion at the carbonyl oxygen favours the development of the ionic forms and hence the contribution of Knorr's structure can be expected to be larger in free antipyrine than in its complexes.

Summary

1-Phenyl - 2,3- dimethyl -5- pyrazolone, commonly called antipyrine, is medically important. It has a large dipole moment and the oxygen atom, being at the electronegative end, acts as the coordinating ligand to different metal ions. Further the proton-accepting nature of the oxygen atom facilitates the formation of hydrogen bonded complexes of antipyrine with proton-donor groups and molecules. A large number of antipyrine compounds of rare earths and actinides, of transition metals, of non-transition metals and of molecular and other hydrogen bonded complexes are classified and discussed. The available crystallographic studies have also been briefly surveyed.

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Recent Developments in the Biosynthesis of Carotenoids

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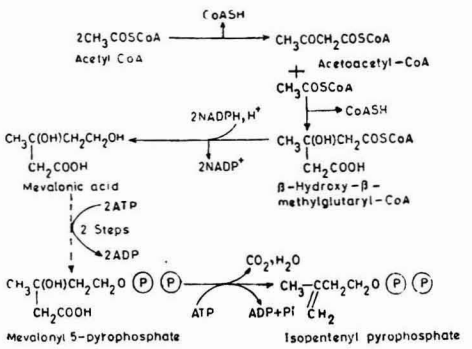
CAROTENOIDS follow the general pattern of terpenoid biosynthesis in that they are derived from the biological isoprene precursor, isopentenyl pyrophosphate (IPP). This compound originates from acetyl-CoA by the pathway indicated in Scheme I[1,2].

Chain elongation begins by isomerization of IPP to dimethyl allyl pyrophosphate (DMAPP), to which further molecules of IPP are added sequentially to form geranyl pyrophosphate (C_{10}) (Schemes II and III), farnesyl pyrophosphate (C_{15}), the precursor of sterols and other triterpenes, and geranylgeranyl pyrophosphate (C_{20}, GGPP), which is the precursor of the carotenoids. Two molecules of GGPP condense with loss of two pyrophosphate groups to form phytoene, the first C_{40} compound formed in the biosynthetic sequence to the coloured carotenoids such as lycopene (Scheme IV). The formation of cyclic carotenoids such as α- and β-carotenes is a late step in the chain and takes place either at the neurosporene or lycopene level (Scheme IV). The insertion of oxygen into the carotene molecules to form xanthophylls is also a late step in the biosynthetic sequence and will not be discussed here[1].

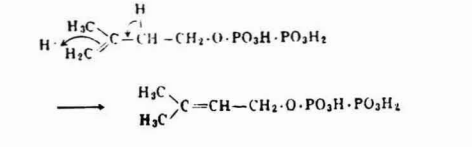
The isomerization of IPP to DMAPP involves the expulsion of a proton which was originally attached to C-4 of mevalonic acid (MVA) (Scheme II). The condensation of a second molecule of IPP with DMAPP to form GPP also involves the loss of a proton from C-4 of MVA (Scheme III); subsequent chain elongation to farnesyl pyrophosphate and GGPP involves at each step the further loss of a proton from the same source. No loss of protons occurs during the dimerization of GGPP to phytoene. Thus, in the formation of one molecule of this carotenoid, eight protons from C-4 of eight MVA molecules are lost.

As there are two hydrogen atoms attached to C-4 of MVA, the question arose as to whether there was stereospecific removal of one hydrogen atom and, if so, which.

Popják and Cornforth[3] and their colleagues synthesized two species of [2-^{14}C] MVA stereospecifically replacing the hydrogen atoms at C-4



SCHEME I



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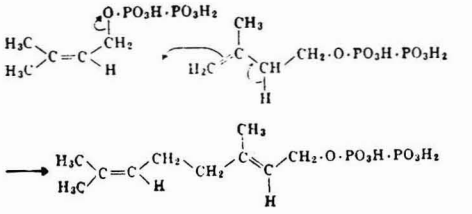

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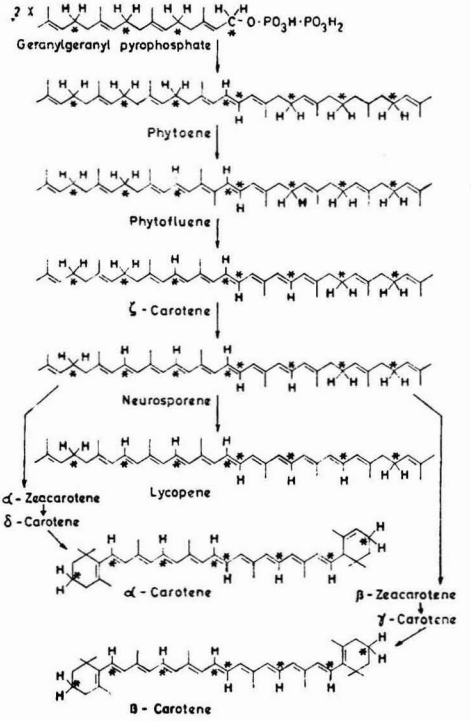
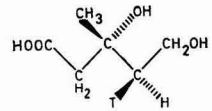
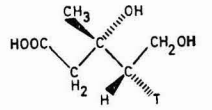


SCHEME IV



[4R-$^3H_1$] Mevalonic acid

I



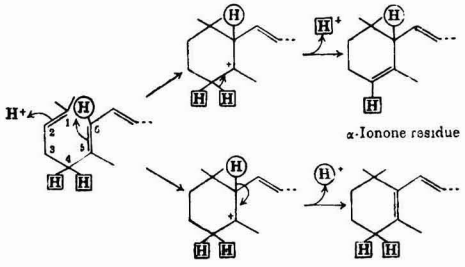
[4S-$^3H_1$] Mevalonic acid

II

(I, II) with tritium, and showed with the use of these substrates that in the formation of squalene, all eliminations of hydrogen from C-4 of MVA molecule are stereospecific and in the same sense; the *pro-R*-hydrogen is retained and the *pro-S*-hydrogen is lost[4,5]. When 3*RS*-[2-^{14}C(4*R*)-4-$^3H_1$] MVA was the substrate, the ^{14}C : ^{3}H ratio, in squalene, was the same as in the starting material, while with 3*RS*[2-^{14}C(4*S*)-4-$^3H_1$] MVA, no tritium was retained in squalene*.

Using the same substrates, the stereospecificity of hydrogen removal from C-4 of MVA molecule in the formation of phytoene in a number of systems has been shown to be the same as in squalene biosynthesis[6-8], i.e. the *pro-R*-hydrogen is specifically retained.

3*RS*[2-^{14}C(4*R*)-4-$^3H_1$] MVA was further used to demonstrate a mechanism for ring closure to form the β-ionone ring system of carotenoids. Phytoene has the same ^{14}C: ^{3}H atomic ratio as MVA, i.e. 8:8 (as 8 moles of MVA are involved in synthesis of one phytoene molecule). In *Phycomyces blakesleeanus* and carrot slices, this ratio falls to 8:6 in β-carotene, indicating that the tritium atom attached to C-6 and C-6′ in phytoene is lost during the cyclization process. A mechanism which accounts for this involves initiation of cyclization by proton attack at C-2 and the resulting intermediate carbonium ion is neutralized by the loss of a proton from C-6 (Scheme V). α-Carotene which has one α-ionone and

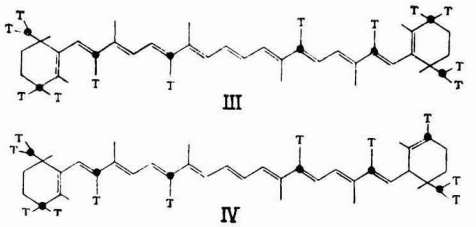


○, Labelled from [(4*R*)-4-$^3H_1$]MVA, □, labelled from [2-$^3H_2$]MVA

SCHEME V

one β-ionone residue has a ^{14}C: ^{3}H ratio of 8:7, indicating that an α-ionone residue cannot arise from a β-ionone residue. This view was substantiated by experiments with *del*-tomatoes, a mutant strain which produces δ-, ε-, γ-carotenes as well as α- and β-carotenes[9]. These experiments did not, however, resolve the problem whether the β-ionone residue can

*R and S indicate the absolute configuration at C-4 in each case.

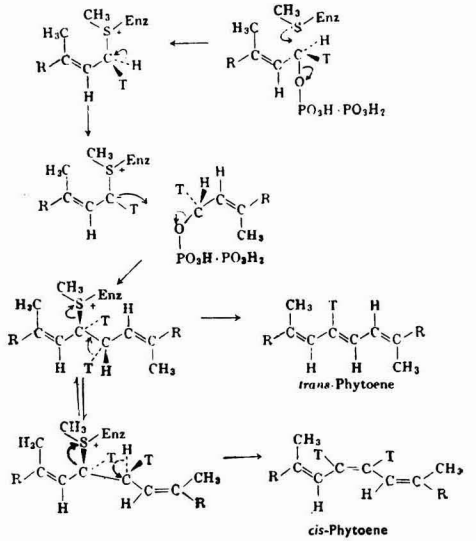


III

IV

arise from an α-ionone ring. This was solved using [2-^{14}C, 2-$^3H_2$] MVA. If the β- and α-ionone rings are formed as indicated in Scheme V, β-carotene should have a ^{14}C:^{3}H atomic ratio of 8:12 (III) and α-carotene a ratio of 8:11 (IV). On the other hand, if the β-ionone ring was formed by isomerization of an α-ionone residue, the ratio for β-carotene should be 8:10. The ratio was 8:11 for α-carotene and 8:12 for β-carotene, indicating that Scheme V is operating; thus it is concluded that the α-ionone and β-ionone rings of the carotene are formed from a common carbonium ion and not by any interconversion of the formed rings.

Further consideration of carotenoid biosynthesis indicates that in the formation of phytoene two hydrogens are lost from the two central atoms which originated from C-5 of MVA (Scheme IV); in addition one hydrogen from C-5 of MVA is lost at each step during the conversion of phytoene into the fully unsaturated carotenes, exemplified by lycopene in Scheme IV. When the stereochemistry of these hydrogen eliminations was studied using (3*RS*)-[2-^{14}C(5*R*)-5-$^3H_1$] MVA and (3*RS*)-[2-^{14}C$_1$(5*S*)-$^3H_2$] MVA as substrates[10], it was observed that in the formation of phytoene, two *pro-R*-hydrogens are retained and the two *pro-S*-hydrogens are eliminated. On the other hand, during the dehydrogenation of phytoene to lycopene (and the cyclic carotenes)



SCHEME VI

one *pro-R*-hydrogen is lost at each stage while the *pro-S*-hydrogen is retained.

These observations with phytoene can be used to put forward a mechanism for its formation and to explain why the central double bond has the *cis* configuration (in Scheme IV, it is indicated as *trans* for simplicity). In Scheme VI, the geranylgeranyl residues are connected via a sulphonium ylide, a mechanism which requires the presence of a thio-ether grouping (e.g. a methionine residue) at the active centre of phytoene synthetase. This thio-ether group displaces the pyrophosphate from a molecule of GGPP by an S_N2 substitution reaction which involves the inversion of C-1 configuration of the geranylgeranyl group to give a sulphonium ion. The hydrogen atoms at C-1, situated between a double bond and S^+, have a tendency to ionize with the formation of an ylide. This alkylates a second molecule of GGPP (again with inversion at C-1) to give a lycopersyl-sulphonium ion (lycopersene is the C_{40} homologue of squalene). The central double bond of phytoene is then introduced by normal *trans* elimination of the -S-enzyme and a proton from the adjacent methylene group. Retention of both *pro-R*-hydrogens at the centre of the molecule will occur only if the configuration of the central double bond which is formed is *cis*. Further, the mechanism does not require the participation of NAD^+ and $NADP^+$ and this has been demonstrated experimentally with non-aqueously prepared chloroplasts which will synthesize phytoene from MVA (ref. 11).

The experiments reported in this communication indicate that the various stereospecifically labelled mevalonic acids are powerful weapons in elucidating the details of carotenoid biosynthesis. They have proved equally effective in studies on sterol biosynthesis[3] and are currently being used to probe into the details of the formation of these fascinating compounds.

Summary

Recent developments in the biosynthesis of carotenoids from the biological isoprene precursor, isopentenyl pyrophosphate, have been reviewed. Various stereospecifically labelled mevalonic acids have been used to show that one hydrogen is stereospecifically lost at each step in the formation of geranylgeranyl pyrophosphate, a carotenoid precursor. It has also been shown that α-ionone and β-ionone rings of the carotenes are formed from a common ion and not by any interconversion of the formed rings. A mechanism for the formation of phytoene has been proposed; this accounts for the *cis* configuration of the central double bond in phytoene.

Acknowledgement

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Transfer Ribonucleic Acid & the Genetic Code

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THE species of nucleic acids which acts as adaptor[1,2] in the translation of genetic information into protein molecules is known as transfer RNA (tRNA). The involvement of an RNA fraction as intermediates in protein synthesis was first shown by Hoagland *et al.*[3,4]. When a cell extract is centrifuged at 100000 *g.*, ribosomal RNA sediments to the bottom of the tube as ribosomes, while tRNA remains in the supernatant fraction. Most of the ribosomal RNA is precipitated in 1·0*M* sodium chloride solution leaving tRNA in solution. Since this species of nucleic acid is soluble under conditions at which the major portion of RNA is insoluble, it has been designated soluble RNA or sRNA. The sRNA fraction combines with amino acids forming aminoacyl derivatives. Hence the term sRNA has been used to mean amino acid acceptor RNAs, although the soluble fraction contains other species of nucleic acids, such as fragments of messenger RNA and 5S RNA. There are specific RNAs for each of the amino acids[5]. The formation of the ester bond between the RNA and the amino acid is brought about by specific enzymes known as aminoacyl RNA synthetases. During protein synthesis the amino acids carried by the RNAs are transferred to the growing polypeptide chains through the interaction of messenger RNA, ribosomes and transfer enzymes. Because of this function this class of RNAs is called tRNAs. The latter terminology is being increasingly used. The terms sRNA and tRNA are used freely in this article.

As there are 20 different naturally occurring amino acids, there must be at least 20 different tRNAs in any organism. This number, however, is usually much more than 20 as in some cases there are more than one tRNA specific for the same amino acid[6]. Probably, the number of tRNAs is 40-50. A tRNA, in addition to its specificity for a particular amino acid, has specificity towards codons on the messenger RNA[7]. The codons form hydrogen bonds specifically with the anticodons on the tRNA and this specific interaction is responsible for the particular arrangements of amino acids in a protein corresponding to a particular messenger RNA. A tRNA can recognize multiple codons[6,8]. Although the code appears to be universal, the tRNAs, which read the code, appear to be different in different species[9].

tRNAs from different sources do not vary much in their molecular weights. Values ranging from 23000 to 30000 have been noted. Because of the comparatively low molecular weight there has been much interest in the purification and study of the structure of specific tRNAs. The structures of 5 tRNAs have been reported[10-13]. In order to draw general conclusions regarding the amino acid specificity and the codon specificity of a tRNA, the structures of several of the species must be known. The announcement of the structures of more tRNAs, therefore, will be awaited with great interest.

Isolation of tRNA

A mixture of all the specific tRNAs may be prepared from any natural source by simple methods. Brown[14] has reviewed in some detail the various methods of preparation. Two general methods employed for the extraction of RNA from the cells are the hot detergent method[15] and the phenol extraction method[16]. Yeast sRNA may be prepared by the hot detergent method as follows. A 2 per cent solution of a detergent, such as sodium dodecyl sulphate (SDS), containing 0·05*M* phosphate buffer and 5 per cent ethanol is heated to boiling and the yeast cells are added little by little to the hot mixture. After addition of the cells, the mixture is kept at 90°C. for a few min. Under these conditions, the cells are broken and the proteins are denatured. The mixture is cooled rapidly in ice and then centrifuged. The RNA in the supernatant solution is precipitated by the addition of 2 volumes of ethanol and collected by centrifugation. The crude RNA is washed twice with 75 per cent ice-cold ethanol and finally with 80 per cent ethanol. RNA, thus prepared, is a mixture of ribosomal RNA and sRNA with polysaccharide contamination. Ribosomal RNA can be removed from the mixture by precipitation with 1·0*M* sodium chloride[15] or with ammonium sulphate[17] and polysaccharide by extraction with 2-methoxyethanol[18]. A more recent method of removing ribosomal RNA and polysaccharides is by adsorption on DEAE-cellulose at 0·1*M* salt concentration and elution with 1·0*M* sodium chloride[19]. The tRNA prepared using hot detergent usually contains a small percentage of degraded RNA.

The method involving extraction with phenol is the one which is widely followed now. The original method has been modified to suit the needs of various investigators. At present, treatment with phenol and the subsequent operations are done at room temperature. In the method employed by Holley[20] for large-scale preparation of yeast tRNA, fresh, pressed bakers' yeast (100 lb.) was stirred with 42·7 litres of 88 per cent phenol and 100 litres of water. The mixture was allowed to settle for a week and the clear aqueous layer was siphoned out. The RNA in the aqueous layer was precipitated and washed with ethanol as indicated above. The crude RNA mixture was dissolved in 0·1*M* tris-HCl buffer and passed through a column of DEAE-cellulose previously equilibrated with 0·1*M* tris-HCl. The column was washed to remove any remaining protein and polysaccharides. The column was next washed with 1·0*M* sodium chloride in tris-HCl to elute the sRNA from the column. It was then recovered from solution by precipitation with 2 volumes of ethanol. The yield was 65 g.

Repeated extraction with phenol is required to remove the adhering proteins completely. Treatment of the extract with 2 per cent SDS solution followed by phenol hastens deproteinization. Klee and Staehelin[21] made a comparative study of the different methods of preparation of yeast tRNA. tRNA prepared by the phenol method was found to be the least degraded. Rammler *et al.*[22] have reported a rapid method of isolation and estimation of tRNA from bacteria involving extraction with an organic solvent which does not interfere in the estimation of RNA by its absorption in the ultraviolet region.

The yield of crude tRNA obtained from different sources varies from 50 to 150 mg. for 100 g. of wet weight of the cells[20,23-25]. Zubay[26] has reported an yield of 5 g. from 2 kg. of wet *Esch. coli* cells. The amount of tRNA in *Esch. coli* is 1 per cent of the dry weight[27]. It constitutes approx. 10 per cent of the total weight of RNA[28]. tRNA preparations invariably contain degraded messenger RNA, ribosomal RNA and DNA. During extraction, if DNA is solubilized, as in the case of liver, it may be removed by fractional precipitation with isopropyl alcohol[24]. DNA and high molecular weight RNA may also be removed by gel filtration on Sephadex G-100 or Sephadex G-200.

General Characteristics of tRNA

The tRNA prepared by any one of the above methods is a mixture of all the specific amino acid acceptor RNAs. It is a white amorphous powder, a solution of which gives strong absorption in the ultraviolet region with a maximum at 260 mμ and a minimum at 230 mμ. An aqueous solution (1 mg./ml.) of tRNA shows an optical absorbancy of approx. 22 at 260 mμ. The ratio of optical absorbancy at 260-280 mμ in different preparations varies from 2·0 to 2·3 and that at 260-230 mμ varies from 2·2 to 2·3 (ref. 27). tRNAs are fairly stable in the absence of nucleases. The amino acid acceptor activity is neither destroyed by heating to 60°C. up to 30 min. nor impaired by precipitation at *p*H 2 (ref. 29). These molecules can withstand a temperature of 100°C. for short periods without losing activity; heating for longer periods may degrade the molecule due to thermal agitation. At 90°C. and *p*H 7·0, a loss of about 63 per cent in activity has been noted after 10 hr. The presence of magnesium ions increases the rate of inactivation[30].

The molecular weight of sRNA obtained from different sources varies from 23000 to 27000 (ref. 31-34). Brown and Zubay[32] have reported that a freshly prepared solution of tRNA from *Esch. coli* had an *S* value between 2·5 and 2·89, while the sample incubated at 30°C. and *p*H 10 had an *S* value of 3·93. The reason for this change in the *S* value is not clear. Specific aggregation[35] as well as non-specific aggregation can give a higher apparent molecular weight.

Each tRNA chain has a common terminal trinucleotide sequence pCpCpA at the end of which the amino acid is attached. Available evidence suggests that the amino acid is attached to the 3′ hydroxyl group of the RNA chain[36]. Under certain conditions the terminal AMP residue is lost *in vivo* without further degradation of the polynucleotide chain[37]. The pCpCpA end is lost by treatment with small quantities of venom phosphodiesterase for a short period. Enzymes, which reintroduce this end on the polynucleotide chain, have been detected in different species[38-40]. At the other end of a tRNA chain, i.e. at the 5′ end, there is invariably a guanine residue to which is attached a 5′ phosphate group. It has been estimated that 78 per cent of the chains have, at the 5′ end pGp, 10 per cent pUp, 7 per cent pAp and 5 per cent pCp[23,41]. Four out of the 5 tRNAs, whose complete structures are known, end in pGp and the fifth one, that of yeast tyrosine RNA, ends in pCp[10-13]. All the tRNA molecules contain small quantities of pseudouridine, ribothymidylic acid and a few methylated bases. Several of these minor bases have been isolated from tRNA preparations and characterized[42-47]. The presence of thiouridine has been demonstrated in *Esch. coli*. It is not present in yeast and rat liver sRNA[48]. A pentanucleotide with the sequence pGpTpΨpCpGp seems to be present in most tRNAs[49]. Serine tRNA I constitutes an exception in possessing a pGTpΨpCpAp sequence instead of the pGTpΨpCpGp sequence[12]. It is believed that the secondary structure for all tRNAs is the same, the clover leaf model[12]. If it is so, it will aid in arriving at the primary structures of tRNAs even without the availability of complete data. It would be possible to eliminate one or more of the many possible primary structures on the basis of the clover leaf model.

Aminoacyl RNA Synthetases

Several investigators have observed the activation of amino acids in the presence of enzymes from the 100000 *g.* supernatant fraction of tissue homogenates[50-54]. Similar enzyme fractions were shown to possess the aminoacyl RNA synthetase activity[55]. It was not certain whether the amino acid activating enzymes and the aminoacyl RNA synthetases were the same enzymes. Berg *et al.*[56] followed the rate of formation of 4 different aminoacyl RNAs in the presence of low concentrations of the appropriate partially purified activating enzymes from *Esch. coli* and found that the rate of formation of aminoacyl RNAs parallels the amino acid-dependent ATP-^{32}PP exchange activities throughout the purification of each enzyme. Clark and Eyzaguirre[57] obtained essentially the same results. These observations provided evidence that amino acid activating enzymes and aminoacyl RNA synthetases constitute the same group of enzymes.

These enzymes are present in the 100000 *g.* supernatant fraction of tissue homogenates. This fraction after dialysis or gel filtration through Sephadex G-25 may be used directly as the enzyme fraction. Yeast enzymes are usually used after dialysis without any further treatment. Quick freezing and storage at low temperature keeps the enzyme activity for several months. For obtaining an RNA-free enzyme, the enzyme fraction may be absorbed on DEAE-cellulose and eluted with 0·35*M* sodium chloride[58] or fractionated with streptomycin sulphate[59-61]. These enzymes are insoluble at *p*H 5·0 and hence it is a usual practice to partially purify

them by precipitation at this *p*H. Such a preparation is called '*p*H 5 enzyme'. Various protein fractionation techniques have been used for the purification of several of these enzymes[24,57,62-68].

There have been conflicting reports concerning the existence of separate specific aminoacyl RNA synthetases and tRNAs for glutamic acid and aspartic acid and their amides. The existence of separate specific activating enzymes and separate tRNAs for glutamic acid and glutamine in mammalian tissues has been reported[69-71]. Zubay[72], on the other hand, could find only one enzyme, the synthetase specific for glutamine in *Esch. coli*, and suggested that *Esch. coli* does not have a synthetase or an acceptor RNA specific for glutamic acid. Two groups of investigators, working with rat liver, yeast and *Lactobacillus arabinosus*, have proved conclusively that separate synthetases and tRNAs exist for aspartic acid and asparagine as well as for glutamic acid and glutamine[73-76]. Magnesium requirements of different enzymes vary considerably and hence in the absence of an appropriate concentration of magnesium, all the enzyme activities may not be observed[76]. Different patterns of amino acid acceptor activities for aspartic acid and asparagine, and glutamic acid and glutamine have been noted with fractions from a DEAE-cellulose column (Cherayil, J. D. & Bock, R. M., unpublished data). It now seems certain that the dicarboxylic amino acids and their amides have separate synthetase and tRNA activities. Separate codons are also assigned for them (Fig. 1).

Studies on the aminoacyl RNA synthetases from different sources show that these enzymes possess species specificity[58,77-83]. Usually, the maximum acceptor activity with an amino acid is observed when the RNA and the enzyme are from the same homologous source. When they are from different sources, usually a reduced acceptor activity or no activity is observed. Relative amino acid acceptor activities of *Esch. coli* and yeast sRNAs when assayed with enzyme extracts from two sources are given in Table 1.

Activity with the heterologous enzyme is lower in all cases. However, this is not always true.

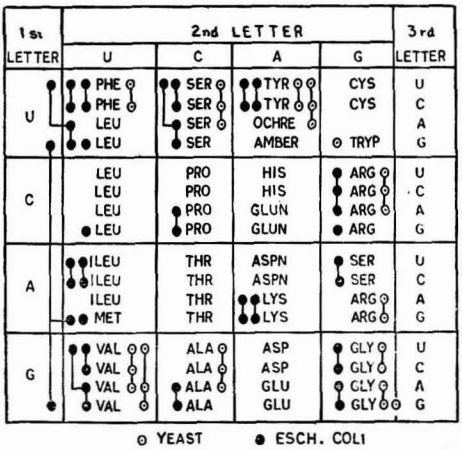

Fig. 1 — Patterns of codon recognition by tRNAs

Table 1 — Relative Amino Acid Acceptor Activities of *Esch. coli* and Yeast sRNAs*

sRNA source	Synthetase source	Acceptor activity				
		Met	Phe	Leu	Lys	Pro
Esch. coli	*Esch. coli*	100	100	100	100	100
	Yeast	37	2	74	59	0·9
Yeast	Yeast	100	100	100	100	100
	Esch. coli	60	12	0·8	70	3

*Data taken from ref. 58.

Table 2 — Relative Amino Acid Acceptor Activities of sRNAs

(Synthetase source, *N. crassa*)

sRNA source	Acceptor activity					
	Ser	Glu	Val	Lys	Leu	Phe
N. crassa	100	100	100	100	100	100
Esch. coli	16	1·6	55	60	340	240

Apparent higher activities may sometimes be noted with heterologous systems. The synthetase from one organism may react with the sRNAs from a source other than homologous. The relative amino acid acceptor activities of sRNAs from different sources are given in Table 2[81].

It is evident from the results given in Table 2 that the amino acid acceptor activity of sRNA from *Esch. coli* is more than that of sRNA from a homologous source in the case of leucine and phenylalanine. It is possible that when the assay is done in the test tube the optimum conditions for each acceptor activity are not met and hence an apparent discrepancy may be observed. At any rate a uniform specificity is not observed with the various species. The presence of multiple aminoacyl RNA synthetases for phenylalanine and aspartic acid in *Neurospora crassa* has been reported[84]. As the presence of only one enzyme for each of the amino acids has been indicated earlier, it is not certain whether multiple enzymes exist in other systems.

Amino Acid Acceptor Assay

For the determination of amino acid acceptor activity, the sRNA is incubated with radioactive amino acid in the presence of aminoacyl RNA synthetase, ATP, magnesium ions, mercaptoethanol and potassium or ammonium ions at a *p*H between 7·0 and 7·5. Under these conditions, the labelled amino acid gets attached to its specific tRNA. The aminoacyl RNA formed in the incubation medium is precipitated out with ethanol or trichloroacetic acid and washed free of the unreacted amino acid and the radioactivity is determined in a counter. A typical assay mixture consists of 50 μmoles of cacodylate buffer (*p*H 7·0), 0·5 μmole of ATP (adjusted to *p*H 7·0), 1 μmole of $MgCl_2$, 2 μmoles of β-mercaptoethanol, 5 μmoles of KCl, 6×10^5 c.p.m. of ^{14}C glycine (specific activity 20 μC/μmole), 0·3-0·5 mg. of sRNA, 0·1 μmole each of the remaining 19 cold amino acids and the enzyme fraction in a total volume of 0·5 ml. (ref. 56). For some of the

synthetases, 5- to 10-fold more of magnesium ions are required for optimum activity[76]. Cytidine triphosphate (CTP), equivalent to one-fourth ATP, is, sometimes, added to the reaction mixture in order to complete the chains that miss the terminal nucleotides[38]. EDTA, equivalent to one-tenth of $MgCl_2$, is also, sometimes, included in the assay mixture.

Simplified Assay Procedure

Removal of the unreacted radioactive amino acid from the incubation mixture requires precipitation of the RNA followed by repeated washing with a solution in which RNA is insoluble. This operation is tedious and time-consuming. The method has been simplified by the use of filter paper disks on which the assay mixture is applied and then the paper disks are washed[85]. The method is suitable for the assay of sRNA samples free from excess salt, urea, organic solvents, etc. It is, however, not suitable for the assay of fractions from a chromatographic column which may contain high concentrations of salt and urea or from a counter-current distribution apparatus which may contain organic solvents. A simple method has been designed to save much of the labour involved in the removal of salt, urea, organic solvent, etc., in large number of quantitative determinations needed in such high resolution methods[86-88]. Aliquots of the chromatographic or counter-current fractions are applied on numbered filter paper disks and washed with 75 per cent cold ethanol in a Buchner funnel to remove the salt, urea or organic solvent. RNA, being insoluble in 75 per cent ethanol, remains embedded in the matrix of the filter paper. After the washing is over, the assay mixture containing the radioactive amino acid, ATP, enzyme, etc., is applied on the paper disks and the incubation is carried out on the wet paper. After incubation, the paper disks are washed free of the unreacted radioactive amino acids in the Buchner funnel, dried and counted in a liquid scintillation counter. The procedure is very simple and dozens of assays can be done with little effort. Development of this assay procedure has contributed much to the success of the fractionation of sRNA on DEAE-cellulose and DEAE-Sephadex columns.

Fractionation of tRNA

The isolation of individual amino acid acceptor RNAs from mixed tRNA preparations is rendered tedious by the similarity of their physical properties, such as molecular weight and base composition. The comparatively low molecular weight and the ease in determining the biological activity created keen interest in finding methods for the purification of this species of RNA. Several methods have been reported for the isolation of individual species of tRNA specific for different amino acids. Because of the inherent difficulties involved, no method of purification which is capable of separating all the specific amino acid acceptor RNAs in a given preparation has been developed. Short reviews on the fractionation of tRNA are available[89,90].

Counter-current distribution — Of all the methods the counter-current distribution (CCD) method has been most successful in the isolation of fairly large amounts of specific amino acid acceptor RNAs needed for structure studies. All the 5 tRNAs whose complete structures are known were isolated by the CCD method, which has been largely developed by Holley and coworkers[91-94]. The method has also been used by other groups of investigators to get specific sRNAs of high purity[95-98]. The partition coefficient of a species of RNA between two immiscible solvents is very sensitive to small changes of temperature and, hence, it is necessary to carry out the distribution in a room in which the temperature is kept constant. In fact, the effect of temperature on the partition coefficient has been made use of in the separation of *Esch. coli* leucine acceptor RNA into 5 peaks[94]. The CCD method, although very efficient in the purification of certain specific transfer RNAs, is not suitable for the purification of many others. Besides, elaborate and expensive equipment are required for the operation. The removal of the solvent from the various counter-current fractions is laborious and time-consuming.

Chemical methods of purification — Chemical methods of isolation depend upon the specificity of a tRNA for its particular amino acid. Mixed sRNA which has been freed of all amino acids is treated with a particular amino acid in the presence of the enzyme, so that the RNA is charged with the amino acid. The aminoacyl RNA formed is separated from the uncharged RNA species by one of the following two general methods. In one method the aminoacyl RNA is made to react with an N-carboxyanhydride of an amino acid which forms a long polymer with the amino acid attached to the RNA. The RNA with the polymer attached to it being very different from other molecules can be easily separated. Mehler and Bank[99] used N-carboxyanhydride of ϵ-trifluorocetyllysine to precipitate charged tRNA species. After separation the polypeptide is removed from RNA by treatment with mild alkali. Three- to fourfold increase in the specific activity of certain sRNAs has been achieved in this method. Simon *et al.*[100] purified tRNA species using β-benzyl N-carboxy L-aspartylanhydride. The RNA was, however, degraded when the polypeptide was removed. The method has been modified and a preparation of 50-60 per cent pure tRNA with an overall yield of 30 per cent has been reported; pronase was used to remove the polypeptide[101].

The second general chemical method of purifying aminoacyl RNA involves treatment of the RNA mixture with potassium periodate. The glycol group at the 3′ end of the RNA chains gets oxidized to a dialdehyde group which being very reactive can be made to combine with a variety of compounds or groups. Valyl sRNA of 90 per cent purity has been prepared by this procedure which involved periodate treatment and a dye addition followed by chromatography on DEAE dextran[102-104]. Zubay[26] used aminoethyl cellulose columns for the removal of the dialdehyde RNA. A water-soluble polymer, polyacrylic acid hydrazide, which reacts readily with a dialdehyde has been used in the purification of valine acceptor RNA[105]. Mirzebekov *et al.*[106] employed this method to further purify the RNA after preliminary purification by the counter-current distribution method. Saponara and Bock[107] employed a phenylhydrazine resin prepared from *p*-aminobenzyl

cellulose by diazotization followed by reduction with sodium borohydride for the separation of the dialdehyde derivative from charged sRNA. The disadvantage of the method involving oxidation with periodate is quite obvious; for the sake of one species of sRNA all the other 19 species must be destroyed.

The success of the chemical methods depends upon the efficiency with which all the amino acids are removed and then recharged with the desired amino acid. The extent to which the reactions can be carried out also determines the overall yield and purity. The usual practice to discharge the RNAs of amino acids was to incubate the sample at *p*H 10 for approximately 1 hr and then separate the RNA from the mixture[108]. Treatment at *p*H 10 usually degrades the RNA to some extent. Sarin and Zamecnik[109] found that tris-HCl at *p*H 8 can be successfully utilized to strip and relabel sRNA without loss of its specific activity. Chemical methods of purification will be very useful in achieving high degree of purity after a preliminary purification has been effected by other methods.

Chromatographic methods of purification—Partition chromatography and ion-exchange chromatography have been used for the fractionation of tRNA. Partition chromatography may be considered as a simpler way of carrying out the counter-current distribution on a column. The column is packed with a material such as Sephadex which has been soaked with one of the immiscible solvents and it is equilibrated with the other solvent which forms the mobile phase. The sample is applied to the column and then eluted with the mobile phase. Various molecular species emerge from the column on the basis of their partition coefficient between the two solvents. Tanaka *et al.*[110] employed this method for the purification of serine sRNA. Muench and Berg[111] modified this method by employing a gradient of the organic phase. Kelmers *et al.*[112] developed a chromatographic system based more or less on the same principle, in which a sodium chloride gradient is used to elute RNA. Using a 8 ft column, they obtained 74 per cent pure phenylalanine sRNA. Partition columns are usually very sensitive to changes of temperature and hence the temperature should be kept constant to get reproducible results.

Several attempts have been made to purify tRNA on a variety of ion-exchange columns. Smith *et al.*[113] used a column of cato-8, a cationic starch exchanger, and obtained partial resolution of leucine and tyrosine acceptor RNAs. The suitability of hydroxylapatite columns in the fractionation of tRNA was indicated by the work of Hartmann and Coy[114]. Marked separation of certain species has been observed by various investigators on this type of columns[115-117]. Only fine particles of hydroxylapatite were found to be suitable[117]. The flow rate on these columns is usually very low. Sueoka and Yamane[118] employed methylated albumin columns for the fractionation of tRNA. The method has found use in certain interesting biochemical studies[119,120]. The capacity of methylated albumin columns is low and hence it is not suitable for large-scale purification of tRNA. An attempt has been made to increase the capacity of the column using silicic acid instead of kieselguhr as support for methylated albumin[121].

DEAE-cellulose and DEAE-Sephadex have several qualities which make them good ion-exchange materials. They have high capacity, have good flow characteristics and are commercially available. The possible use of these ion exchangers for the purification of tRNA was indicated by the studies of Kawade *et al.*[122]. Urea was found to influence the elution profile of tRNA from these columns[87]. In addition, the order of elution of certain tRNA species was found to be reversed when conditions such as *p*H, urea concentration and column matrix type were changed. One of the columns which gives high resolution is a DEAE-Sephadex column at *p*H 4·5 in the presence of 7·0*M* urea developed with a linear sodium chloride gradient. Several tRNAs have been resolved into multiple peaks on this column[9,87,123]. *Esch. coli* glycine RNA, which gives a single peak in counter-current distribution, resolves into two peaks on a similar DEAE-Sephadex column[9]. Chromatographic separation of tRNA on DEAE-cellulose and DEAE-Sephadex has been shown to be suitable for large-scale purification[124]. tRNA species have increased affinity for an ion exchanger at higher temperature[125]. A procedure, in which the effect of temperature on adsorption on DEAE-cellulose and DEAE-Sephadex has been utilized in purification, has been reported[126-128]. Up to 5 multiple peaks are observed for certain species. Unless the temperature gradient is controlled very efficiently, column artefacts are likely to be produced in this method. Paper chromatography of sRNA was performed by Jacobson and Nishimura[129]. Fractionation of *Esch. coli* sRNA on dextran gel has been reported[130]. The latter methods are only of limited use.

Structure of Transfer RNA

Raj Bhandary and Stuart[90] have recently reviewed the literature on the structure determination of nucleic acids. Therefore, only an outline of the procedure used for structure determination is presented here. The essential steps in the determination of the structure are purification of the particular tRNA, digestion of the purified RNA with specific endonucleases and determination of the sequence of the oligonucleotides obtained and finally partial controlled degradation of the RNA to obtain large fragments, which are analysed for their oligonucleotides. From the data the whole molecule is reconstructed. Labelled RNA in the crude mixture or partially purified state has been used for the determination of the nucleotide sequence near the labelled end. Lagerkvist and Berg[131] labelled the 3′ end of sRNA with ^{32}PCTP after pyrophosphorolysis, digested it with endonucleases and separated the radioactive oligonucleotides on DEAE-cellulose. Analysis of these oligonucleotides showed that 69 per cent of the chains had adenine residue near CpCpA end and 20 per cent had guanine; no cytidine was noted in any of the chains. Mixed sRNA labelled with serine, glycine, threonine and alanine has been used to find the sequences near the 3′ end of the respective chains[132,133]. By this method it has been shown that valine sRNA exists in two molecular forms with two different nucleotide sequences near the 3′ end[134]. Young and Khorana[135]

have reported a method for labelling the 5′ end of an RNA chain, and determining the sequence near the 5′ end. However, these methods are of only limited use. Purified sRNA in fairly large amounts is required for the determination of the complete structure. Purification methods have already been discussed. A sample of approximately 70 per cent purity is sufficient for most structural studies, although a sample of 100 per cent purity is preferable. Holley *et al.*[136] used alanine sRNA of 90 per cent (±30 per cent) purity for sequence determination.

Digestion with specific nucleases — The endonucleases widely used in the study of nucleic acid structure are pancreatic ribonuclease and takadiastase ribonuclease T_1, both of which are commercially available. Pancreatic ribonuclease cleaves an RNA chain next to pyrimidine nucleotides, so that the oligonucleotides end in 3′ uridine and cytidine residues. Typical oligonucleotides formed in pancreatic ribonuclease digest are ApCp, GpUp, ApGpUp, GpGpCp, etc. Ribonuclease T_1 cleaves an RNA chain next to guanine and ionosine residues, so that the oligonucleotides formed are UpGp, ApGp, UpIp, CpUpApGp, etc.[137]. For complete digestion of a sample of RNA with pancreatic ribonuclease, the sample is incubated with approximately one-twentieth of the weight of crystalline pancreatic ribonuclease (Worthington) in phosphate buffer of *p*H 7·0 at 37°C. for 12-16 hr[136]. The conditions for digestion with ribonuclease T_1 are essentially the same. Incubation at low temperatures with less enzyme for shorter periods of time results in incomplete digestion, so that larger fragments are formed. Addition of magnesium ions helps to keep certain regions less vulnerable to attack by the nuclease. By this means it is possible to effect specific cleavages at certain points in the RNA chain resulting in large fragments[138]. Degradation into specific large fragments facilitates arriving at the final complete structure. Herbert and Smith[139] have shown that GMP sites nearest the acceptor end are more susceptible to the action of ribonuclease T_1; they carried out the digestion with the nuclease at *p*H 5·4 at which the amino acid remains attached to the RNA.

Two other nucleases used in sequence studies are snake venom phosphodiesterase and spleen phosphodiesterase. The former is an exonuclease which degrades nucleic acid from the 3′ end in a stepwise manner[140]. For the proper functioning of the enzyme, the 3′ phosphate group must be removed from the 3′ end. This may be done with alkaline phosphatase. Complete digestion of the dephosphorylated oligonucleotide with snake venom phosphodiesterase produces one nucleoside and the rest nucleotides as indicated below.

$$ApUpCpG \rightarrow A+pU+pC+pG$$

Identification of the nucleoside establishes the identity of the 5′ end of the oligonucleotide. Partial degradation of the oligonucleotide produces a series of intermediate compounds

$$ApUpCpG \rightarrow ApUpC+pG$$
$$ApUpC \rightarrow ApU+pC$$

If these intermediate compounds are separated and hydrolysed with alkali, the 3′ ending base appears as a nucleoside and the rest as nucleotides with 3′ phosphate groups. For example, ApUpC on hydrolysis with alkali produces

$$ApUpC \rightarrow Ap+Up+C$$

Identification of the nucleoside, therefore, establishes the 3′ end of the intermediate compound. Holley *et al.*[141] employed this method for the determination of the sequence of certain large oligonucleotides obtained from alanine sRNA. Spleen phosphodiesterase degrades RNA from the 5′ end. Its mode of action is complementary to that of venom phosphodiesterase[142].

In the case of certain trinucleotides and tetranucleotides obtained by digestion with one of the two enzymes, pancreatic ribonuclease or ribonuclease T_1, the sequence is easily obtained by treatment with the other enzyme. For example, when an unknown tetranucleotide from the T_1 ribonuclease digest is treated with the pancreatic ribonuclease, if the products formed are two dinucleotides containing Ap and Up in one and Ap and Gp in the other, then the complete sequence of the tetranucleotide is ApUpApGp. In some cases additional treatments as indicated above are necessary. The presence of certain rare bases makes the analysis more difficult[13,46]. However, the presence of minor bases helps to get specific overlaps and arrive at the final unique structure.

Separation of nucleotides and oligonucleotides — Raj Bhandary and Stuart[90] discussed the various principles involved and the methods followed in the separation of nucleotides and oligonucleotides. Column chromatography, paper chromatography and paper electrophoresis are the three methods widely followed in the separation of the various species present in an sRNA digest. For the separation of mononucleotides, chromatography on Dowex-1 resins is the method most widely used[133,143-145]. Katz and Comb[146] developed a method in which Dowex-50 resin is used. Several paper chromatographic systems are being used for the separation of nucleosides, nucleotides and oligonucleotides[147-150].

DEAE-cellulose and DEAE-Sephadex are largely used in the separation of oligonucleotides. The binding of an oligonucleotide to the ion exchanger depends on the net charge as well as the ratio of the purine and pyrimidine residues. Tomlinson and Tener[151] developed a chromatographic system involving the use of 7·0*M* urea at *p*H 7-7·5, which separates oligonucleotides largely on the basis of their charge. By lowering *p*H it is possible to achieve separation on the basis of the base composition also[152,153]. The oligonucleotides may be first separated on the basis of charge or chain length and each of the peaks may then be subjected to chromatography at a lower *p*H to get separation on the basis of the base composition. Rechromatography under a different set of conditions usually gives resolution of species which are not resolved under a given set of conditions. Apgar *et al.*[154] separated two large pieces obtained from alanine sRNA by rechromatography at 50°C. Sanger *et al.*[155] developed an elegant two-dimensional paper electrophoretic method for the separation of oligonucleotides.

The present method for the determination of the complete sequence of a tRNA requires 0·5-1·0 g. of a specific tRNA of 80-90 per cent purity. A large number of precise quantitative determinations go into the final structure. It may become necessary to develop new methods to overcome the difficulties caused by the presence of certain rare bases or a particular oligonucleotide sequence[13]. The development of micro techniques of analysis or simplification of the present methods of purification of tRNA will make structure studies easier and less time-consuming.

Secondary and Tertiary Structure

The secondary structure of a polymer depends upon the primary sequence of monomer units and their resultant interaction among themselves. In the case of RNA, adenine can interact with uridine and guanine with cytosine forming hydrogen bonds. In alanine tRNA there are no long complementary sequences that could give A paired with U and G paired with C. In fact, the longest complementary sequences contain only 5 nucleotides so that double-stranded regions are relatively short[10]. If the alanine tRNA chain is twisted in such a way that the maximum number of base pairs is formed, then the molecule falls into the shape of a clover leaf. This may be done with all the other tRNAs whose structures are known[12,13]. It is tempting to speculate that all tRNAs have this common secondary structure. The model of alanine tRNA is shown in Fig. 2.

Under certain conditions some species of tRNA lose their amino acid acceptor ability. Possibly under these conditions the conformation or the tertiary structure of the molecule somehow changes. In some cases it has been shown that aggregation of the molecules is the cause for the loss of acceptor ability[35,156]. Yeast glycine tRNA resolves into three distinct species upon chromatography on DEAE-Sephadex. Only one of the species aggregates[6,35]. This type of specific aggregation is noted with other species of tRNAs also. Lindahl *et al.*[157] observed loss of acceptor activity under certain conditions. They opined that it is not due to aggregation but due to some conformational changes in the molecule. Gartland and Sueoka[158] reported a similar observation. At present the nature of these various forms and the cause for their formation are not known.

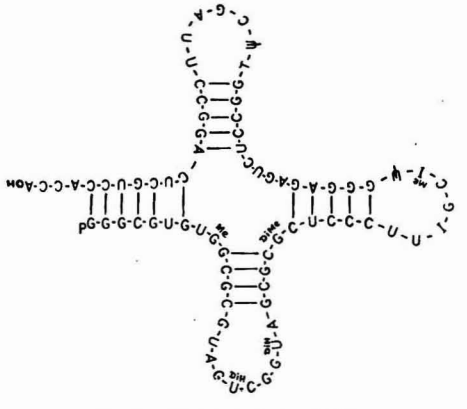

Fig. 2 — Model of alanine tRNA

Codon Specificities of tRNA

The function of tRNA in protein biosynthesis has been discussed in a number of reviews[159-161]. tRNAs carrying amino acids get attached to specific triplets on the mRNA through hydrogen bonds between the codon on the messenger RNA and anticodon on the tRNA, the binding being stabilized by ribosomes which have specific sites for tRNA attachment. When two aminoacyl RNAs are held together on the ribosomes, the two amino acids carried by the tRNAs come close together so that they are linked together by an enzyme in peptide bond. The tRNA which loses the amino acid, having less affinity for binding, is released from the mRNA-ribosome complex. Another tRNA carrying an amino acid gets attached to its specific codon near the tRNA which now carries the dipeptide. The third amino acid carried by the incoming tRNA gets linked to the dipeptide forming a tripeptide and the process continues[162].

In the first step of protein synthesis, tRNA attaches itself to its specific codon on the mRNA through its anticodon. There are 64 theoretically possible triplet codons. It now seems certain that all the 64 codons are meaningful codons[163,164]. Study of the codon recognition of a tRNA by a simple technique developed by Nirenberg and Leder[165] and also cell-free incorporation of amino acids into polypeptides performed with ribopolynucleotide messengers of known sequence[166] show that while there is strict specificity of tRNA for the first two letters of a codon a tRNA can recognize multiple codons differing in the third letter only[9,123,167]. The general patterns observed are as follows:

(1) A tRNA species recognizes both U and C in the third position: Several examples of this type of recognition have been observed. Purified yeast phenylalanine tRNA recognizes the trinucleotides UpUpU and UpUpC (abbreviated UUU and UUC). One species of yeast as well as *Esch. coli* glycine tRNA is preferentially bound to GGU and GGC. Similarly, a species of purified serine tRNA from *Esch. coli* recognizes UCU and UCC. *Esch. coli* isoleucine tRNA responds to AUU and AUC.

(2) A tRNA recognizes both A and G in the third position: Examples of this type of recognition observed are yeast glycine tRNA I as well as *Esch. coli* glycine tRNA I (GGA and GGG), yeast arginine tRNA II (AGA and AGG), *Esch. coli* valine tRNA I (GUA and GUG) and *Esch. coli* serine tRNA II (UCA and UCG).

(3) A tRNA species recognizes U, C and A in the third position: Yeast alanine tRNA (GCU, GCC and GCA), yeast valine tRNA I (GUU, GUC and GUA) and yeast arginine tRNA I as well as *Esch. coli* arginine tRNA II (CGU, CGC and CGA) belong to this class.

(4) A tRNA species recognizes only G in the third position: Yeast glycine tRNA III (GGG), *Esch. coli* arginine tRNA I (CGG), *Esch. coli* leucine tRNA I and III (CUG and UUG respectively), *Esch. coli*

TABLE 3 — WOBBLE HYPOTHESIS

Third letter in the anticodon	Possible third letters in the codon
U	A or G
G	C or U
C	G
A	U
I	C, U or A

methionine tRNA (AUG) and tryptophan tRNA (UGG) belong to this class.

The general patterns of multiple codon recognition observed are consistent with the postulates of Crick's 'wobble hypothesis'[8] and Bock's 'assisted tautomeric shift' model[168]. According to the wobble hypothesis, the first two letters in the codon and anticodon pair in the usual manner (Waston-Crick pairing). In the case of the third letters, however, there is some kind of 'wobble' or 'play'. The possible third letters in the codon corresponding to the various bases in the anticodon of the tRNA are shown in Table 3. A tRNA which is specific for U alone in the third position of the codon has not so far been discovered. All the species so far purified recognized the codons XYU and XYC and not XYU alone.

One experimental result that goes against Crick's wobble hypothesis is that one species of valine tRNA recognized the codons GUA, GUG and GUU[167]. Here the multiple recognition is for A, G and U in the third position. Two other species, leucine tRNA and serine tRNA, show this type of specificity (Bock, R. M., personal communication). Bock's assisted tautomeric shift model can explain this type of specificity. It is assumed that whenever the base in tRNA, which recognizes the third codon base, is a 6-keto base (G or U), the environment in sRNA or the ribosome will assist a tautomeric shift to the enol form. Thus, GU and UG pairs will have resultant stability comparable to GC and UA. It is further assumed that inosine reacts exactly like guanosine but can, in addition, pair with adenosine, which has rotated 180° around its glycosidic bond, permitting N-7 and 6-amino group to pair with inosine. When the base is pseudo-U or 4-thio-U, a single H bond with the tautomeric form of the base will permit recognition of U in addition to the A and G recognized when the third base in tRNA is U. This model may be considered an improvement over Crick's wobble hypothesis.

tRNA Redundancy

There are three species of glycine tRNA in yeast, two of which recognize the same codon, GGG. Glycine tRNA I recognizes GGA and GGG and glycine tRNA III recognizes GGG, i.e. both species code for GGG. Several cases of such redundant tRNAs have been noted[9,169]. Two species of isoleucine tRNA isolated from *Esch. coli* code for the same triplets AUU and AUC[9]. The biological need for redundancy at the tRNA level is not clear. It may be assumed that it is a safeguard against mutation.

Chain Initiation

There are two methionine tRNAs in *Esch. coli*. Only one of them can be converted to its N-formyl derivative[170]. The species that can be formylated is supposed to be the chain initiator in protein synthesis. The codon recognition pattern of this species is different from that of the other tRNAs[171,172]. It recognizes the triplet AUG and in addition two other triplets UUG and GUG which are the codons for leucine and valine respectively. In this case it would appear that the specificity for codon recognition has been lost. However, it is likely that UUG and GUG are recognized by leucine and valine tRNAs when they occur in an internal position in the mRNA but recognized by N-formylmethionine tRNA at the 5′ terminal position. According to this, formylmethionine must be the N-terminal amino acid in every protein. In contrast to this several others occur as N-terminal amino acids in various protein molecules. It is possible that formylmethionine is removed by a specific enzyme from the nascent protein once it is formed. Alternately, there could be several chain initiating tRNAs or the environments in the ribosome could make any tRNA a chain initiator. In this connection, the occurrence of N-acetyl amino acids in various proteins is of interest.

Chain Termination

It has been found that when a synthetic messenger like polyuridylic acid is used in a cell-free protein synthesizing system, the polyphenylalanine formed is attached to the 50 S ribosomes[173]. However, when a natural messenger is used free polypeptide is released into the medium[174]. This suggests that natural messengers possess the mechanism of spontaneous chain termination which is lacking in synthetic messengers. When certain polynucleotides containing A and U are used as messengers, some free polypeptides are released into the medium[174,175]. This suggested that the signal for chain termination is contained in certain specific trinucleotides. In fact, premature termination of a protein chain has been noted in a class of mutants known as amber and ochre mutants[176,177]. Using mutagens whose mutational mechanism is known and analysing the pattern of base substitutions in these mutants the amber and ochre codons have been identified to be UAG and UAA respectively[176,178]. These are known as nonsense codons. UGA also has been shown to be a nonsense codon[179]. Mutation in the gene introduces these triplets in the mRNA, so that the polypeptide chain terminates prematurely at these points forming incomplete proteins. In suppressor mutation the effect of the earlier mutation is partially suppressed[180-183]. In a few cases examined it has been found that suppression leads to insertion of serine at the site replacing glutamine or tryptophan in the protein of the wild type[181]. It is assumed that serine is put at the site of mutation by a tRNA which carries serine. Such a species of tRNA has been termed a suppressor tRNA. The anticodon of the suppressor tRNA is modified such that it is able to recognize the nonsense triplet. It has been shown in one case of suppressor mutation that both UGA and UAA specify tyrosine[184]. It is not known

whether there exists a single species of tRNA which recognizes all the three nonsense triplets. The codons for the various amino acids are shown in Fig. 1. The patterns of codon recognition for yeast and *Esch. coli* are shown with circles joined with lines. One species of tRNA codes for all the codons connected by the circles.

Missense Suppression

tRNAs prepared from certain strains of micro-organisms are capable of introducing a wrong amino acid into a polypeptide chain in response to a particular triplet. According to Carbon *et al.*[185] glycine is introduced to the extent of 5 per cent into a polypeptide chain in response to AGA which is a codon for arginine. This effect is seen with tRNA from only a particular strain. It would appear that the mutation introduces some change in the tRNA which results in a low level mistake. In a similar ' missense suppression ' Gupta and Khorana[186] have noted the incorporation of glycine in response to a codon for cysteine to the extent of 2 per cent. The suppressor tRNA in the latter case has been enriched 10-fold by counter-current distribution. Since the mistake is only to a very small degree it must be assumed that no major change has taken place at the anticodon of the suppressor RNA. Probably some subtle changes have taken place at the anticodon or near the anticodon. In addition to the modification of tRNA species other factors also may be involved. Capecchi and Gussin[187] have discussed the various factors that may be involved in suppression. In this connection the effect of methylation on tRNA function is an important factor to be considered. Six different enzymes which specifically methylate the various bases in a tRNA have been isolated from *Esch. coli*[188,189]. Similar methylating enzymes are present in other species also[190]. Methylation of a base at the anticodon or a base near the anticodon can alter its coding properties. Actually such changes have been observed[191,192].

The binding affinity of a tRNA to its codons varies depending upon the triplet as well as the organism from which the tRNA is isolated. In some species certain codons are used more in preference to others. At present it is not clearly known how these variations affect the rate of synthesis of proteins on the mRNAs in an organism. Factors such as temperature and salt concentrations, especially the concentration of divalent metal ions also affect the affinity of a tRNA for its specific codons[193,194]. How these factors affect protein synthesis in various organisms which have quite different environments of growth has not been explored. Viruses multiply only in certain host cells. Do the host cells contain specific tRNAs or do the cells possess the potentiality to synthesize certain specific tRNAs which can code efficiently with the message carried by the virus ? How does the degree of methylation affect the overall rate of protein synthesis ? The code appears to be universal, but the tRNAs which read the code seem to be different in different species[9,195]. Was the code universal or was it evolved ? A number of theories on the evolution of the code have been put forward[196-200]. These are some of the questions, answers to which will be awaited with keen interest.

Cytokinin Activity of tRNA

One of the minor bases that has been isolated from sRNA preparations is N^6-(γ, γ dimethylallyl) adenosine, concentration of which is 0·05-0·1 mole per cent in different samples[47]. As the proportion of this compound is low, only certain species of tRNA can be expected to contain it. Serine tRNA is one of the species in which the presence of this compound has been demonstrated[201,202]. This nucleotide promotes cell division, growth and organ formation in tobacco callus test[203]. It is 10 times more active than kinetin, a plant growth hormone. sRNA preparations from yeast, bacteria, rat liver and monkey liver, muscle and brain possess this activity in varying degree (Bock, R. M., personal communication). When cytokinin activity of tRNA fractions from a DEAE-cellulose column was tested by the tobacco callus assay, it was found that certain fractions had more activity than the others; purified valine and arginine tRNAs had no activity at all[204]. This suggested that only certain species of tRNA possessed this activity. As N^6-(γ, γ dimethylallyl) adenosine has been shown to act as cytokinin it must be assumed that the species which contain this moiety alone are active. It is not known in what state, whether in the macromolecular form or as nucleotide or as nucleoside, it is active. In yeast serine tRNA whose complete base sequence is known, the adenosine with the dimethylallyl group is near the anticodon[12]. The mechanism by which this nucleotide acts as a cytokinin in growth and morphogenesis is not clear at present. The presence of this unusual nucleotide in certain tRNA species, however, adds special importance to its study.

Summary

The review deals with the isolation, chemical characteristics and biological role of transfer RNA. The various methods of isolating mixed tRNA from different sources with an outline of the large-scale preparation from yeast are given. Methods for the preparation of tRNA's specific for a particular amino acid are discussed in detail and the general procedure for the determination of the structure of a purified tRNA is outlined. The enzymes which attach amino acids to the ends of tRNA chains are discussed and a convenient micro-scale assay for tRNA is given. The ' wobble hypothesis ' and the ' assisted tautomeric shift model ' are presented in connection with the discussion on the biological role of tRNA.

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Unesco Conference on the Application of Science & Technology to the Development of Asia

The Council of Scientific & Industrial Research, in collaboration with the UN Economic Commission for Asia, will be organizing this conference (CASTASIA) at New Delhi during 9-20 August 1968. This would be an inter-governmental conference at ministerial level, aimed at furthering the application of science and technology to the development of Asia.

CASTASIA would be the third of a series of regional conferences for the benefit of the less developed areas. The first, dealing mainly with the study and utilization of natural resources, was held in Lagos, Nigeria, in 1964 while the second, on the application of science and technology to the development of Latin America, was held in Santiago, Chile, in 1965.

About 300 delegates from over 30 countries are expected to participate in the conference. Besides the participating countries, observers from various governmental and non-governmental organizations and interested private foundations from different countries would attend the conference.

Mutation: An Appraisal of the Methodology Used in Bacterial Genetics

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ONE of the most powerful tools available to the student of bacterial genetics is the use of mutants deficient in one or another enzymatic activity. Mutant analysis has been of incalculable value in studies aimed at (i) the definition of fine structure and functioning of genes; (ii) the establishment of *in vivo* operation of the genetic code; and (iii) the clarification of how gene activity is governed by induction and repression.

The first step in the study of genetics of any organism employing mutation is the production of mutants in which either one or more specific characteristics originally present in the wild type are missing, or a new characteristic which was not originally present in the wild type now manifests itself. In bacteria this generally takes the shape of a nutritional deficiency, so that one has to add specific amino acids or vitamins or similar factors to the growth medium before the mutant grows, or of acquiring resistance to toxic agents like antibiotics and chemotherapeutic drugs. If the mutation results in damage to a structural component of the cell like a protein or a nucleic acid where it is not possible to add external agents in order to grow the mutant, mutants are selected in which this structural damage exhibits itself only under specific conditions, e.g. higher temperature. Barring the drug-resistant mutations all the other mutations studied in bacteria are lethal mutations in the sense that under conditions in which the original wild types grew, the mutants will not grow. In the first type of mutants lethality is overcome by the addition of specific nutrients to the medium, and in the second type by changing the conditions of growth. The second type of mutants are called conditional lethal mutants.

The essential process in mutation is to treat a culture of wild type bacteria with some mutagenic agent. The commonly used mutagens are ultraviolet light and chemicals like 2-aminopurine, ethane, methyl sulphonate, or N-methyl-N-nitroso-N'-guanidine. Since ultraviolet light is absorbed efficiently by various constituents of complex media (such as purines, pyrimidines and aromatic amino acids), the bacteria should be suspended in phosphate buffer for irradiation for obtaining the best results. The bacterial suspension is placed, preferably with constant stirring, by means of a magnetic stirrer, in a petri dish under the ultraviolet lamp and covered. The time of exposure is governed by covering and uncovering the dish. Samples are withdrawn after exposure to various doses of radiation and plated for survivors. The exposure time is so chosen that 0·1-1 per cent survival is effected. It is necessary to transfer each sample immediately after irradiation to a foil-wrapped flask to prevent photoreactivation.

Among the chemical mutagens, N-methyl-N-nitroso-N'-guanidine (K. & K. Laboratories and Aldrich Chemical Co., USA) appears to be the most promising mutagen. Adelberg *et al.*[1] found that N-methyl-N-nitroso-N'-guanidine induces at least one mutation per treated bacterial cell under conditions permitting over 50 per cent survival. The procedure consists in centrifuging off log-phase cells from the nutrient broth or minimal media, washing them and treating them with 100 μg. N-methyl-N-nitroso-N'-guanidine per ml. buffer at *p*H 6·0 for 15-30 min. Prior to plating, the cells are washed and diluted into nutrient broth. They are permitted to undergo two division cycles to permit segregation. The yield of auxotrophs (organisms requiring growth factors) under these conditions is about 11 per cent. N-methyl-N-nitroso-N'-guanidine has to be handled with great care since it is the most powerful mutagen so far known and it is, therefore, likely to be a potent carcinogen.

The small proportion of auxotrophs which arise by mutation either by ultraviolet or by a chemical mutagen is now isolated and recognized and, in the case of defective mutants, their specific nutritional requirements are identified. The isolation of drug-resistant mutants is fairly simple, as only such mutants will grow if the population is plated on a medium containing the drug. In the case of nutritionally deficient mutants, isolation is generally carried out by the replica-plating method of Lederberg and Lederberg[2]. The mutated culture is diluted and spread on master plates of nutrient agar; the colonies which come up are adsorbed carefully on to a velvet pad and printed on to replica plates containing minimal agar (agar incorporated with minimal medium in which the original wild type grows) and another master plate. When the second master and replica plates are compared, colonies of auxotrophic mutants growing on the master plates are recognized by their absence from the replica plates when the two are superimposed. The auxotrophic mutants must then be purified by replacing, checked for auxotrophy, and their specific growth requirement finally determined by replica plating on minimal agar supplemented by various combinations of amino acids, B group vitamins and purine and pyrimidine bases[3]. As a rule, only a portion of the so-called auxotrophic isolates, identified by their initial failure to grow on minimal agar, turn out to be stable mutants.

The isolation of auxotrophic mutants was greatly facilitated by the introduction of a method based on the mode of action of penicillin[4,5]. Penicillin rapidly kills growing bacteria, but is innocuous to those which are not metabolizing. If, therefore, a mixture of wild type and auxotrophic bacteria is well washed to remove all nutrient material and then incubated in minimal medium containing a lethal concentration of penicillin, the wild types will be killed as soon as they begin to metabolize, but the auxotrophs being unable to grow will remain viable. Thus, a proportionate increase in the number of

auxotroph colonies will be found on subsequent plating. Although penicillin is primarily effective against gram-positive organisms, it is also lethal for a wide range of gram-negative bacteria, such as *Escherichia* and *Salmonella*, if used in fairly high concentration, and especially in synthetic media.

Reliable results with the penicillin-selection method are obtained only by rigorous attention to detail. It is essential that penicillin treatment be preceded by adequate growth of the mutagen-treated cultures in nutrient broth to allow full segregation and expression of the mutants; subsequent starvation of the bacteria in a nitrogen-free medium is desirable, in addition to washing, to ensure metabolic inertness of the auxotrophs. The primary action of penicillin is to induce protoplast formation by interfering with cell wall synthesis[6]; the protoplasts then burst osmotically, releasing nutrients into the minimal medium. If this happens, the auxotrophic mutants also start to grow and they also become susceptible to the action of penicillin. For this reason, the treated bacteria should be restricted to not more than about 10^7 bacteria per ml., the concentration of penicillin should be high (about 1000 units per ml. in the case of *Esch. coli*) and the duration of treatment as short as possible (about 15 hr). The use of hypertonic minimal medium, containing 20 per cent sucrose to prevent the bursting of protoplasts, has been reported to increase greatly the auxotroph yield[7].

As an example of the use of penicillin technique, the isolation of a methionine-requiring mutant of bacteria may be considered. If the mutagen-treated culture is incubated in minimal medium containing methionine instead of in broth, methionine-requiring mutants, but no other type of auxotrophs, can grow and keep pace with the wild type population. The bacteria are harvested from this medium and suspended in a penicillin-minimal medium supplemented with all amino acids except methionine; only methionine-requiring mutants will be unable to metabolize and will be spared by penicillin. After about 15 hr, aliquots of this culture are plated on to nutrient agar for the selection of methionine-requiring colonies of bacteria by the replica-plating method. For the success of this method, highly purified amino acids should be used. It is always preferable before a particular sample of amino acid is used to analyse it by paper chromatography to ensure that no contaminating amino acids are present even in trace quantities.

Suppressor Mutations

When a mutant reverts to wild type as a result of another mutation at a different genetic locus, the second mutation is called a suppressor of the first. If the original mutation imposed a requirement for, say, methionine (met), a suppressor of this mutation is designated as 'su-met', and does not require methionine for growth, just like the wild type. Revertants due to suppressor mutations are usually phenotypically distinguishable from true wild type, as well as revertants due to back mutation at the original site, particularly in displaying a lower grade of function. For example, if suppressors of methionine mutation are to be isolated, a methionine auxotroph should be treated with a mutagen and it should be plated on minimal agar which does not contain methionine. Suppressors of methionine yield colonies on this medium, and these grow more slowly and produce smaller colonies on minimal agar than does the wild type; when the enzyme itself can be estimated, this may be found to be synthesized at a lower rate than normal. Suppressor mutations are of great interest in molecular biology at the present time because of their reported identity with mutations in sRNAs of specific amino acids. Suppressor mutations may be specific to one specific auxotrophic mutation, or it may affect more than one auxotrophic mutation, so that if the original mutation had imposed a requirement for more than one amino acid the suppressor of this mutant does not require any of the amino acids. The latter type of mutations are termed super-suppressors.

Conditionally Expressed Mutations

The above methods can be used only when the blocked reaction can be bypassed nutritionally, either by supplying to the cell the product (or end product) of the blocked reaction or by employing conditions where the product is not essential for growth. However, if an essential reaction in a cell which cannot be bypassed nutritionally is to be blocked, as, for instance, aminoacyl sRNA synthetase, the task is almost impossible, since aminoacyl sRNA preparations cannot be supplied exogenously to bacterial cells, and are indispensable.

In the past few years, much progress has been made in developing variations of mutant technology that offer the possibility of extending the usefulness of this tool to the study of indispensable enzymes. These advances are based on the concept of conditionally expressed mutations, i.e. mutations that exert their full phenotypic effect under one environmental condition (the restrictive condition), but not under another (the permissive condition).

In the study of bacterial enzymes, two selective environmental conditions can now be used: temperature and streptomycin.

The biological activity of proteins depends on the tertiary and quaternary structure of their constituent polypeptide chains. The native or active configuration of a protein is just one of the many possible configurations for its polypeptides, and is probably the most stable one *in situ*. Many of the forces contributing to this configuration are non-covalent and individually weak. Usually they can generate and maintain the appropriate folding only over a narrow temperature range, and the lower range of temperatures at which proteins are inactivated overlaps the range at which bacteria such as *Esch. coli* operate. Epstein *et al.*[8] were probably the first to use this technique for isolating bacteriophage mutants blocked in their essential reactions at a temperature of 42°C., but not at 30°C. Yanif *et al.*[9] employed a similar procedure for isolating mutants with temperature-sensitive aminoacyl sRNA synthetases. Temperature-sensitive mutations have also been used to study other cellular processes[10-12].

Coming to streptomycin-conditional mutations, it is now known that the phenotypic effects of some mutations are partially corrected by growth

in the presence of sub-inhibitory concentrations of streptomycin. The correction is particularly effective in cells possessing certain streptomycin-resistant alleles, and appears to be the result of translational infidelity induced by the interaction of this antibiotic with the ribosome[13]. This situation offers the possibility of isolating mutants that can produce a particular indispensable enzyme, such as aminoacyl RNA synthetase, in a functional form in the presence of streptomycin, but not in its absence. Though no streptomycin-dependent cell has so far been identified as having an altered aminoacyl sRNA synthetase, mutants with other altered enzymes have been isolated[14] through the use of streptomycin.

The task of finding conditionally expressed mutants in a particular enzyme may be divided into three processes. The first involves the production (usually by mutagenesis) and isolation (by penicillin selection) of mutants that grow poorly or not at all under one of the restrictive conditions described above (42°C. or absence of streptomycin), but well under the usual conditions. The second process involves the identification of the specific enzyme lesion in the mutants isolated by one of the above procedures. This process is difficult, but much has been learnt from the behaviour of mutants already identified. In brief, conditional mutants are shifted from permissive to restricted conditions, and kinetics of DNA, RNA and protein synthesis are followed. On the basis of their behaviour in this test, mutants are assigned to various classes. For example, to know which mutants are likely to have primary lesions in protein synthesis, appropriate additional tests (behaviour in the presence of chloroamphenicol, response to bacteriophage infection, response to carbon and energy source shifts, and other nutritional stresses) are carried out on each class. These are then surveyed for mutations in enzymes involved in protein synthesis. The third process is mutant verification. When potent mutagens like N-methyl-N-nitroso-N'-guanidine are employed, it is essential to establish that the particular enzyme alteration detected *in vitro* is in fact the cause of the changed growth. This verification is done in the case of *Esch. coli* by infecting the mutant at low multiplicities with Pl bacteriophage previously grown on normal cells. Transductants[15] are selected for ability to grow at the restricting temperature (in the case of temperature-conditional mutations) or in the absence of streptomycin (in the case of streptomycin-conditional mutations), and then their levels of the altered enzyme are examined. If the particular enzyme alteration detected *in vitro* is in fact the cause of the changed growth, it should have come back to normal in the transductant. Instead of using transductants, recombinants[16] from appropriate bacterial mating experiments can similarly be examined for this purpose.

Mutations Conferring Analogue Resistance

Adelberg[17] developed a method for obtaining derepressed mutants by selecting for strains of bacteria resistant to analogues of the end product. This method has been made use of in studying regulatory mutations in bacteria[18,19]. For example, if mutants in which the regulation for phenylalanine biosynthesis is altered are to be isolated, first mutants resistant to fluorophenylalanine are selected. Some of these mutants have become resistant by virtue of the fact that phenylalanine is now synthesized in unregulated quantities and can compete with the fluorophenylalanine added. The mutation may be in a regulatory gene or an operator gene, and these may be distinguished by appropriate recombination experiments.

Some mutations affect an enzyme in such a way that it has a greatly diminished affinity (higher K_m) for a particular analogue. Historically, it was this fact that led to the first isolation of a mutant with an altered aminoacyl sRNA synthetase. A *p*-fluorophenylalanine-resistant mutant was isolated[20,21] and was discovered to have a phenylalanyl sRNA synthetase which could still competently attach phenylalanine to sRNA, but which differed from the normal enzyme in having a greatly reduced ability to attach *p*-fluorophenylalanine to sRNA.

Summary

Isolating bacterial mutants is of prime importance as the first step towards the study of the various aspects of bacterial genetics. In this review the different methods currently in use for isolating bacterial mutants have been described and the precautions to be used in the methodology have been discussed. These methods can be used for isolating auxotrophic mutants, suppressor mutants, conditionally expressed mutants and mutants resistant to analogues.

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REVIEWS

INTERFEROMETRY by W. H. Steel, Cambridge Monographs on Physics (Cambridge University Press, London), 1967. Pp. ix+271. Price 60s.

This monograph on interferometry gives a lucid account of the theory of interferometry and a description of the techniques applicable to all regions of the electromagnetic spectrum. Thus, the applications dealt with extend from the interferometers using visible light to infrared interference spectroscopy and radio astronomy. The book is divided into 11 chapters and most of the relevant mathematics has been given in the earlier chapters so that one will find the later chapters on the interferometric instruments essentially descriptive. In the description of the instruments, the author has chosen to include a comprehensive range of techniques so that the reader will get a broader view of the whole subject and may in fact find other applications for the instruments. The topics covered in the various chapters include the optical foundations, coherence theory, two and multiple beam interferometers, measurement of phase variations, interference spectroscopy and interference imagery. The bibliography of over 400 references at the end of the book includes, besides original papers, treatises and monographs on optics and mathematics which can serve for further detailed study. As the technique of interferometry is today widely employed by spectroscopists, astronomers, optical designers and meteorologists, solid state physicists and aeronautical engineers, a book like the present one, which seeks to treat the subject with emphasis on the principles and methods, should be found extremely useful in enabling the research workers in these fields to choose the interferometers best suited to their needs.

P. S. NARAYANAN

THE TRIPLE STATE (Cambridge University Press, London), 1967. Pp. x+537. Price 90s.

This volume contains the proceedings of the First International Conference on the Triple State, covering a wide variety of physical phenomena which are known to be associated with molecules having orbital electrons with parallel spins. The conference, organized by the American University of Beirut in February 1967, was attended by about 90 leading workers in this field from all parts of the world and the papers presented at the conference have been well documented in the present volume.

The topics dealt with include spin-orbit coupling, phosphorescence of the triple state, magnetic resonance, magnetic interactions, photochemistry, radiationless transitions, triple excitons, delayed fluorescence, and the triple state in biology. This conference report covers practically all aspects of the triple state and much of the theoretical progress and new techniques that have been evolved since the pioneering works of Lewis, Kasha, Williams and others. Nevertheless, as should be one of the objectives of any conference, the present one highlights the many gaps that still exist and points the way to further work on molecular properties involving the triple state. The extensive coverage of topics and the review papers make the volume a very valuable reference book. The organizers and the editorial board should be congratulated for bringing out the proceedings early so that those interested in triple state research may benefit by the conference.

R. S. KRISHNAN

OPTICAL ROTATORY DISPERSION AND CIRCULAR DICHROISM IN ORGANIC CHEMISTRY, edited by G. Snatzke (Heyden & Son Ltd, London), 1967. Pp. xvi+416. Price $ 13.50

This book is the record of the 23 principal lectures delivered by 17 invited speakers at a summer school on ORD and CD, held at Bonn during the last week of September 1965.

Of the 23 chapters, Chapters 2, 3, 4, 7, 10, 14 and 17 (totalling 186 pages) had already appeared in various scientific journals. Of the remaining chapters, Chapters 1 and 8 (by Prof. P. Crabbe) give a brief introduction to ORD and CD and a comparison of these two techniques. These chapters serve as an excellent introduction to a person who is not already familiar with the subject. Chapter 5 (by Dr Woldbye) describes the instrumentation involved in these methods. An excellent treatment of applications to saturated carbonyl compounds, carboxylic acids and lactones has been given by Prof. W. Klyne in Chapters 9 and 12. Unsaturated ketones have been covered in Chapter 13 in a masterly manner by Prof. G. Snatzke. A valuable chapter (Chapter 11) is on the ORD and CD of chromophoric derivatives of transparent compounds. Solvent effects and temperature dependent CD have been described in Chapters 16 and 18 respectively. Chapter 15 (by Prof. Lavisalles) highlights some of the limitations of these techniques. Various other chapters have been devoted to organometallic compounds, high polymers, chlorines, sulphur containing chromophores and epi-sulphides. Chapter 22 deals with magnetic CD, a technique which holds great promise for non-chirale substances. The last chapter describes how ORD and CD have been employed with singular success in structure determination, in certain cases from the terpene and steroid field.

The book, on the whole, provides excellent material both for the beginner as well as for those interested at research level. The format is beautiful and errors appear to be few (however, there are some, e.g. p. 301, two methyls in structure II missing!).

SUKH DEV

HIGH ENERGY BATTERY by Raymond Jasinski (Plenum Press Inc., New York), 1967. Pp. xv+313. Price $ 17.00

Raymond Jasinski has made an excellent contribution to the science and technology of batteries by writing this highly informative book on high energy batteries,

a subject on which literature is widely scattered and highly inaccessible. The subject of batteries entails the intricate principles of all the different branches of electrochemistry, namely electro-deposition and electro-crystallization, corrosion, passivation, anodic oxidation, solid state reactions, electro-kinetics and electrochemical equilibria, etc. The author has spared no pains in dealing with them lucidly from the basic as well as technological points of view.

The book contains 8 chapters preceded by a short preface and followed by publication and American patent references and the subject index.

After briefing the growth of battery literature in the post-war years, the factors which control the performance of the batteries have been enunciated in the preface. In Chapter 1 these various factors, viz. electrochemical efficiency, faradaic inefficiencies and weight efficiency of the battery materials, have been discussed from the fundamental point of view. Chapters 2, 3 and 4 cover the aqueous electrolyte, non-aqueous inorganic (including fused salt) electrolyte and the non-aqueous organic electrolyte systems respectively. The various anodic and cathodic materials, their performance characteristics and the physico-chemical properties of the electrolytes have been elaborately discussed in these three chapters under aqueous and non-aqueous electrolyte systems. The author has surveyed all the electrode systems potentially useful for battery designs. Information furnished on liquid ammonia, liquid sulphur dioxide, fused salt and several organic electrolyte systems is highly useful for developing new batteries to meet certain specialized requirements.

Chapters 5, 6 and 7 deal with the technological aspects of fabrication and the performance characteristics of batteries. Chapter 5 discusses the electrochemical efficiency and the influence of low temperature, dendrite growth, etc., on the performance of a battery. Weight efficiency and design aspects in relation to the structure and performance efficiency of electrodes has been taken up in Chapter 6. Different types of reserve batteries such as water activated, sea water activated, ammonia activated, automatic activated, fused salt batteries, and dry tape batteries have also been discussed in this chapter. The problems of battery charging and the dependence of the performance of the battery (secondary battery) upon the morphology of electrocrystallization and dendrite growth have been explained in Chapter 7. Various charging circuit systems and controls are also described in this chapter. Chapter 8 is entitled 'State of the art — Performance' and deals mainly with some of the high energy density battery systems such as zinc/silver oxide, magnesium cells, hydrogen oxygen cells, ammonia activated batteries, zinc/oxygen cell, thermal batteries, and organic electrolyte batteries which have already been commercialized or have reached an advanced stage of development. Highly specialized and stringent requirements of high energy density, high efficiency at low temperatures, long shelf-life and short time of activation, etc., of the modern sophisticated equipments which cannot be met by the conventional batteries are attainable in battery systems described in the last chapter.

All the chapters in the book contain very useful data on the components and performance characteristics of the high energy battery systems. The book is an asset for all electrochemists in general and battery technologists in particular.

P. B. MATHUR

THE PRINCIPLES OF HETEROCYCLIC CHEMISTRY by A. R. Katritzky & J. M. Lagowski (Methuen & Co. Ltd, London), 1967. Pp. xvi+183. Price 45s.

The chemistry of heterocyclic compounds has occupied the centre stage of synthetic and degradative organic chemistry for several decades owing to their occurrence in numerous natural and medicinal products. This vast area has often seemed a despair and challenge to the novice in the field from a lack of systematization of the theoretical principles involved in heterocyclic chemistry. Prof. Katritzky and Dr Lagowski achieved a great measure of success in this direction with their monograph *Heterocyclic chemistry* published in 1960. They spotlighted the subject with such clarity that beginners in this branch of organic chemistry need no longer despair of mastering the fascinating behaviour of heterocyclic systems. The book under review is an abridged version of the successful earlier monograph. Within the short space of 175 pages, the authors have successfully conveyed to the reader, " that an encyclopaedic memory is not a prerequisite for the acquisition of a working knowledge of the subject . . .".

The book deals largely with six- and five-membered ring systems containing one or more than one hetero atom. Three- and four-membered rings are allotted a brief space of six pages. The wide range of reactivity patterns of the different heterocyclic systems within the above category are well delineated with excellent formulae and equations.

The reviewer commends this book highly to undergraduate students as a happy introduction to the subject.

B. S. THYAGARAJAN

NEWER METHODS OF NUTRITIONAL BIOCHEMISTRY WITH APPLICATIONS AND INTERPRETATIONS: Vol. 3, edited by Authony A. Albanese (Academic Press Inc., New York), 1967. Pp. xv+527. Price $ 18.50

The book has nine chapters. In Chapter 1, the nutritional and metabolic implications of changes in urinary amino acid levels are reviewed. The now well-known terms used in chromatography have been discussed. In Chapter 2 which is on the nutritional aspects of protein reserves, a subject of much controversy, dietary implications among other aspects are emphasized. Chapter 3 is on *in vitro* methods for the evaluation of protein quality with greater emphasis on PDR index because it was developed in the author's (Sheffner's) laboratory. In Chapter 4 on (nutritional) availability of plant proteins by M. Swaminathan, there are a number of repetitious statements on protein malnutrition (pp. 197 and 220) and on the effects of heat treatment (pp. 209 and 210). While the text (p. 203) cites reference 6 as one of the sources for the data compiled in the unduly long Table VI (pp. 204-5), this reference is not given under the table. There are some obscure statements like: " In view of its high fibre content, coconut

can be used only in small amounts as a protein supplement, and it has therefore been incorporated as one of the ingredients in protein foods by certain workers" (p. 228). In reference 128 (p. 239), relating to a group of workers, the name of the same author has been repeated twice. Chapter 5 on lipoprotein transport reviews broadly "the participation of classes of lipid-protein complexes in the transport of fats, and the relationship of lipid transport to the development of certain disease states". Chapter 6 on chemical assay of adrenocorticosteroids aptly presents and evaluates this methodology. This chapter could have been prefaced, advantageously, with more information on the role of these hormones in metabolism. Chapter 7 gives "a proper perspective to current views on the problem of zinc metabolism and nutriture in animals", especially developments in this direction since 1960. Chapter 8 is "concerned mainly with recent developments in our knowledge of biochemistry of folate compounds with emphasis on folate deficiency in man". Chapter 9 is entitled 'Functional evaluation of nutritional status: Thiamine'.

From the title of the book, one would expect a major emphasis on methods and methodology. Such is not the case in many of the chapters. Thus, the scope being not well defined, coverage is free, and includes reproduction of literature data *in extenso* where a reference or representative data would have sufficed. The publication, however, serves as a source book of information for research workers and the copious collection of references at the end of each chapter is very useful.

Being an effort of the Academic Press, the production is up to their standards of high quality.

M. SRINIVASAN

BOOK NOTE

COMPARED ACTIVITIES OF THE MAIN ABSTRACTING AND INDEXING SERVICES COVERING PHYSICS, CHEMISTRY AND BIOLOGY DURING THE YEAR 1965 (ICSU Abstracting Board, Paris), 1967. Pp. 83. Price $ 5.00

This report issued by the Abstracting Board of the International Council of Scientific Unions gives detailed information about the main abstracting and indexing services which are members of the board: Referativniy Zhurnal, Bulletin Signaletique, Chemical Abstracts Service, Biological Abstracts, Physikalische Berichte, Chemisches Zentralblatt and Astronomischer Jahresberichte. The number of periodicals scrutinized, non-periodical literature covered, number of abstracts published, abstracting and indexing practices, use of computers, etc., are described, compared and commented upon.

PUBLICATIONS RECEIVED

DESIGN OF BYPRODUCT RECOVERY UNITS OF COKE PLANTS by I. E. Korobchanskii & M. D. Kuznetsov, translated by S. Sarkar (Asia Publishing House, Bombay), 1967. Pp. viii+293. Price Rs 28.00

MULTICHANNEL TIME SERIES ANALYSIS WITH DIGITAL COMPUTER PROGRAM by Enders A. Robinson (Holden-Day Inc., San Francisco), 1967. Pp. xxiii +298

NETWORK ANALYSIS AND TRANSMISSION LINES by George J. Konnully (Asia Publishing House, Bombay), 1967. Pp. vii+339

COMBUSTION OF PULVERIZED COAL by M. A. Field, D. W. Gill, B. B. Morgan & P. G. W. Hawksley (British Coal Utilization Research Association, Leatherhead, UK), 1967. Pp. xv+413. Price 50s. or $ 7.00

DIMENSIONAL ANALYSIS AND HYDRAULIC MODELS TESTING by H. M. Raghunath (Asia Publishing House, Bombay), 1967. Pp. v+112. Price Rs 15.00

TOPICS IN SEVERAL PARTICLE DYNAMICS by K. M. Watson & J. Nuttall (Holden-Day Inc., San Francisco), 1967. Pp. viii+121. Price $ 7.50

HIGH SPEED PULSE TECHNOLOGY: Vol. II, by Frank Früngel (Academic Press Inc., New York), 1965. Pp. xiii+477. Price $ 21.50 or 176s.

SCIENTIFIC EXPERIMENTS FOR MANNED ORBITAL FLIGHT edited by Peter C. Badgley (American Astronautical Society, Washington DC), 1965. Pp. xiv+358

PHYSICS OF THE MOON edited by S. Fred Singer (American Astronautical Society, Washington DC), 1967. Pp. xi+248

SHELL ROOF ANALYSIS by A. Paduart, translated by F. H. Turner (Oxford & IBH Publishing Co., Calcutta), 1967. Pp. 97. Price Rs 26.00

NOTES & NEWS

New coaxial cable system

The L-4 coaxial cable system, using the 20-coaxial cable, is a new system for long-haul toll transmission which follows the earlier L-1 and L-3 systems. It furnishes economical, high quality long-haul telephone circuits with the advantage that it provides for twice as many circuits per pair of coaxials as the L-3 system, with apparently less noise. Using simple solid state repeaters designed for mounting in underground repeater stations, L-4 is intended to be installed as a hardened system that will survive natural disasters or even close nuclear blasts and provide continuous service. A field trial of the L-4 system was completed and the first commercial service between Washington DC and Miami, Florida, has started.

The L-4 system design centres on a simple basic repeater which has very good frequency range, load-carrying capacity, reliability, and modulation performance. One-way repeaters for four coaxials are mounted in a cylindrical, waterproof case of 15·5 in. diameter and 26 in. length. A ten by six feet underground station accommodates five cases, serving 20 coaxials. The overall plan of equalization, regulation and monitoring allows all maintenance except for physical replacement of equipment to be performed from manned main stations every few hundred miles along the routes. Power from the line comes from d.c. to d.c. converters using solid state silicon devices. With the 24 V. batteries at main stations as the source, the converters supply a constant 0·5 amp. at up to 1800 V. to the centre conductors of the coaxials. The use of d.c. line power effects a substantial reduction in costs and engineering efforts. Three major categories of equipment designed for the L-4 system are: (i) the line and main station repeaters; (ii) a remote control system, used for line equalization and fault location; and (iii) a new master group multiplex.

The L-4 system, transmitting 3600 two-way telephone channels over a pair of coaxials, would be able to cope with the traffic on the major long-haul telephone routes which is growing by about 2500 circuits per year. The present capacity of an L-4 route is 32,400 circuits. Since coaxial cables are free from radio interference, the L-4 would also be valuable in areas already crowded with microwave radio systems [*Bell Labs Rec.*, **45** (1967), 211].

Identification of atom on a surface

Secondary electron spectroscopy is a new analytical technique for identifying atoms at the surface of various materials. The technique makes use of the phenomenon known as Auger electron emission to detect atoms at (or several atomic layers below) the surface of materials. It is non-destructive and uses no harmful radiation.

Secondary electron spectroscopy is useful for the analysis of all elements except hydrogen and helium and is particularly well suited for the detection and identification of surface atoms of the higher elements such as lithium, beryllium, boron, carbon, sulphur and oxygen. Atoms in surface concentrations of less than one-tenth of a monolayer can be detected. It can also detect bulk impurities less than 0·01 per cent of a sample.

In this method, a solid sample placed in the analytical apparatus is bombarded by a beam of relatively low energy electrons. The sample emits a number of other secondary electrons which are collected by the apparatus according to their various energies. Among the secondary electrons, certain electrons in a specific energy range are known as Auger electrons. An Auger electron has an energy characteristic of the atom from which it originates. When the number of secondary electrons emitted at each different energy is recorded, the total will be higher than expected at certain energies, indicating thereby the presence of Auger electrons. These increases can be enhanced electronically to provide a characteristic identification for atoms of each element at the surface of the material [*Science, N.Y.*, **3** (10) (1967), 13].

New multipurpose indicators

The possible use of 4-(4′-hydroxybenzylidyl)-1-phenethyl-2,3-dioxopyrrolidine (I) and 4-(3′-methoxy-4′-hydroxybenzylidyl)-1-phenethyl-2,3-dioxopyrrolidine (II) as indicators covering wide ranges of *p*H and selective reagents has been described [*Chemy Ind.*, (1967), 1322]. The *p*H dependent colour changes are attributed to the tautomeric change involving the hydroxyl group in the 4-position of the benzylidyl aromatic ring. The colour changes with variations in *p*H for 10 per cent (wt/vol.) test solutions are: (I) yellow, below *p*H 5·8; brown, between 6·2 and 7·0; pink, between 7·2 and 8·2; and red, above 8·4; (II) yellow, below 7·0; orange, between 7·2 and 7·6; and red above 7·8. The colour changes are reversible and the presence of the colloidal matter does not cause any change in the *p*H at which the changes occur, suggesting the use of (I) as a soil indicator.

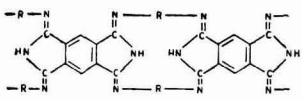
Both the compounds react with halogens and oxidizing agents like hydrogen peroxide. The addition of iodine solutions containing potassium iodide to alkaline solutions of both (I) and (II) gives an intense blue-green colour, which fades away after a few minutes leaving a yellow colour. This reaction is sensitive to 50 p.p.m. of iodine compared to starch mucilage which is sensitive to more than 100 p.p.m.

The red colour of the alkaline solutions of both (I) and (II) is readily discharged by hydrogen peroxide, even in the presence of common organic solvents; 5-6 p.p.m. of the peroxide destroys the colour. This reaction is irreversible if the mixture is warmed to 60°C. and is not hindered by the presence of common anions.

New class of thermally stable polymers

A new class of thermally stable polymers composed of repeating macrocyclic units and in which the size of the ring can be modified

within wide limits have been synthesized at the National Physical Laboratory, Teddington, UK [*Chemy Ind.*, (1967), 1254]. The polymers have been obtained by condensing together pyromellitonitrile (1,2,4,5-tetracyanobenzene) (0·002 mole) and *m*-phenylenediamine (0·04 mole) in boiling 2-methoxyethanol (150 ml.), in the presence of methoxyethanolate (0·005 mole) as catalyst. The reaction is followed by estimating the ammonia liberated. On completion of the reaction the insoluble polymer is filtered off and extracted with ethyl alcohol and benzene and dried to leave a dark red polymeric powder. On the basis of elemental analysis and IR absorption data the following probable structure has been suggested for the polymers:



Thermal gravimetric analysis in air has shown a 10 per cent loss in weight of the polymer at 540°C. In argon the polymer is stable up to 600°C. Specimens obtained by moulding the polymer under pressure at 350-400°C. have tensile strengths up to 13,000 lb./sq. in. Other diamines, *p*-phenylenediamine, 4,4′-diaminodiphenyl ether, 4,4′-diaminophenylmethane and hexamethylene diamine, have also been successfully used for synthesizing similar polymers. Polymers have also been obtained from sediamines and tetra-iminobenzo-*bis*-pyrrole.

Insect juvenile hormone

The juvenile hormone (JH), which plays an important role in the growth and development of insects, has been isolated by Dr Herbert Roller and coworkers of the University of Wisconsin. The natural JH, thought to be a farnesol derivative, has been shown to be methyl 10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate. Identification of JH is of importance to both basic and applied biochemical research; for instance, it would lead to a better understanding of hormone action and effective methods of pest control.

The tridecadiene derivative (300 μg.) has been isolated from an extract of adult male cecropia moths, by a five-stage method involving low temperature precipitation, molecular distillation, thin layer chromatography (twice) and gas-liquid chromatography. All the hormonal activity associated with secretions of the corpora allata is attributed to the compound isolated. All attempts to separate this into different components have failed. The JH isolated is $1{\cdot}25 \times 10^5$ times as active as the moth extract.

The mass spectrum of the hydrogenated JH (in ethanol with palladium black) gives a molecular ion at m/e 284 and suggests an empirical formula $C_{18}H_{36}O_2$. The most abundant fragments at m/e 101 and 74 and the ion at M = 31 show the presence of a carboxymethyl group on an aliphatic chain with a methyl at C-3; ions of relatively high intensity at m/e 143, 185 and 153 indicate a methyl or dimethyl branch at C-7. The mass spectrum of JH gives a molecular ion at m/e 294 and fragments at M = 18, 31 and 32. This in conjunction with the mass spectrum of the hydrogenated product suggests an empirical formula $C_{18}H_{30}O_3$ for JH. When cleaved by osmium tetroxide and periodic acid, JH gives levulinic aldehyde and its homologues. All the above facts and the NMR spectrum establish the structure of JH as 10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate, with *trans*-configurations for the double bonds [*Chem. Engng News*, **45** (No. 16) (1967), 48].

A simple device for obtaining synchronous cultures of algae

A simple device for obtaining synchronous cultures of algae has been developed at the Hydrobiological Institute, Netherlands. To obtain synchrony, a random culture of algae is subjected to regular alteration of light and dark periods of adequate duration. The device consists of a well-stirred thermostat made solely of glass (36 × 23 × 26 cm.) in which two culture vessels (flat bottles of 1 litre capacity) can be placed. Each bottle is provided with two air inlets, one outlet and one tube, adjustable at variable depths to collect the samples and to dilute the suspension. The cultures are illuminated by four fluorescent lamps through each of the two longer sides of the water bath, giving a light intensity of 15000 lux from each side. To get more light a high pressure mercury fluorescent lamp is to be placed over each culture, which gives from above a light intensity of 5000 lux in the centre of the thermostat. Three thermostats can be placed between the lamps which make it possible to operate at three different temperatures and also to culture six different organisms at the same time under the same light conditions, to yield about 900 ml. of a dense suspension from each bottle.

Completely synchronized cultures of *Scenedesmus obliquus*, *Ankistrodesmus falcatus* and *Chlorella vulgaris* at temperature between 25° and 30°C. have been obtained using culture solution of Lorenzen [*Synchrony in cell division and growth*, edited by E. Zeuthen (Interscience, New York), 1964]. The light-dark rhythm was 14 hr light: 10 hr dark, and aeration was maintained with sterile air enriched with 3 vol. per cent CO_2 [*Nature, Lond.*, **213** (1967), 527].

A new method for determining the true ultimate strength of concrete

A new method has been proposed for the determination of the true ultimate strength (C_{TU}) of concrete. The method consists in studying the variation of rate of strain with applied load from the results of a short-time stress test. The differences in the deformational behaviour of concrete in the second and third stages of the stress test are very pronounced. From this curve the critical stress at which breakdown of the internal structure of the material starts can be found out and this critical stress corresponds to C_{TU}.

The experimental work involved in this method is very simple; only a record of the longitudinal deformation of a sample with respect to applied load and a rate of strain versus applied load plot are necessary. The time required for testing is smaller compared to that for the direct or the creep test

method. The method can be used independently or in combination with the log stress-log strain method [*Materials Research & Standards*, 7 (1967), 486].

Special biochemicals unit

The unit established by the Council of Scientific & Industrial Research, in early 1966, at the Vallabhbhai Patel Chest Institute, for undertaking the production of specialized biochemicals of authenticated purity standards needed for teaching and research has just released its second list of products available. It is gratifying that within a brief span of two years the unit has been able to market seventy biochemicals including enzymes, coenzymes, nucleotides, phospholipids, phosphatides, sugar derivatives, etc., not only for internal consumption but also for export. The list gives details regarding packings in which various biochemicals are available as well as their prices. A further list of biochemicals to be added to the production line shortly is also appended. Enquiries regarding the availability of biochemicals should be addressed to the Officer Incharge, Biochemicals Unit, V.P. Chest Institute, University of Delhi, Delhi 7.

Journal of Functional Analysis

This new journal, started by the Academic Press Inc., New York, from May 1967, publishes original research papers in all branches of science in which functional analysis plays an essential role. Papers published deal with new developments in functional analysis, applications and examples of functional analysis in other parts of mathematical science, and novel problems in and conceptional challenges to functional analysis. The annual subscriptions for Vol. 1 (1967) comprising 4 issues are $ 16.00 and $ 10.00 for institutions and individuals respectively.

Actinides Reviews

Elsevier Publishing Co., Amsterdam, is to shortly start publishing this new journal which will carry review articles on the actinide elements series. The actinide elements, lying beyond uranium, offer not only challenging research possibilities in many areas of physics and chemistry but also comprise the source materials for nuclear energy. Each volume of the journal comprising 4 issues is priced at $ 21.00.

Laboratory Animals

This new journal started by the Laboratory Animal Science Association, UK, began appearing in April 1967. The journal publishes research papers, reviews and communications on all aspects of laboratory animal science. Two issues, in April and October, are published per year. A single issue is priced 30s. and the annual subscription is 50s.

Journal of Optimization Theory and Applications

This new bimonthly journal, started in July 1967, covers mathematical optimization techniques and their applications to science and engineering. It presents theoretical treatment of direct methods, calculus of variations, dynamic programming, gradient methods, linear programming, game theory, inequality theory, boundary value problems, and numerical methods. The annual subscription is $ 18.00 in the USA and 10 per cent higher elsewhere.

European Organization for Nuclear Research

The annual report (1966) of the organization reports its activities in the fields of theoretical and applied physics, proton synchrotron, accelerator research, etc.

It has been found that more information regarding fast particle-proton collisions can be obtained if fast particles are made to collide with polarized rather than unpolarized protons. The spin effects in $\pi^{\pm}$ and p-p high energy elastic scattering have been studied by measuring the paramter P_0 with a polarized proton target. The results have shown that the absolute values of the polarization parameters for π^+ and π^- are approximately equal but of opposite signs at a momentum transfer of about 0·2 $(\text{GeV./c})^2$, thus confirming the predictions of Regge pole theory. A set-up of spark chambers placed in magnetic fields has been developed and used to produce a large number of photographs of η decay for analysis of possible asymmetries in the decay.

Experiments on the CP violating decay mode of the K^0 hyperon provided additional information on the rate of decay of the long-lived K^0 to $2\pi^0$. Studies on the annihilation of anti-protons at rest and at low energy (1·2 GeV./c), using an 80 cm. bubble chamber, have yielded interesting results, viz. (i) the clarification of the KK system at masses close to the threshold, (ii) confirmation of the existence of the resonance $E(KK\pi)$ with an energy of 1425 MeV. including all its quantum numbers $I^GJ^P = 0^+0^-$, and (iii) a test of C and CP conservation in strong interactions.

The 'two-body reactions' have been studied, using the bubble chamber technique, to record complete pictures of the complex reactions following the collision, with a view to elucidating the type of the original collision and the involved fundamental processes.

The main lines of research under the nuclear structure research programme were the study of the properties of μ-mesic and π-mesic atoms, formed by the capture of slow mesons into atomic orbits around the nucleus of a target atom; the formation of nuclei in highly excited states by single and double charge exchange or annihilation of bombarding π mesons; and the production of isotopes by direct proton bombardment. During studies on X-ray transitions in π^--mesic and μ^--mesic atoms, the π-mesic $2p$-$1s$ transitions have been observed for nuclei ^{6}Li, ^{7}Li, ^{9}Be, ^{12}C, ^{14}N, ^{16}O, ^{18}F and ^{21}Na. The $(\pi^+, 2p)$ and $(\pi^-, 2n)$ reactions on ^{6}Li have been studied, the first by the absorption of the pion by either the deuteron or a quasi-deuteron in the α-particle (^{6}Li disintegrates into an α-particle and a deuteron) and the second by the absorption of the pion at rest. The $(\pi^+, 2p)$ reaction has been found to be of direct nature.

The main areas of research in theoretical physics were: group theory, algebra of currents, high

energy collisions of hadrons, phase-shift analysis, electromagnetic and weak interactions, analyticity and axiomatics, and nuclear structure. It has been shown that the use of unitarity makes it possible to extend considerably the analyticity domain of the scattering amplitudes deduced from axiomatic theory. In the algebra of currents, an analysis of the relations between ' chiral and non-chiral ' $U(3) \times U(3)$ algebras, a new sum rule relating nucleon magnetic moments and pion photoproduction cross-sections, and the proof for the possible existence of new families of sum rules have been obtained.

Studies on various aspects of the intersecting storage rings project, which has passed from the design to the construction stage, have been carried out to fix the parameters and narrow the limits of possible variations. The basic requirements of the design and technical details for the construction of the 300 GeV. proton synchrotron have been confirmed.

Department of Atomic Energy

The main research activities of the various institutes associated with the Department, including the Bhabha Atomic Research Centre (BARC) and the Tata Institute of Fundamental Research (TIFR), are presented in its annual report for 1966-67.

The three reactors at BARC — Apsara, CIRUS and Zerlina —have been fully utilized for research and isotope production. Operated on a round-the-clock schedule, Apsara has produced a power output of 2·69 million kWh, which is the highest since it first became critical in 1956. Isotopes and labelled compounds produced at BARC have found a market in Australia, Ceylon, Czechoslovakia, East Germany, France, Hong Kong, and the Philippines, apart from meeting the requirements of the country. The production of nuclear grade uranium has been maintained to meet the needs of CIRUS and Zerlina.

A number of high purity chemicals worth about a lakh of rupees have been prepared saving valuable foreign exchange. Preparation of organic and inorganic compounds labelled with heavy hydrogen has effected a saving of about Rs 28,000 in foreign exchange. Another notable achievement has been the preparation of a plutonium-beryllium neutron source from plutonium-239 produced at the Trombay plutonium plant.

An angular correlation goniometer for the charged-particle gamma ray coincidence experiments in nuclear reactions has been fabricated and (d, p, Υ) reactions studied. Theoretical work has been carried out to explain some unresolved features applying the idea of Markov process of random walk to nucleons.

The production of standard vacuum components like diffusion pumps, control instruments, vacuum gauges, etc., has been increased. New techniques for the fabrication of field emission microscopes and cathode ray tubes have been developed. An important achievement was the development of a d.c. plasma gun capable of operating at a power level of 60 kV. and producing temperatures in the range 5000-30,000°C. The gun can be used for welding and cutting thick sheets of metals and for ceramic spraying. The fabrication of control equipment worth more than Rs 10 lakhs for the Tarapur Atomic Power Project has been started.

The achievements of the Tata Institute of Fundamental Research have been the design of an on-line data processor; the development of a meta-theoretic model which can cope with programming languages and computers within a uniform framework; the building, testing and calibrating of a speech spectrum analyser; the development of a picture processing language (COMPAX) used for simulating a hand-printed English letter generator; and the development of a language (COMPANION) for generating animated picture sequences.

The Physical Research Laboratory, Ahmedabad, has installed a cosmic ray telescope to facilitate studies on the time variation of cosmic rays. The laboratory has also designed, fabricated and tested a rocket payload for use at Thumba.

Investigations have been carried out at the Tata Memorial Hospital, Bombay, and the Indian Cancer Research Centre, Bombay, on some peculiarities in the growth-pattern of cancer among Indians. New methods have been tried out for the treatment of cancer of the gullet, cheek and tongue.

Investigations on biochemistry and food technology have been chiefly concerned with (i) fundamental and applied aspects of cell metabolism and (ii) food irradiation procedures of possible practical application.

Work has been continued on the three nuclear power projects at Tarapur, Rajasthan and Madras respectively.

Announcements

▪ *International Powder Metallurgy Conference* (1970) — The Third International Powder Metallurgy Conference will be held at the Waldorf-Astoria, New York, during 12-16 July 1970. Papers on high temperature P/M materials, including superalloys, refractory and reactive metals; P/M parts and products; theories of compacting and sintering; a review of the ' state of the art ' including compacting, sintering, and powder preparation; and P/M composite materials and heavy metals will be presented and discussed. A technology exhibition of P/M products and metal powders representing the latest industrial developments will be held during the conference.

▪ *Budapest Corrosion Week* — The 41st Manifestation of the European Federation of Corrosion will be organized by the Scientific Society of Mechanical Engineers and the Hungarian Chemical Society, Budapest, during 7-12 October 1968. The latest theoretical and practical results of studies on the corrosion protection of metals will be discussed. An international exhibition on corrosion protection will also be organized simultaneously. The subjects of the lectures include: (i) Electroplating and anodic oxidation; (ii) Hot galvanization and ceramic coating; (iii) Corrosion protective paint coatings and systems and plastic coatings; (iv) Temporary protection of metal surfaces; and (v) Theoretical corrosion problems and results. Details regarding the event can be had from the Secretariat of

the Corrosion Week, Budapest V, Szabadsag ter 17, III. 305.

▪ *International Symposium on Macromolecular Chemistry* — The International Union of Pure & Applied Chemistry will hold this symposium in Toronto, Canada, during 3-6 September 1968. The major theme of discussion will be the structure and properties of macromolecular system and will embrace synthetic, natural and biological polymers. The topics to be discussed are: molecular structure and properties; crystallization and morphology; properties of macromolecular solutions; structure and physical properties of solid macromolecular compounds; and systems, structure and function of biopolymers. Further details may be obtained from the Organizing Committee, Box 932, Terminal A, Toronto, Canada.

▪ *The Fifth International Congress on Photobiology*, sponsored by the Comité International de Photobiologie, will be held under the auspices of the National Academy of Sciences, USA, during 26-31 August 1968. The venue of the congress will be Dartmouth College, Town of Hanover, New Hampshire, USA.

It is planned to have introductory lectures on various areas of photobiology at the beginning of the morning and afternoon sessions followed by contributed papers and discussions. The congress will emphasize, in particular, on topics like photochemistry, bioluminescence, photochemistry of macromolecules, photodermatology, photomorphogenesis and tropism, vision, rhythms, instrumentation and action spectra, and repair mechanisms.

The registration and abstract forms are available from the Secretary General, Fifth International Congress on Photobiology, Argonne National Laboratory-202, Argonne, Illinois 60439, USA.

▪ *International Symposium on Mixed Dust Pneumoconiosis* — This symposium will be organized by the British Occupational Hygiene Society in London in September 1970. Further particulars can be obtained from Dr J. S. McLintock, National Coal Board, Hobart House, Grosvenor Place, London SW 1.

▪ *International Congress on Pharmacology* — The Fourth International Congress on Pharmacology will be held at the University of Basel, Switzerland, during 14-18 July 1969. Papers on all aspects of the recent state of pharmacological science will be presented and discussed. Further information may be obtained from Dr Frank James Bové, Pharmakologie-Kongress 1969, Postfach 30, Basel 4, CH-4000 Basel, Switzerland.

▪ *International Symposium on Conformational Analysis* — This symposium will be held at Brussels during 9-12 September 1969. Papers on organic chemistry, biochemistry and medicinal chemistry will be presented. Further information may be obtained from the Executive Secretary of the Symposium, 49 Square Marie-Louise, Bruxelles 4, Belgium.

▪ *The Fourth International Congress on Metallic Corrosion* will be held during 7-14 September 1969 at Amsterdam, Netherlands. The congress will deal with two main themes: (i) Corrosion processes and (ii) Protection against corrosion. Further particulars can be obtained from the Secretary, Organizing Committee, 4th International Congress on Metallic Corrosion, Postbus 52, Delft, Netherlands.

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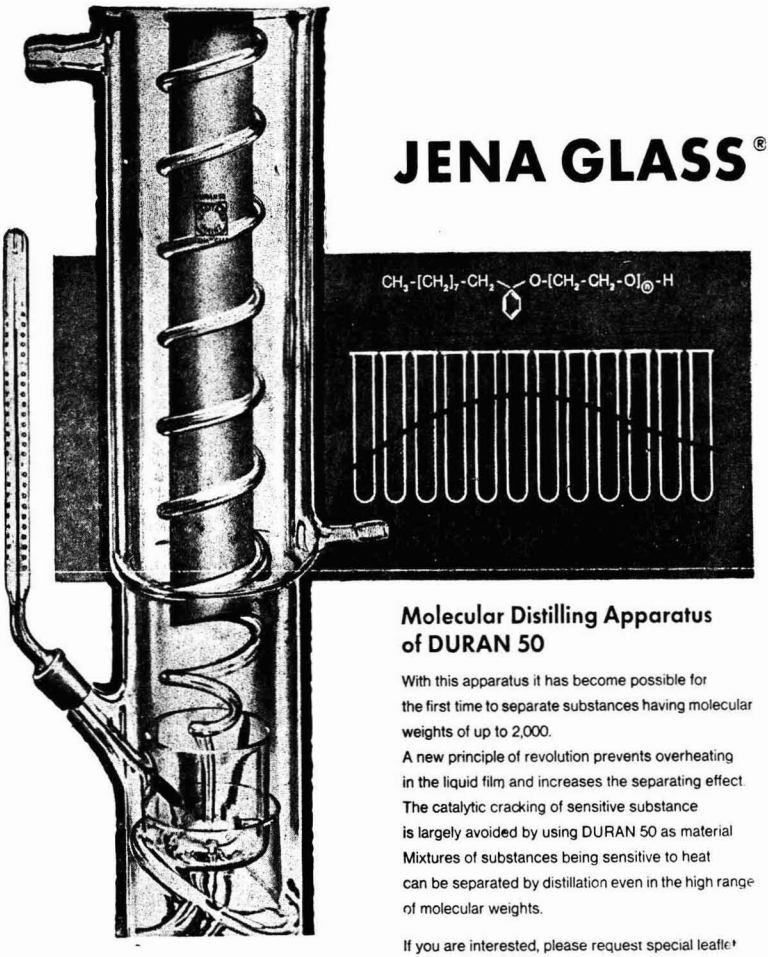

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