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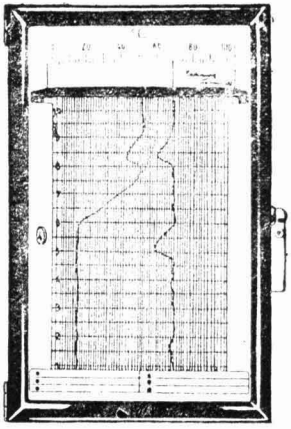

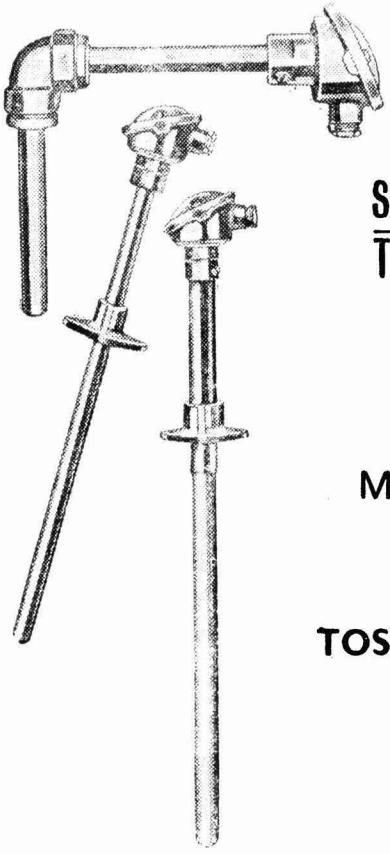

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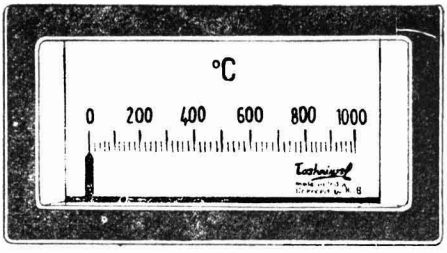

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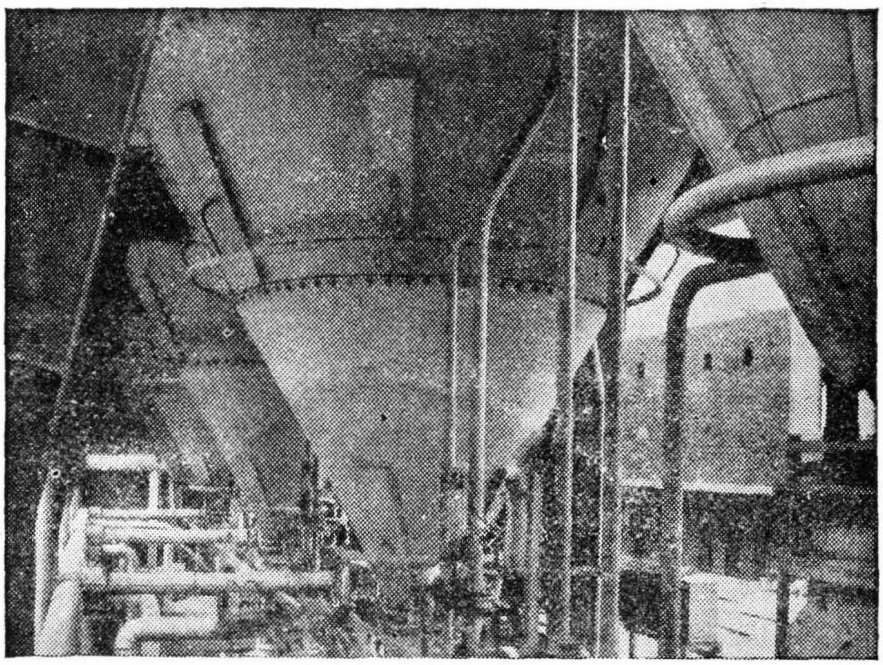

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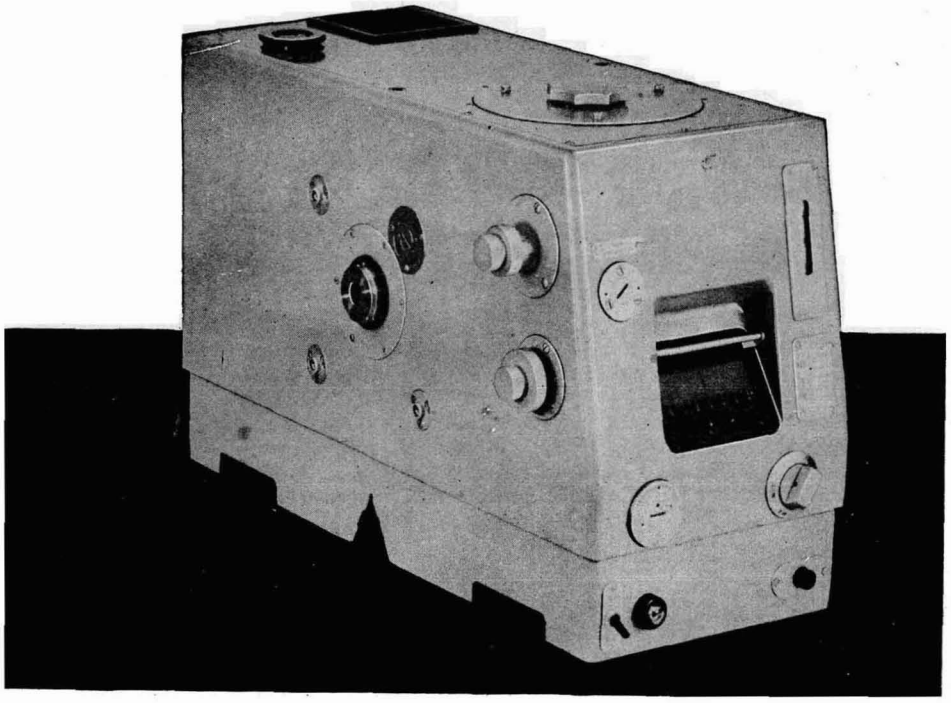

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INDEX TO ADVERTISERS

Current Topics

Improvement of Scientific & Technical Publications

FOR the first time in the annals of scientific and technical publication activity in the country, technical writers, editors and publishers, drawn from publishing establishments of learned societies, research councils and others, were provided a forum by the Indian Standards Institution (ISI) to discuss measures for improving the quality of scientific documents published in the country. One of the sessions of the Tenth Indian Standards Convention held at Ernakulam (30 December 1966) and presided over by Shri B. N. Sastri, former Chief Editor, Publications & Information Directorate, Council of Scientific & Industrial Research, was devoted to this important subject. Fifteen papers dealing with topics such as preparation and processing of manuscripts, matters of style — preparation of illustrations, symbols, abbreviations, units of measurement, layout and typography — professional qualifications of technical writers and editors, working manuals for editors, and training of technical writers and editors were presented and discussed. The papers presented and the discussions that ensued revealed the chief reasons for the poor standard of the majority of scientific and technical publications produced in the country and resulted in the formulation of many useful recommendations for producing properly planned, well written and edited, and well produced documents.

In his opening remarks, the Chairman of the Session, Shri B. N. Sastri, rightly observed, "Publications are regarded as 'ambassadors' of science, for the quality of the scientific achievements of a country is reflected in its publications, and the world at large gets to know the achievements through its periodicals, monographs, and other scientific and technical publications. And the maintenance of high standards of excellence in publication work is a matter of highest importance." A majority of the scientific and technical publications produced in the country are neither well written and edited nor well produced. This is a matter for concern.

One of the contributory factors responsible for this situation, according to Shri B. N. Sastri, is that the yardstick for measuring the work of a scientist for appointment, promotion or preferment is based on the number of publications. This results in documents lacking in scientific quality or merit continuing to burden scientific literature. Urgent measures have to be taken to correct this undesirable tendency if scientific documents of real merit are to be published and trivial ones eliminated. Much can also be done at the origin — the institutions from which documents originate — to discourage publication of substandard documents and eliminate them.

Two other factors are also responsible for poorly written and edited documents, as well as the continued publication of substandard ones. One of them is the lack of formal technical writing courses in the Indian universities and higher institutes of technology as part of graduate or postgraduate science curricula. Another is the non-availability of well qualified scientists and technologists, trained in the arts of technical writing, editing and publication mechanics, to supervise the publication of scientific documents. At the editorial level documents of doubtful value could be weeded out and documents of scientific merit but poorly written given proper shape, provided the editors possess the necessary subject qualifications and are well trained in editorial work. It is essential that urgent steps are taken to provide facilities for the training of technical writers and editors. It is not enough if the above impediments which come in the way of publication of quality documents are removed. The basic problem — how to attract well qualified scientists to take up careers in the publication field — still remains. This can be tackled only by recognizing the professions of technical writers and editors as distinct professions, and according them a place of importance and a status they deserve in regard to service conditions.

Some of the recommendations formulated at the session were specially designed to promote building up of sound traditions in publication activity and raising the standard of scientific and technical documents. These are: (i) Formulation of standards for technical writing and editing positions, and preparation of a code of practice for scientific and technical publications; (ii) Technical writing and editing be recognized as distinct professions and measures be taken to train technical writers and editors. The research councils and the learned societies to be requested to provide on-the-job training facilities. Concurrently, the University Grants Commission be requested to organize technical writing courses in the universities; (iii) Style manuals be prepared by the publication units of the research councils, learned societies and other publishers of scientific and technical publications. Based on this, a national style manual be prepared by ISI for scientific and technical documents; and (iv) Technical writing and editing position standards formulated by ISI be circulated to research councils, the Atomic Energy Establishment, the Union Public Service Commission and others to ensure that only qualified persons are appointed to technical writing and editing positions.

These recommendations merit the serious consideration of all those concerned with scientific and technological education and research. The learned societies and the research councils have a particularly important role in raising the standard of scientific and technical publications in the country, and guidance in matters concerning communication of scientific information and improvement of scientific and technical publications must come from them. The universities and the higher institutes of technology have an equally important and basic role to play in the training of technical writers.

Symposium on Proteins

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THE following is a report on the Symposium on Proteins which was held at the Department of Chemistry, Osmania University, Hyderabad, during 22-24 September 1966. Dr A. Sreenivasan, President, Society of Biological Chemists, India, welcomed the gathering of participants, and the symposium was formally inaugurated by Prof. N. V. Subba Rao, Dean of Science, Osmania University.

The proceedings of the symposium consisted of research papers which were presented in six sessions, and four general lectures. Two of the sessions were devoted to topics of physical and chemical content, two others to nutrition, goat haemoglobins and milk proteins, and the last two sessions to protein synthesis, immunology, amino acid metabolism and enzymes. One of the sessions on nutrition was jointly sponsored by the Nutrition Society of India and was held at the Nutrition Research Laboratory, Hyderabad.

Speaking of the general lectures — one on some mechanistic and structural features of crystalline tryptophanase from *Esch. coli*, by Esmond E. Snell (and Y. Morino), covered the various α,β-eliminations and β-replacement reactions catalysed by the enzyme, the role of pyridoxal phosphate therein, the nature of the rate-limiting step, the subunit structure and molecular weight of the enzyme, and the differences in spectra of the holoenzyme in the presence of the activators, K^+ or NH_4^+, and, in contrast, Na^+ which does not activate. The lecture on phytotoxins by P. S. Sarma (and S. L. N. Rao) covered the distribution of these toxins in seeds (edible), and particularly the role of β-N-oxalyl-L-α,β-diaminopropionic acid, present in *L. sativus* seeds, as a neuro-lathyrogen. It was pointed out that adult birds and animals seemed to possess a significant haemoencephalic barrier to this toxin. Intrathecal administration into the adult monkey, intracisternally into adult mice, or provision of an acidotic condition by oral feeding of $CaCl_2$ or NH_4Cl prior to intraperitoneal injection of the toxin helped induce experimental neuro-lathyrism in animals. The significance of these studies in relation to human lathyrism was pointed out. The lecture on human protein nutrition by C. Gopalan covered factors which weigh in the assessment of the daily protein requirement of the individual, and the role of protein malnutrition in certain of the well-known diseases such as kwashiorkor in children. The prevalence of this condition in rural parts of India, where protein nutrition was faulty, was illustrated by slides. An adequate prenatal nutrition at the prenatal stage was pointed out as having a material influence on the postnatal growth rate of the infant. K. S. V. Sampath Kumar, in his lecture on the active sites of enzymes, pointed out the importance of selecting a proper assay method in evaluating the changes in activity deriving from selective modification of groups, etc. The lecture highlighted current knowledge on the structure of several enzymes in relation to the active site.

Of twenty-nine invited research papers all but two were presented. B. M. Braganca (and N. T. Patel) reported on the fractionation of cobra venom to yield a carcinolytic factor which behaved as a protein of molecular weight 10,500. A phospholipase activity with high affinity for phosphatidylethanolamine and serine was present in the fraction, and this may be related to the preferential cytotoxicity displayed by the protein towards Yoshida sarcoma cells *in vivo*. Growth of Ehrlich ascites tumour could be inhibited by the factor. G. Dimitrov, R. C. Kankonkar and S. S. Rao reported on the fractionation of Russel's viper venom by gel filtration. Phospholipase A was easily separated from the phosphatidases on Sephadex G-75, and the peaks so obtained were further chromatographed on cellulose ion-exchangers and the final fractions tested for purity. S. Saraswathi (and B. K. Bachhawat) found brain alkaline phosphatase to be separated by chromatography on DEAE-cellulose into two fractions showing different affinities for pyridoxal phosphate. One was richer in bound neuraminic acid, and removal of part of this using neuraminidase affected the elution behaviour of the enzyme. Removal of some of the bound sugar did not, however, alter the affinity towards pyridoxal phosphate and *p*-nitrophenylphosphate.

In studying the component of egg yolk responsible for gelation of yolk on freezing and thawing, S. Mahadevan observed that yolk plasma treated

with 98 per cent formic acid and subsequently dialysed could be fractionated to yield 'gelled' and 'soluble' fractions which were sorted out by a centrifugation procedure. Extensive analysis of these fractions revealed many similarities, as well as dissimilarities. Ultracentrifugal studies by M. W. Pandit (and M. S. Narasinga Rao) on the molecular weight of silk fibroin, as a function of the number of washes given to the silk fibre with boiling 2 per cent Na_2CO_3 solution, reveal that the fibroin is of molecular weight $2\text{-}4\times10^4$, and that aggregation of these subunits promoted by the presence of contaminating sericin is responsible for higher (viz. $1\cdot2\times10^6$) molecular weight components which are more conspicuous in specimens isolated from fibres subjected to fewer washes. The silk fibre is grossly constituted of aggregates of fibroin subunits of molecular weight 2×10^4 and sericin. Dissociation of the aggregates occurs on removal of sericin. M. B. Patil and G. D. Kalyankar reported on an interesting series of photodegradations of amino acids in aqueous solution in the presence of TiO_2. The reactions were completely dependent on the presence of oxygen. The influence of the free amino group on the course of photodegradation could be noted, with α-amino and ω-amino acids yielding respectively α-amino and ω-amino products. Sindhu V. Joshi (and K. S. Kangaonkar) reported on irradiation effects on monolayers of poly-L-tyrosine and poly-DL-alanine. Side chain groups were more susceptible to radiation than the peptide bond, as noted in earlier studies.

A. S. Balani (and J. Barnabas) found two blood samples, amongst many, from mixed breed goats which contained a haemoglobin different from the two known types. All were purified and various properties such as electrophoretic mobility, alkali denaturation rate, fingerprints of derived peptides, etc., were studied. The various haemoglobin types appear to have similar α-chains and probably differ in their β-chains. R. B. Mawal reported that tryptic-chymotryptic peptide patterns of cow α-lactalbumins A and B were strikingly similar except for a basic and a neutral peptide, whereas that for buffalo lactalbumin was significantly different. Analysis of the peptide patterns of the S-sulphonated derivatives of the β-lactoglobulins of the cow and the buffalo revealed some differences in the mobility of acidic peptides on chromatography. Bhavani Belavady (and P. Udayasekhara Rao) reported that protein concentration in milk from Indian women from low socio-economic groups was normal, but that xanthine oxidase activity of the milk is increased when the diets are supplemented with skim milk. The stage of lactation had a significant effect on the concentration of the casein fractions, and one protein having the same mobility as serum albumin reached a maximum in one month after the start of lactation. In later stages of lactation the whey portion of milk contained a basic protein, positively charged at *p*H 8·6, a counterpart to which was not discernible in the donor's serum.

A paper by Shantoo Gurnani dealt with the action of trichloroacetic acid on lysozyme. The enzyme treated with 5 per cent TCA at 0-20°C. for up to 1 hr had not lost any activity as measured after removal of the TCA. Most physical properties and the rate of hydrolysis by trypsin remained unchanged after such treatment. The optical rotatory dispersion characteristic (a_0 and b_0 values) of the material were significantly different, and indicated a stable, significant conformational change in the molecule. T. G. Rajagopalan (and W. H. Stein and S. Moore) reported on the rapid inactivation of pepsin when it reacted with diazoacetyl-DL-norleucine methyl ester in the presence of Cu^{2+} ions. In the absence of Cu^{2+} ions, in a slow reaction more than one mole of norleucine is incorporated but inactivation remains incomplete. The single residue of norleucine incorporated into pepsin, in the presence of Cu^{2+}, appears to be esterified to a single carboxyl group at the active site of pepsin and is removable by hydroxylamine. A novel colour reaction specific to N-formyl-N'-2,4-dinitrophenyl-hydrazine, which is observed in the presence of a base and acetone, and the applicability of this colour reaction for the determination of ester formyl and formamido groupings in polypeptides were reported on by L. K. Ramachandran.

Y. G. Deosthale and P. G. Tulpule found that the total and specific activity of ornithine transcarbamylase of liver was significantly increased in rats treated with antibiotics, the effect being greater with chlortetracycline relative to phthalylsulphathalazole and tetracycline. It was suggested that antibiotic medication increases the rate of urea synthesis in the liver. B. S. Narasinga Rao observed that, in rats, increased urinary excretion of certain tryptophan-niacin metabolites, particularly quinolinic acid and N-methylnicotinamide, resulted from a dietary excess of leucine, but not of other amino acids. In young rats such a dietary excess is followed by increase in the tryptophan pyrrolase and 3-hydroxyanthranilic acid oxidase activities of liver. S. K. Nayak and B. N. Mashelkar presented a study of the amino acid requirements for young cultures of mouse fibrosarcoma cells and a line of cells (MFS_{83}) derived therefrom. U. K. Vakil (and H. R. Adhikari) found that free retinol in liver increased, and serum levels fell, in protein-depleted rats. Retinol palmitate hydrolase activity increased, and retinol esterase decreased in the liver, pancreas, and intestinal mucosa of such animals. Protein regeneration reversed these effects. It was suggested that the albumin fraction of serum is directly concerned in retinol mobilization from liver.

That supplementation of poor Indian diets with lysine results in increased growth of experimental rats, and higher protein efficiency was reported by S. Venkata Rao. Incorporation of threonine along with lysine led to further increases, and maximal effects were observed when the diets were adequate with respect to vitamins and minerals. Analysing the food value of leaf proteins, Narendra Singh commented that lucerne or alfalfa cultivation would lend to protein yields 3-5 times higher as compared to conventional food crops which yield about 500 kg. protein per hectare. Protein concentrates from alfalfa on analysis showed deficiency

only in regard to methionine. Studies on rats with this leaf protein indicated lysine-adequacy, and when methionine was added it was nutritionally adequate. As a supplement to poor rice diets, added at a 5 per cent level this protein was as efficient as similar supplementation with skim milk powder. Incorporated into Indian dishes to provide about 20 per cent of the daily protein intake, the leaf protein was found to be generally acceptable.

Stimulation by light of the incorporation of several labelled amino acids into proteins of the occipital cortex was observed by U. B. Singh, S. B. Chopra, B. K. Goel, B. D'Monte and G. P. Talwar. The influence of intensity, wavelength, flicker frequency and duration of incidence of the light on such incorporation was studied. B. R. Das and S. R. Das observed that soluble proteins (0·005*M* phosphate; *p*H 7·2; 80730 *g* supernatant) of the grey matter of the brain of the monkey (*Macacus mulatus*) contained four antigens common to blood and two specific to brain. No region-specific soluble antigen was detected in this study. H. G. Madhwa Raj and N. R. Moudgal outlined a radioimmunoassay for sheep pituitary interstitial cell stimulating hormone (ICSH). Using ^{131}I-labelled ICSH and rabbit antiserum, 300-600 μg. of ICSH could be determined with a 1: 500 dilution of antiserum, and the 100-200 μg. range was handled with a 1: 1500 dilution of the antiserum.

A. Bhaduri presented the correlations between the enzyme activity and the fluorescence of yeast UDP-galactose-4-epimerase and chemical modifications of this enzyme. A model for the epimerization brought about by this DPN-containing enzyme was presented. N. George Kurian and A. N. Radhakrishnan reported that urinary hydroxypyroline consisted of one fraction retained by a cation exchange resin, and another of a low molecular weight glycopeptide not retained by the resin. ^{3}H-proline (100 μC.) administered to subjects excreting 5-12 mg. hydroxyproline/day was largely accounted for by the first 24 hr specimen of urine and much of that by the glycopeptide fraction. The ratio of specific activities of hydroxyproline/proline in the glycopeptide fraction was 1·4 and about 0·5 in the cationic fraction. Proline destined to form hydroxyproline does not seem to be in equilibrium with the proline pool. P. R. Adiga, K. Sivarama Sastry and P. S. Sarma reported that cystathionine accumulated in *N. crassa* in cysteine toxicity. The major defect caused by excess cysteine is a conditioned deficiency of methionine, most probably through cysteine inhibiting cystathionase II. Both methionine and S-methylcysteine reverse cysteine toxicity, by affecting the uptake of cysteine and by counteracting the inhibitory effect of that amino acid on cystathionase.

Studies on amino acid incorporation into proteins of mitochondrial fractions, differing in size and density, were reported by S. S. Katyare and P. Fatterpaker, and the results analysed for the significance of the 'light' and 'fluffy' fractions in mitochondrial genesis. The effect of physiological doses of thyroxine in modifying the composition of the mitochondrial population was utilized in examining protein turnover in mitochondria. S. M. Hadi and C. R. Krishna Murti observed *de novo* synthesis of enzymes such as ribonuclease, deoxyribonuclease and a phospholipase in seeds of *Cicer arietinum* soon after the water imbibition stage. A preparation of ribosomes, homogeneous in a sucrose gradient at 50000 *g*, from growing seedlings actively incorporated amino acids, and this was further stimulated on addition of the 108000 *g* supernatant from homogenates of the seedlings. Uncoupling agents, streptomycin, chloramphenicol and, curiously, penicillin inhibited the incorporation.

Meaningful discussion followed the presentation of each paper, and the cooperation of the participants assured the even flow of the proceedings. The symposium brought together investigators interested in the chemistry, physical chemistry, immunochemistry, metabolism and nutritional aspects of the study of proteins and amino acids. And, it would be no exaggeration to say that the symposium had something to offer to each.

Analysing the general content of papers presented at the symposium, two glaring lacunae in biochemical research in India can be recognized. One is that the area interlinking the chemistry and metabolism of the nucleic acids to the chemistry and metabolism of proteins remains neglected. Second, the field of protein structure which has witnessed dramatic developments in the past decade finds few adherents. In both instances the lack of research activity is not entirely due to want of skilled investigators. These advancing frontiers of biochemical research should not remain neglected for long in this country, and it would be well if major institutions supporting research such as the Council of Scientific & Industrial Research, Atomic Energy Establishment, Indian Council of Medical Research and the University Grants Commission devoted some attention to fostering such activity.

The symposium on proteins was sponsored by the Society of Biological Chemists, India. The symposium owes its success largely to the ceaseless efforts of the secretaries of the society, in particular, Dr S. Mahadevan. Some of the expenses were met from a grant sanctioned by the University Grants Commission. The enthusiastic assistance which was always forthcoming from his colleagues, particularly Dr T. N. Pattabiraman and Dr K. Sivarama Sastry, the joint conveners, made the task of organization and conduct of the symposium an easy one for the convener.

Symposium on Coastal & Nearshore Oceanography

R. JAYARAMAN
National Institute of Oceanography, New Delhi

A TWO-DAY Symposium on Coastal and Nearshore Oceanography, organized jointly by the Indian National Committee on Oceanic Research and the National Institute of Oceanography, Council of Scientific & Industrial Research (CSIR), was inaugurated on 4 November 1966 by Dr K. L. Rao, Minister of State for Irrigation and Power, Government of India, at the Central Institute of Fisheries Operatives, Ernakulam. Dr M. S. Krishnan, former Director of the Geological Survey of India and the National Geophysical Research Institute, CSIR, presided over the symposium. Dr N. K. Panikkar, Director, National Institute of Oceanography, was the convener of the symposium. Dr E. C. Lafond, Emeritus Professor of Oceanography, Andhra University and at present Director, Marine Environment Division of the US Navy Electronics Laboratory, San Diego, California, USA, was among the distinguished scientists participating in the symposium.

In his inaugural address, Dr K. L. Rao stressed the need for studying the fundamental problems of land-sea interaction, particularly with reference to the movement of sand along the coasts of India which cause silting in harbours and beach erosion along the Kerala coast. He hoped that the discussions between the oceanographers and coastal engineers during the symposium would help to highlight the important factors in the land-sea interaction processes which are responsible for many of the coastal phenomena.

Geological Evolution of Indian Coasts

Dr M. S. Krishnan, in his presidential address, gave a detailed account of the various phases in the evolution of both the east and west coasts of India from the original Gondwana land of which India was a part. The eastern coast of India began to take a definite shape in the upper Jurassic. The earliest marine formations are of the Neocomian age as indicated by the fossils in the Bengal basin and in the Godavari and Guntur areas. He also referred to the marine transgressions which took place over the east coast of India over the later period and the separation of Ceylon from India in the upper Jurassic or early Cretaceous. The present eastern coast, according to him, has been well defined since the Cretaceous, although a major regression took place in the Oligocene and a major transgression in the Mio-Pliocene.

From geological evidence, the island of Madagascar, which had been contiguous with southwest India, would have separated during the upper Cretaceous, leading to the formation of the Indian west coast, which subsequently underwent faulting. Reference was made to the origin of Bombay-Gujarat coast from the arm of the Tethys and also to the marine transgressions which took place over the greater part of the western coastal region in the Eocene and Miocene, with a well-marked regression during the Oligocene. The final shape was given to the west coast of India in the late Pliocene, and the coast is now marked by the almost straight edge of the continental shelf. Dr Krishnan concluded his address by laying stress on more detailed studies of the Pleistocene deposits all along the coast for gaining an idea of the relative movements of land and the sea during the last one million years.

Scientific Sessions

In the four scientific sessions which followed the presidential address, 60 papers were presented under the following five major groups: (1) Waves, tides, currents, sea level variations and storm surges; (2) Hydrography of shallow waters and estuaries; (3) Shore processes, beach material and sediment transport; (4) Nearshore sediment, geomorphology of Indian coasts and harbour problems; and (5) Biological and miscellaneous studies.

Beach Erosion

The topic which was discussed in great detail was beach erosion along the west and east coasts of India and the various physical and geological factors which contribute to the erosional processes. There are two groups studying these problems: (i) the group at the Andhra University who have carried out beach studies of the Visakhapatnam-Waltair coast, and (ii) the group studying the beach processes along the Kerala coast. The maximum number of papers (15) was on the beach processes of the Kerala coast and the contributions were from the Physical Oceanography Division of the National Institute of Oceanography at Ernakulam led by Dr V. V. R. Varadachari and the Coastal Engineering Division of the Kerala Engineering Research Institute, Peechi, Kerala, led by Shri K. K. N. Nambiar. The topics presented by these groups ranged from physical oceanographic factors, such as waves, tides, long shore currents, etc., associated with the beach erosion, and regional and seasonal variations in beach profiles and littoral movements along the different areas of the coast, to coastal protection works and marine structures. Discussions mostly centred round the direction of movement of sediments and quantitative aspects of erosion. An interesting point brought out during the discussions was that in a few places the movement of the bottom sediments has been found to be opposite to the coastal current prevalent during that period. The importance of wave refraction and the nearshore water movements set up by these waves undergoing refraction was stressed by a number of speakers who participated in the discussion. The paper on the radioactive tracer studies at Cochin, presented by Gole and Tarapore from the Central Water & Power Research Station, Poona, gave some

data on the sediment movements, particularly the dredged material from the harbour in the Cochin area and related these movements to the tidal influx in the Cochin backwaters. Gole and his colleagues presented another paper on the beach erosion in the Ratnagiri region of the west coast of India, wherein they discussed the effects of the sea wall on the various processes. The coastal and nearshore processes along the Waltair-Visakhapatnam coast and hydrological features of the Godavari estuary were discussed in a number of papers presented by teams from the Andhra University led by Prof. Ramanadham and Dr Poornachandra Rao.

Hydrography of Shallow Waters and Estuaries

Among the several papers presented under this section, the paper on 'Shallow-water oceanographic studies in southern Californian coast' by Katherine G. Lafond and E. C. Lafond deserves special mention. These authors presented the results of their investigations carried out from US Navy Electronics Laboratory's Oceanographic Research Tower off Mission Beach, San Diego, California. The data included all aspects, such as temperature structures, internal waves, tides, currents and ambient sound of biological origin. This paper had a special interest in that it gave a complete description of the oceanographic tower which has been set up as a special facility for making time-series observations of various oceanic parameters using the most sophisticated equipment. The papers by the participants from the Marine Biological Research Station, Porto Novo, Annamalai University, presented data relating to the hydrology of the Vellar estuary. Instrumentation in nearshore oceanography and the setting up and operation of shore-based wave recorders were discussed by the group from the Indian Naval Physical Laboratory, Cochin.

Biological and Miscellaneous Studies

Dr Panikkar presented a paper on the 'Distribution of coral reefs along the Indian coasts'. He made particular reference to the oceanographic conditions in the different areas along the coasts and showed how these conditions are not favourable for the extensive formation of coral reefs along these coasts. A number of interesting papers on radioactive and trace element studies, with particular reference to their uptake by marine organisms in the Bombay harbour region, were also presented and discussed by the scientists from the Health Physics Division of the Atomic Energy Establishment, Trombay. A paper on 'Solar radiation and light penetration in Cochin backwaters' was presented by Dr Qasim and his group from the Biological Oceanography Division of the National Institute of Oceanography.

Among the papers of general interest, mention may be made of the one on 'Ionic relationships of Gujarat coastal waters' by Sharma and Dave of the Central Salt & Marine Chemicals Research Institute, Bhavnagar, and the other on the 'Characteristics method in the wave analysis' by S. Vasudev of the College of Engineering, Trivandrum.

Indo-US Agreement on Exchange of Scientists

An agreement between India and the United States providing for exchange of scientists and engineers was signed in New Delhi on 14 February 1967. The agreement gives practical recognition to the growing importance being given in the two countries to a continuing exchange of scientific personnel and information. The plan calls for scientists and engineers from each country to visit the other for periods varying from two weeks to several months.

The new programme differs from the existing one in that it covers persons of a more advanced professional level. On the Indian side there will be emphasis on certain aspects of applied science to help in solving important national problems.

The two governments have agreed that the agencies responsible for carrying out this exchange programme within each country will be the Council of Scientific & Industrial Research for India and the National Science Foundation for the United States.

The proposed exchange of scientists will substantially augment the currently available means of exchanging the most up-to-date information between the scientific communities of the two countries.

Luminescence in Liquids Irradiated by Sonic Waves

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SONOLUMINESCENCE is the emission of light from engassed liquids cavitated by intense sound waves. The phenomenon was observed by Zimakov[1] and Marinesco *et al.*[2,3] while investigating the effect of ultrasonic vibrations on photographic plates submerged in ultrasonically irradiated water. The term 'sonoluminescence' was introduced by Fresnel and Schultes[4] who developed the photographic technique to record this phenomenon.

Cavitation is the formation and collapse of cavities in liquids filled either with a gas or vapour. It can be produced as a result of thermal, chemical or mechanical means as also by sonic waves. Cavitation originates from high negative pressures produced within the liquid. The magnitude of the negative pressure required is given by the expression

$$p = 2\sigma/R$$

where σ is the surface tension of the liquid and R is the radius of the cavity formed. A negative pressure of the order of 10^4 atm. is required to form cavities of molecular dimensions in pure water. This threshold decreases if impurities are present in the liquid. It is the presence of impurities which generally gives rise to cavitation in liquids by sonic waves because the high pressure otherwise required cannot be easily generated over a finite region in the liquid.

It is of importance to study the mechanism, nature, localization, spectral composition and intensity of sonoluminescence because, generally, emission of light from irradiated liquids is accompanied by chemical and physico-chemical effects.

Experimental Technique

Earlier investigations were made mostly with unaided dark-adapted eye. This qualitative method permitted determination of the emission or lack of emission of light intense enough to produce an effect on the retina as also to give a rough estimation of the relative intensities emitted from the examined liquids. Later, photographic plates were used to record and estimate the intensity of sonoluminescence. Recent quantitative experiments on the measurements of sonoluminescence are, however, being made with the help of sensitive photomultiplier tubes.

Experimental Observations

A good deal of experimental work has been done to investigate the intensity, spectral composition, localization and phase relationship of sonoluminescence with respect to incident acoustic waves. The main results obtained by various workers engaged in this field are discussed in the following paragraphs.

Intensity of Sonoluminescence

Liquids such as glycerol, nitrobenzol, ethylene glycol, *o*-nitrotoluol, aminophthalic hydrazide (luminol), isoamyl acetate, *n*-butyl phthalate, dimethyl phthalate, dibutyl phthalate, water, *n*-butyl alcohol, corn oil, propyl alcohol, isopropyl alcohol and ethyl alcohol show luminescence[5-10]. According to Chambers[5,6], glycerol and nitrobenzene emit intense luminescence, visible even without dark adaptation, and ethyl alcohol emits light hardly visible even under the most favourable conditions. Laufer and Srinivasan[9] and Negishi[11], however, have observed glycerol to emit intense luminescence in the presence of water only; pure glycerine is known to emit only weak luminescence. Similarly, in the case of nitrobenzene, while Jarman[10] has reported more luminescence, Griffing and Sette[12] reported weaker luminescence as compared to that in the presence of water, and Levshin and Rzevkin[7], Laufer and Srinivasan[9] and Kling[13] did not detect any luminescence.

Pure luminol and luminol saturated with nitrogen or neon have been found to give the same faint luminescence as observed in water, but luminol saturated with carbon dioxide and hydrogen has not been seen to show any luminescence[8]. Whereas Bresler[14] finds aqueous solutions of luminol to give rise to a fairly intense luminescence detectable by the unadapted eye, Negishi[11] observes that neutral solutions give the same faint luminescence as observed in water but alkaline solutions (addition of sodium carbonate) enhance the luminescence by as much as 1000 times as compared to water.

Other organic liquids such as carbon tetrachloride, carbon disulphide, *m*-xylol, benzol, toluol, ethyl ether, amyl acetate, ethyl acetate, methyl acetate, ethyl butyl acetate, ethyl amyl acetate, *n*-tetradecane, aniline, acetaldehyde, acetone, methyl salicylate, mesitylene, oleic acid, cottonseed oil, chlorobenzol, methanol, ethanol, propanol, isobutanol and transformer oil have not been seen to show luminescence[5-7,9,12]. Carbon disulphide and carbon tetrachloride in the presence of water, however, show an increased intensity of luminescence[10,12,15,16] to that observed in water.

The presence[17,18] of propyl, butyl, amyl and capryl alcohols, amyl acetate, ethyl ether, benzene and dioxane in water suppresses the sonoluminescence otherwise observed in water; the presence of toluene, thiophene, petroleum ether and aniline shows weak luminescence and the presence of xylene, nitrobenzene, benzyl alcohol, chloroform, bromoform and bromobenzene does not affect the intensity of sonoluminescence of water.

Degassed water[4], pure sulphuric acid[7] and water saturated[8,11,17-20] with ether, carbon dioxide or hydrogen do not show luminescence. On the other hand, water saturated[8,9,17,21-25] with air, oxygen, bromine, neon, nitrogen, helium, argon, krypton and xenon shows an increase in the intensity of sonoluminescence.

Harvey[8] observed that the presence of inorganic substances in water does not affect its luminescence.

Similarly, Jarman[10] observed that the impurities not chemically reactive with the liquid do not affect the intensity of luminescence. However, Levshin and Rzevkin[7], Laufer and Srinivasan[9] and Negishi[11] and Prudhomme[24] find that the presence of some of the inorganic salts, carbon disulphide or carbon tetrachloride in water enhances sonoluminescence.

Sonoluminescence[5,6,8,22,23,25] is more pronounced at lower temperatures, and with increase in temperature the intensity is reduced to a certain temperature beyond which, depending upon the liquid, no luminescence is observed even with the best possible techniques. Quantitatively, Günther *et al.*[23] have found an exponential decrease of the luminescence intensity with increasing temperature. Studying sonoluminescence intensity in distilled water in the temperature region ranging from 3° to 50°C., Alfredsson[26] has observed that the luminescence intensity increases with a rise in temperature from 3° to about 25°C., beyond which it starts decreasing, a result similar to that found by Jarman[10].

Griffing and Sette[12] and Parke and Taylor[25] have found that the intensity of luminescence increases with increase in acoustic power. Negishi[11], on the other hand, has reported that the luminescence at first increases with increase in acoustic power, but then suddenly decreases at about 2 W./cm.^{2} acoustic power. Rosenberg[27] has found that the luminescence intensity increases with increase in acoustic power to a strongly marked maximum and then decreases. The intensity reaches the maximum value corresponding to the state when $\tau = t/2$, where τ is the time of cavitation bubble annihilation and t is the period of the ultrasonic vibration. Alfredsson[26] has shown that the initial intensity varies linearly with acoustic power and that the intensity of luminescence at constant acoustic power increases with time to a maximum after which it decreases and attains an approximately constant value.

Chambers[5,6] has advanced an empirical law according to which the intensity of luminescence in pure liquids is directly proportional to the product of the viscosity coefficient and the molecular dipole moment of the liquid (Table 1), and that the intensity of luminescence in solutions of nitrobenzol in toluol varies with the change in the concentration of the polar substance (Table 2). Jarman[10], on the other hand, has shown that although the product of the viscosity coefficient and the dipole moment of the liquid gives a good correlation with the intensity of sonoluminescence for the liquids studied by Chambers, a detailed study of this relationship as applied to other liquids does not give a good correlation (Table 3). It is observed that the intensity of sonoluminescence is directly proportional to the square of the surface tension of the liquid and inversely proportional to the vapour pressure of the liquid. The experimental results of observed relative intensity of luminescence as obtained by different workers do not, however, agree, and as such it is not possible to check on the universal applicability of the various empirical relations advanced to account for the change in luminescence intensity in relation to the various physical properties of the liquid.

In the case of aqueous solutions of liquids, Negishi[11] has found that liquids having relatively larger density, lower boiling point and smaller solubility give more intense luminescence (Table 4) and in the case of water saturated with a gas, Prudhomme[24] has found that the intensity of sonoluminescence depends on the ionization potential and the solubility of the gas. The lower the ionization potential of the gas and greater its solubility, greater is the intensity of sonoluminescence (Table 5). The ratio of the two specific heats of the gas does not affect the luminescence. In case of aqueous solutions of electrolytes, Günther *et al.*[22,23] have found that the intensity of sonoluminescence is dependent on the atomic radius of the cations — the sonoluminescence increases with increase in the atomic radius of the cations of the same valency type.

Studying the effect of the yield of the chemical product on the intensity of sonoluminescence, Prudhomme and coworkers[15,16] have found that, in water saturated with inert gases and in aqueous

TABLE 1 — DEPENDENCE OF SONOLUMINESCENCE ON THE DIPOLE MOMENT AND VISCOSITY OF THE LIQUID AS OBSERVED BY CHAMBERS[5,6]

(Values of viscosity except where indicated have been measured at 25°C.)

Liquid	Dipole moment μ Debyes	Viscosity coefficient η 10^{-2} poise	$\mu\eta$	Luminescence intensity relative units
Nitrobenzol	3·90	2·013$^{20°C.}$	7·85	5
o-Nitrotoluol	3·75	2·0	7·5	5
Isoamyl alcohol	1·85	3·865	7·15	4
Water	1·87	1·005	1·88	3
n-Butyl alcohol	1·74	2·822°C.	4·87	3
Propyl alcohol	1·66	2·23$^{20°C.}$	3·70	2
Isopropyl alcohol	1·78	1·95	3·47	2
Ethyl alcohol	1·63	1·192$^{20°C.}$	1·94	1
Chlorobenzol	1·52	0·751	1·14	0
Methyl alcohol	1·64	0·591$^{20°C.}$	0·97	0
Acetone	2·70	0·3	0·81	0
Methyl acetate	1·67	0·478°C.	0·80	0
Ethyl acetate	1·74	0·442°C.	0·77	0
Chloroform	1·10	0·564$^{20°C.}$	0·62	0
Acetaldehyde	2·68	0·223	0·60	0
Ethyl ether	1·22	0·226	0·28	0
Toluol	0·52	0·552	0·29	0
Benzol	0·06	0·649$^{20°C.}$	0·04	0
m-Xylol	0·06	0·595	0·04	0
Carbon disulphide	0·06	0·367$^{20°C.}$	0·02	0
Carbon tetrachloride	0·00	0·962$^{20°C.}$	0·00	0

TABLE 2 — DEPENDENCE OF SONOLUMINESCENCE INTENSITY ON THE CONCENTRATION OF THE POLAR LIQUID AND ON THE TEMPERATURE OF THE SOLUTION AS OBSERVED BY CHAMBERS[5,6]

Percentage of nitrobenzol in toluol	Relative emission of light observed at 28°C.	Variation of relative light emission with temperature
100	Very bright	Somewhat dimmed at 63°C.
80	Bright	Somewhat dimmed at 57°C.
60	Less bright	Dim at 55°C.
40	Dim	Barely perceptible at 52°C.
20	Very dim	No light at 52°C.
0	None	—

TABLE 3 — SONOLUMINESCENCE FLUX AS A FUNCTION OF THE PHYSICAL PROPERTIES OF THE LIQUIDS AS OBSERVED BY JARMAN[10]

Liquid	Sonoluminescence flux relative units			Vapour pressure mm. Hg			Surface tension at 20°C. dyne cm.$^{-1}$	Dipole moment at 20°C. μ Debyes	Viscosity coeff. at 30°C. η 10^{-3} poise	$\mu\eta$
	25°C.	40°C.	55°C.	25°C.	40°C.	55°C.				
Dimethyl phthalate	16	6·6	2·4	0·02	0·04	0·07	60	2·2	Large	Large
Ethylene glycol	12	3·4	0·5	0·2	0·5	1·0	60	2·28	do	do
Tap water	3·6	1·0	--	24	55	115	73	1·87	0·80	1·50
Chlorobenzene	0·84	0·43	0·20	12	26	53	33·3	1·69	0·71	1·20
Isoamyl alcohol	0·54	0·28	0·18	4·6	10	25	24·3	1·85	2·96	5·48
o-Xylene	0·36	0·24	0·14	6·6	16	32	30·2	0·51	0·69	0·35
Butan-2-ol	0·30	0·17	0·086	19	45	110	23·5	1·65	3·18	5·25
Benzene	0·23	0·06	0·01	94	180	310	28·9	0·00	0·57	0·00
n-Butyl alcohol	0·21	0·10	0·03	7·0	19	45	24·6	1·74	2·27	3·95
n-Propyl alcohol	0·21	0·076	0·038	20	50	115	23·7	1·66	1·72	2·85
Isobutyl alcohol	0·17	0·088	0·046	12	30	68	23·0	1·72	2·88	4·95
Toluene	0·15	0·074	0·050	28	60	130	28·5	0·34	0·53	0·18
Isopropyl alcohol	0·054	0·028	0·012	44	106	--	21·4	1·78	1·77	3·15
Ethyl alcohol	0·04	0·018	—	44	130	280	22·3	1·70	0·99	1·68
tert-Butyl alcohol	—	0·050	0·025	42	102	224	19·6	1·66	3·32	5·51

TABLE 4 — SONOLUMINESCENCE INTENSITY AS RELATED TO THE VARIOUS PHYSICAL PROPERTIES OF THE LIQUIDS OBSERVED BY NEGISHI[11]

Liquid	Intensity of luminescence relative units	Density g./cc.	Boiling point °C.	Vapour pressure at 20°C. mm. Hg	Solubility in water at 20°C. wt %	Ratio of sp. heats of vapour γ
Carbon disulphide	11	1·27	46·3	298	0·217	1·63
Bromine	10	3·14	58·7	173	3·41	1·32
Methyl iodide	7	2·29	45·0	331	1·40	1·28
Carbon tetrachloride	3·5	1·57	76·7	91	0·08	1·13
Trichloroethylene	2·7	1·47	87·0	60	0·1	—
Bromobenzene	1·0	1·50	155·9	5·67$^{30°C.}$	0·045$^{30°C.}$	—
Aniline	0·75	1·02	184·3	2·45$^{50°C.}$	3·49	—
Water	0·40	1·00	100·0	17·54	—	1·33
Benzene	0·25	0·88	80·5	74·7	0·072	1·4

TABLE 5 — DEPENDENCE OF SONOLUMINESCENCE INTENSITY ON THE NATURE OF GAS PRESENT IN WATER AS OBSERVED BY PRUDHOMME[15]

Gas	Ionization potential V.	Solubility in water at 20°C. wt %	Atomic weight	Ratio of sp. heats γ	Amount of H_2O_2 produced 10^{-6} g./ml.	Luminescence intensity relative units	Ultraviolet pulses/min.
Helium	24·58	13·8	4·0	1·65	1·0	1	1
Neon	21·56	14·7	20·8	1·64	7·0	1	18
Argon	15·75	37·9	39·9	1·65	21·5	3	64
Krypton	14·00	73·0	83·8	1·67	24·0	4	226
Xenon	12·30	110·0	131·3	1·67	27·5	5	448
Air	–	29·2	—	—	8·0	1	—
Oxygen	12·50	31·6	—	—	13·5	2	—
Nitrogen	15·51	23·3	—	—	2·5	1	—

solutions of carbon disulphide, carbon tetrachloride or chloroform, along with the increase in the intensity of sonoluminescence, the yield of chemical product (hydrogen peroxide in the case of water saturated with inert gases, hydrogen sulphide in case of aqueous solutions of carbon disulphide and chlorine in case of aqueous solutions of carbon tetrachloride and chloroform) also increases. In systems having water, carbon tetrachloride and gas not chemically reactive with the system, it has been seen by Griffing and Sette[12] that there is a linear relationship between sonoluminescence intensity and yield of free chlorine (Table 6) and by Fitzgerald *et al.*[28] that the yield of this free chlorine increases with the increase in the ratio of the heat capacities and decreases with the increase in the thermal conductivity of the gas (Table 7). It is further seen from the data presented in Table 7 that the rate of formation of free chlorine is highest in solutions saturated with monoatomic gases, comparatively lower in solutions saturated with diatomic gases and lowest in solutions saturated with polyatomic gases. The intensity of sonoluminescence in these solutions is also seen to vary in the same way as the rate of the yield of free chlorine. This shows that sonoluminescence depends on the yield of the chemical product in the systems investigated.

TABLE 6 — COMPARISON OF THE INTENSITY OF LUMINESCENCE AND CHEMICAL YIELD FOR THE H_2O-CCl_4-GAS SYSTEMS (GAS NOT CHEMICALLY REACTIVE WITH THE SYSTEM) AS OBSERVED BY GRIFFING AND SETTE[12] AT A SONIC FREQUENCY OF 2 Mc/s.

Gas present in the system	Qualitative estimate of the intensity of sonoluminescence	Yield of free chlorine after an irradiation by sonic waves for 6 min. millicquivalent per litre
Argon	Strong	0·888
Neon	do	0·690
Nitrogen	Medium	0·534
Carbon monoxide	Weak	0·330
Sulphur hexafluoride	do	0·276
Freon 114	Very weak	0·060
Hydrogen	None	0·00*
Carbon dioxide	do	0·000†

*Cavitation was present.
†Cavitation was not present.

TABLE 7 — RATE OF FORMATION OF FREE CHLORINE AS A FUNCTION OF THE THERMAL CONDUCTIVITY AND THE RATIO OF THE SPECIFIC HEATS OF THE DISSOLVED GASES IN H_2O-CCl_4-GAS SYSTEMS (GAS NOT REACTIVE CHEMICALLY WITH THE SYSTEM) AS OBSERVED BY FITZGERALD *et al.*[28]

Gas present in the system	Initial rate of formation of free chlorine millicquivalent/min.	Ratio of heat capacities of the gas (γ)	Thermal conductivity (σ) 10^{-3} cal.sec.$^{-1}$ cm.$^{-1}$°C.$^{-1}$
Argon	0·148	1·66	0·0387
Neon	0·115	1·66	0·1104
Helium	0·096	1·66	0·344
Oxygen	0·094	1·39	0·0573
Nitrogen	0·089	1·40	0·0566
Carbon monoxide	0·055	1·43	0·0514
Sulphur hexafluoride	0·046	—	0·0336
Freon 114	0·010	1·088	—

Spectral Composition

Using a spectrophotometer, Pasunoff[29] has found that in water saturated with oxygen, luminescence lies in the visible region, being intense in the wavelength range 4450-5580 A. In case of aqueous solutions of luminol, Bresler[14] has found that the spectral band extends from the red to the violet region with a flat-topped peak in the region 4100-4400 A. Along with the visible spectrum, weak ultraviolet components[15,16,19] having a maximum intensity around 2300 A. have also been observed.

Srinivasan and Holroyd[21,30] have found that for water saturated with argon or helium, the spectral energy distribution curve corresponds very nearly to the black body radiation curve at 11000°K.; that for water saturated with oxygen or nitrogen, the corresponding black body temperature is 8800°K.; and that in the presence of other inert gases, the energy distribution curve resembles the corresponding curve for a black body at a temperature of 6000°K. These values conform to the Noltingk and Neppiras' theory[31,32].

Günther *et al.*[22,23] and Heim[33] have found that the luminescence spectrum is independent of the gas or electrolyte present in the solution and consists of broad structureless bands (continuum) extending from the infrared to the ultraviolet with a peak at 4250 A. In concentrated solutions of salts of alkali and alkaline earth metals, the luminescence spectrum, in addition to continuum, shows characteristic lines of the metal present. These characteristic lines are suppressed by the addition of radioactive isotopes which, however, do not materially affect the continuum.

Localization

According to Pasunoff[29], luminescence comes from the nodes of stationary waves and no luminescence is possible at the pressure antinodes since at these spots water is not subjected to ultrasonic degassing. This observation has been supported by Pinoir and Pouradier[34] on the basis of fogging of photographic plates at the pressure nodes. However, it is now considered that this fogging can also be in part due to the cavitation of the medium even when no luminescence is present.

Laufer and Srinivasan[9] observed that in case of a progressive wave, luminescence arises at the liquid-air interface, i.e. in the fountain region, while in case of standing waves, luminescence occurs at a certain distance from the reflector. According to them, sonoluminescence appears even when resonance pulsation of cavitation bubbles does not occur.

Phase of Sonoluminescence

Using a photon counter and the Debye-Sears effect[35,36], Macleay and Holroyd[37] have shown that photon emission in the sonoluminescence process takes place during the compression phase of the sonic field. Günther *et al.*[38-40], while taking measurements of sonoluminescence emission in water and in some organic liquids in the presence of the diffused gases, observed that light flashes occur at the end of the ultrasonic compression phase and that the duration of this flash is less than 10^{-7} sec.

Negishi[11,41], working at a sonic frequency of 28 kc/s. against a dark field illumination of the cavitating bubbles, has obtained a curve representing the instantaneous bubble volume by displaying the scattered light from the bubble on the oscilloscope through a photomultiplier. Keeping the triggering conditions of the oscilloscope the same, he also observed sonoluminescence as discrete flashes of light occurring once in each sound cycle and determined the phase relation between the two.

The double exposure oscillogram of bubble volume and sonoluminescence in fresh tap water supersaturated with air (Fig. 1) shows that luminescence pulses appear at about 1/5 period before the phase of minimum bubble volume, attain a maximum at the minimum bubble volume, i.e. at the instant of complete collapse of the cavity, and thereafter fade out rapidly. In water of appreciably reduced air content, however, luminescence pulses appear almost at the moment of the bubble collapse. The sound pressure waveform and change in bubble volume is shown in Fig. 2. It is seen that at the minimum of the bubble volume, a large pressure peak is produced due to the presence of superposed shock waves. In Fig. 3 is reproduced a secondary flash occurring

shortly after the main flash. This is visible only at high sonic pressures. In phase with the secondary flash a small dip is seen to appear in the curve of the bubble volume. This is interpreted to signify that some of the bubbles rebound and collapse shortly afterwards emitting a secondary flash. Similar observations, i.e. luminescence pulses occurring at the moment of collapse of the bubbles, have also been made by Kuttruff *et al.*[42-45].

Wagner[46], investigating the phase of luminescence in krypton-saturated water at a sonic frequency of 200 kc/s., observed that luminescence occurs as single discrete flashes, once every sound cycle within a tenth of a period of the sound pressure minimum (Fig. 4). At a reduced temperature the occurrence of the secondary flashes is also noticed. Jarman[10], irradiating various liquids by sound waves of frequency 16·5 kc/s., has also seen that sonoluminescence generally occurs as single discrete flashes once every sound cycle and does not coincide with the collapse of the cavitation bubble (Figs. 5 and 6). A secondary flash is also seen to occur shortly before or after the main flash. The main flash appears to occur within a tenth of a period at the sound pressure minimum in case of non-volatile liquids and shortly before or at the sound pressure maximum in case of volatile liquids. In general, the phase of the main flash tends to move towards that for water as the liquid becomes less volatile.

Finch[47,48], examining the occurrence of sonoluminescence as discrete flashes once every cycle of the acoustic field, observed that in a single sweep oscillogram (Fig. 7), sonoluminescence occurs as pulses always at roughly the same phase of the sound field but not with every cycle of the sound wave. Secondary flashes are also seen adjacent to the main flash. The probability of the sonoluminescence pulse occurring during a given cycle of the sound field is seen from this oscillogram to be about one-fifth. In the many-sweep oscillogram (Fig. 8), where an averaging effect appears, sonoluminescence is seen to occur as a flash with every sound cycle — an observation made earlier by other workers[10,11,41-46]. The appearance of a flash, once in about five cycles, has been attributed

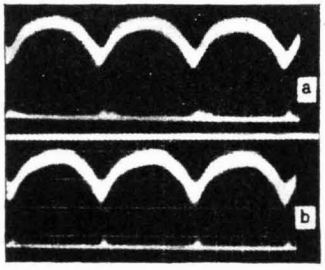


Fig. 1 — Oscillograms of volume change of cavitating bubbles (upper trace) and sonoluminescence (lower trace) as recorded by Negishi[11] [(a) in fresh tap water; and (b) in water of appreciably reduced air content]

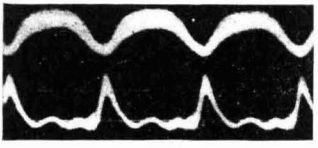


Fig. 2 — Variation of bubble volume (upper trace) with respect to sound pressure waveform (lower trace) as observed by Negishi[11]

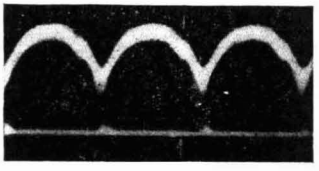


Fig. 3 — Small dips in volume change of cavitating bubbles (upper trace) indicating corresponding secondary flashes (marked by arrows in lower trace) as observed by Negishi[11]

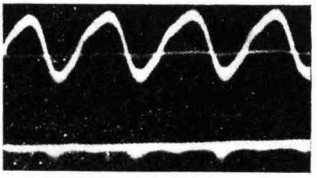


Fig. 4 — Simultaneous display of the acoustic waveform (upper trace) and sonoluminescence intensity as observed by Wagner[46]

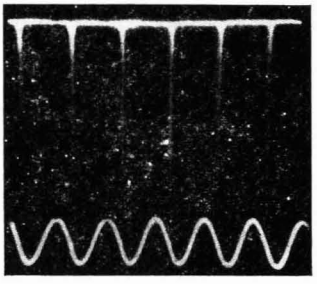


Fig. 5 — Sonoluminescence flashes in water saturated with carbon disulphide (upper trace) and a.c. input to the transducer (lower trace) displayed on the double beam oscilloscope (under condition of simultaneous triggering) at an acoustic power of about 20 W. as observed by Jarman[69]

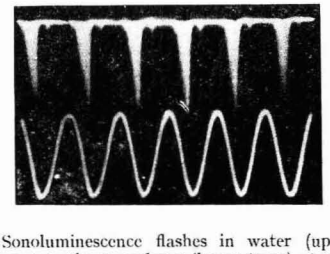


Fig. 6 — Sonoluminescence flashes in water (upper trace) and a.c. input to the transducer (lower trace) at an acoustic power of about 45 W. as observed by Jarman[70]

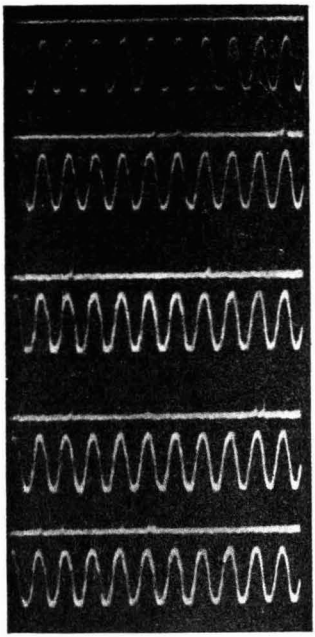


Fig. 7 — Oscillogram of the pick-up voltage of the acoustic waveform (upper trace) and photomultiplier anode voltage indicative of sonoluminescence intensity (lower trace) observed simultaneously in a single sweep by Finch[47,48]

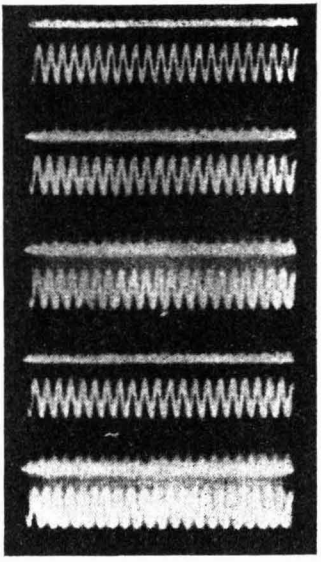


Fig. 8 — Oscillograph trace of the pick-up voltage of the acoustic waveform (upper trace) and photomultiplier anode voltage indicative of sonoluminescence intensity (lower trace) simultaneously observed in many-sweep oscillogram by Finch[48]

to the availability of a suitable nucleus in the medium. The presence of the secondary flash has been attributed to the simultaneous presence of two nuclei and to the rebound or disintegration of a single nucleus.

Theories of Origin

There are several hypotheses of the origin of sonoluminescence. These hypotheses are closely connected with the mechanism of the chemical action of ultrasonic waves and cavitation[49-51]. No conclusive evidence in favour of any of these theories is yet available.

Electrical Microdischarge Theory

The electrical microdischarge theory proposed by Frenkel[52] postulates that thin non-spherical lens-shaped cavities of molecular dimensions are abruptly produced in the liquid due to the effective tearing of the liquid brought about by negative pressures developed in the liquid on the passage of an acoustic wave. Due to the statistical fluctuation of charge distribution in the liquid, high Lenard potentials[53] of opposite charges are generated on both sides of the cavities. (These are charges which separate out when a liquid-gas interface is torn apart.) These potentials increase as the cavities grow into spherical shape with the result that, at a certain potential, an electrical breakdown, accompanied by visible and ultraviolet radiations, occurs between the walls of the cavity. The discharge takes place through the low pressure gas or vapour in the expanding cavities at the pressure antinodes with the production of light and the formation of active oxygen. This active oxygen may be further responsible for the observed chemical effects on ultrasonic irradiation in liquids.

The electrical potential developed is given by the expression

$$E = \frac{4e}{R}(\pi n_o d)^{\frac{1}{3}}$$

where E is the strength of the electric field in the cavity; d, the initial diameter of the cavity; e, the electric charge of a single ion; n_o, the average number of ions of each sign per unit volume of the liquid; and R, the radius of the cavity.

Chambers[5,6] has suggested triboluminescence, observed in the breakdown of certain crystals, as responsible for sonoluminescence (triboluminescence is the luminescence produced due to the discharge of frictional electric charges generated as a result of strong grinding or rubbing). It is presumed that when the quasi-crystalline structure of the liquid is fractured by cavitation, triboluminescence occurs.

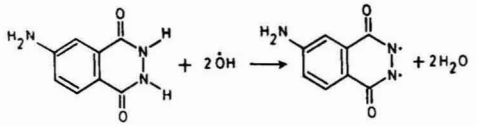
Harvey[8] has ascribed sonoluminescence to a phenomenon similar to that assumed by Frenkel. Unlike the lens-shaped cavities, it is assumed that electric charges appear on the surface of bubbles in analogy to Lenard potentials on liquid drops suspended in gases. The potential of these charges rises as the cavity collapses and ultimately gives rise to luminescence on discharge.

Chemiluminescence Theory

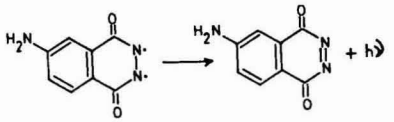
It has been found by a number of workers[54-61] that the phenomena of triboluminescence, ballo-electric charges and sonoluminescence are closely associated with the chemical reactions which take place at the nascent surfaces of the cavities. At the same time, the origin of sonoluminescence from electrical discharge between two electrodes cannot be assumed because of the difference in behaviour of the molecules present in a discharge tube and those dissolved in water and exposed to an ultrasonic field. It has been, therefore, suggested that light may be emitted by photochemical recombination of free ions produced

due to the mechanical dissociation of molecules at the nascent surfaces of the growing cavities. Impurities such as carbon tetrachloride and other non-polar organic molecules can play a special role in this process because of acting as cavitation nuclei. These cavitation nuclei provide discontinuities in the structure of water and thereby affect the tensile strength of water.

The observed glow in aqueous solutions of luminol has been similarly treated by Bresler[14]. Luminol in an ultrasonic field may be oxidized by the OH^- radicals produced due to the decomposition of water as



The resulting compound, being unstable, undergoes reordering emitting photons as



The emission of these photons is taken to be responsible for the observed sonoluminescence.

Griffing *et al.*[12,28], while attributing the emission of sonoluminescence to photochemical recombination of free ions, have argued that sonoluminescence due to electrical microdischarge is not possible because cavitation and luminescence can be observed at intensities of the sound waves far below the intensity required to overcome intramolecular forces. It is assumed by these workers that the free ions required for photochemical recombination are produced due to the thermal dissociation induced by sonic waves in collapsing cavities.

Jarman[10] has suggested that both photochemical recombination of dissociated molecules at the nascent surfaces of the bubbles and electrical microdischarges occurring within the cavitation bubbles may be causing sonoluminescence. In the case of volatile liquids, it has been suggested that cavitation bubbles need to be large to make the combined pressure of the gas and vapour in the bubbles sufficiently low for a discharge to take place causing luminescence flashes to occur at a later stage of the sound cycle.

Hot Spot Theory

Noltingk and Neppiras[31,32], while investigating the behaviour of a gas bubble in an acoustic field of pressure,

$$P = P_h - P_o \sin wt$$

where P_h is the hydrostatic pressure of the liquid and P_o is the acoustic pressure amplitude, assumed that during the time preceding the bubble collapse when the acoustic pressure amplitude is small, the gas in the bubble is compressed isothermally, while in the collapse phase, where the acoustic pressure amplitude is maximum and the velocity of motion of the bubble walls is very large, the compression is adiabatic. This adiabatic compression of the gas at the time of collapse of the cavity increases the temperature of the gas inside the cavity sufficiently high till it reaches a stage where the gas begins to glow emitting black body radiation.

The expression for the surface velocity of collapse of the bubbles as derived by Noltingk and Neppiras[31] by improving Rayleigh's expression[62] and assuming pressure of the acoustic field to be constant during the time of collapse of the bubble is given as

$$v^2 = \frac{2P(\gamma-1)}{3P\gamma}\left[\frac{P(\gamma-1)}{Q\gamma}\right]^{1/(\gamma-1)}$$

where γ is the ratio of the heat capacities of the gas and Q is the pressure of the gas in the bubble at its maximum radius. At a constant value of the maximum bubble radius R_m, Q is proportional to $(P_h+2\sigma/R_o).R_o^3$, where σ is the surface tension of the liquid and R_o is the initial bubble radius.

The time of collapse of the bubble according to this view should be extremely short and Rayleigh's expression for this time in case of a hollow cavity is given as

$$\tau = R_m\left(\frac{3P}{2P_h}\right)^{1/2}\int_\beta^1 \frac{\beta^{3/2}}{(1-\beta^3)^{1/2}}.d\beta$$

where β represents the ratio of the bubble radius R just before the collapse and maximum bubble radius R_m. For a case where β is zero, i.e. where a hollow cavity completely collapses, this expression further simplifies to

$$\tau = 0{\cdot}915R_m(P/P_h)^{1/2}$$

This fast collapse of the cavity is accompanied by a shock wave as has been observed by Harrison[63], Mellen[64], Bohn[65], Günther *et al.*[39] and Negishi[11]. The shock pressure $P_{max.}$, which is equal to the pressure of the gas inside the bubble, is given as

$$P_{max.} = Q(R_m/R)^{3\gamma}$$

This expression shows that shock pressure depends on the maximum bubble radius which is controlled by the acoustic frequency, hydrostatic pressure and acoustic pressure amplitude. With increase in acoustic pressure amplitude and decrease in hydrostatic pressure and acoustic frequency, the value of the maximum bubble radius increases.

The temperature obtained inside the bubble at its minimum radius under adiabatic compression is approximately given as

$$T \simeq T_o(P/3Q)^{3(\gamma-1)}$$

where T_o is the absolute temperature of the liquid surrounding the bubble. This expression has been improved by Güth[66]. A rough calculation shows that the temperature of the gas at the minimum bubble radius under adiabatic compression rises to 10000°K. for a case where T_o is 300°K., pressure P is 1 atm. and Q is equal to 0·01 atm.

Macleay and Holroyd[37] have made calculations of the intensity of sonoluminescence in terms of the phase angle distributions on the basis of Noltingk and Neppiras' theory, and have found that theoretical values agree well with the experimentally determined

distributions. Günther *et al.*[38-40], on the basis of observations of phase relationship of sonoluminescence with the acoustic cycle, also assume that adiabatic compression of the gas in cavities is responsible for luminescence. Negishi[11,41], on the basis of the observation that sonoluminescence at first increases with increasing sound intensity but decreases subsequently rather suddenly at high sound intensities, and that luminescence occurs at the moment of collapse of the bubble, has inferred that sonoluminescence is thermal in origin as assumed by Noltingk and Neppiras. Negishi explains the disappearance of sonoluminescence at very high sound intensities on the assumption that cavities successively collapse in order of their expanded sizes emitting flashes of increasing intensity, till they grow too large to collapse.

Wagner[46], however, has found that luminescence occurs within a tenth of a period of the sound pressure minimum. On the basis of this observation, he has suggested that heating of the gas bubbles due to adiabatic process cannot cause sonoluminescence.

Validity of the Various Theories

In the microdischarge theory, Frenkel assumes the formation of lens-shaped cavities but, as pointed out by Negishi[11], there is no experimental evidence in support of cavities of this shape.

The chemiluminescent theory implies the existence of a chemical reaction simultaneous with light emission. In practice a chemical reaction is not always detected along with sonoluminescence[9,21,30].

The hot spot theory, however, may account for the observed luminescence as indicated by the resemblance of the continuous spectra of sonoluminescence from engassed water with black body radiation between temperatures 6000° and 11000°K. as observed by Srinivasan *et al.*[21,30] and Günther *et al.*[38-40]. This view is also supported by the observation that sonoluminescence spectrum from concentrated solutions of sodium chloride contains line spectrum of sodium along with the continuum[22,23], and that generation of heat and the subsequent ignition of the gas as assumed in the hot spot theory by the impact of shock waves is possible as has been shown by the experiments conducted by Soloukhin[67]. However, it is to be noted that sonoluminescence may not be purely a black body radiation. It is likely that some ionic processes or dissociation of neutral molecules as observed in high current density arcs[68] may also contribute to the observed sonoluminescence[11]. Further, it has been pointed out by Jarman[69,70] that spectra of some of the chemiluminescent, particularly bioluminescent, liquids resemble that of black body radiation and that such a spectrum occurs even in the case of electrical discharge in the rarefied atmosphere of a gas bubble.

Jarman[70] has suggested that sonoluminescence can arise from microshocks occurring within the cavities during their final stage of collapse. This hypothesis is based on the close resemblance of intensity and spectral quality as produced in sonoluminescence as also in the luminescence produced either behind a shock front or at the point of reflection of a shock front. Firstly, both luminescences are very intense in the presence of rare gases in water[23,71,72]; secondly, inorganic impurities (sodium and calcium) in water produce similar lines and bands in the spectra[23,33] in both the cases; and thirdly, the addition of carbon tetrachloride or carbon disulphide to water results in a considerable increase in the light emission[7,24,73]. Jarman and Taylor[74] have recently reported that water flowing past the constriction of a Venturi tube induces vaporous cavitation due to a fall in pressure and emits light at the collapse of the cavities. The spectrum of this light has been found to be similar to that observed for sonoluminescence with its intensity several orders of magnitude less than that observed for sonoluminescence and that the intensity is enhanced with the addition of a small quantity of carbon disulphide in water in the same way as observed for sonoluminescence.

The validity of these theories to explain the observed results can be further scrutinized on the basis of the flash of light appearing either at the initial instant of development of the gas cavity in the case of Frenkel's theory or at the instant of collapse of the cavity in the case of Noltingk and Neppiras' theory. In regard to this, Wagner[46] and Jarman[10] find the phase in favour of Frenkel's theory, whereas Macleay *et al.*[37], Negishi[11,41] and Kuttruff *et al.*[42-45] find results in favour of Noltingk and Neppiras' theory. However, this difference in the observed phase relationship as pointed out by Macleay *et al.*[37] and Kuttruff *et al.*[45] may be reconciled in view of the fact that liquids contain nuclei differing in size which require different times for the formation and subsequent collapse of the cavities, resulting in luminescence flashes during a considerable portion of the wave period. Further, according to the theory of adiabatic heating of the gas, the brightest light pulse corresponds to minimum bubble diameter, whereas according to the theory of gaseous electrical discharge, the brightest light pulse corresponds to the initial stage of evolution of the cavity. This leads to a time difference between the two luminescent pulses to a small fraction of the acoustic wave period. This time difference may be more discernible if the experiments are conducted at lower frequencies.

Further progress in this area of knowledge depends very much on an improvement in the methods of registration of ultrasonic luminescence whose intensity is very low. The actual identification of the spectral lines and bands, especially in the ultraviolet part of the ultrasonic luminescence, is of no less importance. Further experiments to confirm the phase relationship between sonoluminescence and pressure of the sonic waves are also needed. This will provide new opportunities for elucidation of the mechanism of induction of luminescence and the corresponding chemical reaction.

Summary

The present status of the experimental work on the phenomenon of sonoluminescence is discussed with special reference to experimental techniques employed, observations made on the mechanism, nature, localization, spectral composition and intensity of sonoluminescence and the various theories advanced to explain the phenomenon are reviewed. A critical appraisal of the available data suggests that the determination of the phase of sonoluminescence in relation to the cavitation bubbles needs further

study to establish the exact relationship between the two. Also improvements in the method of registration of sonoluminescence, especially in the ultraviolet part of the spectrum, are required because the intensity of sonoluminescence in this region is very low. These studies will provide new approaches towards the elucidation of the mechanism of induction of luminescence.

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Convention of Biochemists

A convention of biochemists sponsored by the Biochemical Society, UK, and the Society of Biological Chemists, India, will be held at Bangalore during 4-9 September 1967. About 50 British and American, and 20 Indian biochemists will be invited to present papers. Further particulars regarding the symposium can be had from the Hon. Secretaries, Society of Biological Chemists, Department of Biochemistry, Indian Institute of Science, Bangalore 12.

Metabolism of Vitamin A

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IN 1964, on request from the Indian Council of Medical Research a comprehensive summary of the work carried out in this laboratory was prepared by Ganguly and Mahadevan[1]. Since then, considerable progress has been made in our programme of work in this laboratory and much of our work has been confirmed and further extended in other laboratories. Therefore, it was felt that the earlier review should be revised and brought up to date. In doing so, no attempts have been made to review all related work reported from other laboratories of the world, but only those reports that have confirmed and extended our work have been highlighted here. There have been some conflicting reports in a few areas. Attempts have been made critically to analyse these and, wherever possible, to interpret them.

Work carried out in this laboratory during the past decade can be classified under the following broad headings: (I) Mechanism of absorption of glycerides, carotenoids, cholesterol and vitamin A; (II) Storage and transport of vitamin A; (III) Biological formation of vitamin A active compounds; (IV) Mode of action of vitamin A; and (V) Metabolism of vitamin A in protein malnutrition.

MECHANISM OF ABSORPTION OF GLYCERIDES, CAROTENOIDS, CHOLESTEROL AND VITAMIN A

Enzymes Regulating Absorption

Hydrolysis of Retinyl and Cholesterol Esters

Several years ago Ganguly[2] observed that a homogenate of hog pancreas, when incubated with crude retinyl esters of fish liver oil, catalyses the hydrolysis of the retinyl esters giving rise to retinol. A few years later retinyl ester hydrolase activity was demonstrated in rat liver homogenates by McGugan and Laughland[3]. Immediately after, Ganguly and Deuel[4] and Ganguly[5] confirmed this observation and further showed that the retinyl ester hydrolase activity of rat liver is localized in the microsomal fraction of the liver homogenates. In continuation of this work, Seshadri Sastry *et al.*[6] showed that many tissues of the rat, like liver, intestine and pancreas, possess the capacity to hydrolyse retinyl esters. The liver enzyme is very active against short-chain fatty acid esters like the acetate, while the pancreatic and intestinal enzymes are able to hydrolyse both short- and long-chain fatty acid esters of retinol with equal efficiency.

Liver — The liver has been known for a long time to contain an esterase that acts on the short-chain aliphatic esters, like ethyl acetate, ethyl butyrate, etc. Therefore, it became obvious that the question of the specificity of the rat liver retinyl ester hydrolase should be investigated. Upon testing identical samples of enzyme preparations from rat liver against retinyl acetate and ethyl butyrate in the presence of a variety of inhibitors, like metal ions, arsenicals, etc., significant differences in the sensitivity of the two activities towards the inhibitors were noticed[6]. Moreover, the retinyl ester hydrolase activity was more labile towards heat treatment and ageing. These observations, therefore, suggested that the retinyl ester hydrolase of rat liver is probably different from the common esterase.

Investigations on the intracellular distribution of the retinyl ester hydrolase, the common esterase and the cholesterol ester hydrolase in rat liver showed that all the three activities are quantitatively localized in the microsomal fraction of the homogenate. In contrast, in the chicken liver, the three enzyme activities, in addition to being present in the microsomal fraction, were found to be associated with the nuclear fraction also. In this work the purity of the cell fractions of chicken liver was established by determining the relative distribution of the activities of enzymes, like acid and alkaline phosphatases, succinic dehydrogenase and glucose-6-phosphatase, which are characteristic of the different cell fractions, and also from the relative distribution of the nucleic acids in all cell fractions. It was thereby concluded that the three esterase activities in the nuclear fraction of chicken liver homogenates are not due to contamination[7].

Attempts to solubilize the microsomal enzymes (common esterase, cholesterol ester hydrolase and retinyl ester hydrolase) of rat liver by acetone drying, followed by extraction with water or buffer, led to a total loss of the latter two esterase activities, leaving the common esterase almost unaffected[6]. Even though solubilization could not be effected, these experiments proved that the common esterase is different from the other two esterases. As will be seen shortly, the pancreatic and intestinal enzymes are readily solubilized by this procedure. Treatment of the microsomal fraction of rat or chicken liver with various agents, like detergents, butanol, etc., commonly used for solubilizing enzymes from particulate materials of tissue homogenates, failed to bring the enzyme activities into solution[8].

Success in solubilizing the liver enzymes ultimately came from the observations of Seshadri Sastry and Ganguly[9] that, in contrast to the microsomal enzymes, the three esterases of chicken liver nuclei are readily solubilized by treating the nuclear fraction with Lissapol N. Clear solutions could be obtained by shaking the chicken liver nuclei with an 1 per cent solution of the detergent for 10 min., followed by high-speed centrifugation. Ammonium sulphate fractionation of such a solution resulted in a clear separation of all the three enzyme activities, and electrophoresis on starch gel showed that the three activities move with three distinctly separable protein bands[9].

It was thus established that rat and chicken livers contain three distinct enzymes that can catalyse the hydrolysis of short-chain fatty acid esters of simple aliphatic alcohols, of retinol and of cholesterol.

Pancreas and intestine — Similar thorough investigations were carried out with the pancreatic and mucosal enzymes of rats[10]. The pancreatic enzymes were markedly more active than the corresponding mucosal enzymes, and here also the activities were found to be localized in the microsomal fraction of the homogenates. Though they were active against both long- and short-chain esters of retinol, as well as of cholesterol, hydrolysis of the higher esters required the presence of bile salts. Soon after this, Murthy and Ganguly[11] showed that, unlike the liver enzymes, they are resistant to acetone treatment; acetone powder, prepared from either tissue, on extraction with distilled water, buffer or physiological saline, readily yielded water-soluble enzymes. Treatment of the aqueous extracts of acetone powder of rat pancreas with calcium phosphate gel resulted in a clear separation of the cholesterol ester hydrolase from the retinyl ester hydrolase[11] (Table 1). This confirmed our earlier results obtained with chicken liver that these hydrolytic activities are due to different enzymes.

In the more recent experiments of David *et al.*[12], where everted intestinal sacs of rats were incubated with cholesterol oleate or cholesterol plus oleic acid in the medium, rapid hydrolysis, but no synthesis, of the sterol ester was noticed in the medium. Homogenization of the whole mucosa, however, showed the presence of significant hydrolytic and synthetic activities. Moreover, when the brush border was separated from the rest of the mucosal cells, 90 per cent of the hydrolytic and only 33 per cent of the synthetic activities of the whole mucosa were found to be localized in the isolated brush border, the remaining portions being present in the rest of the cell. We have, therefore, suggested that the hydrolytic enzyme is situated on the brush border facing the lumen of the small intestine, while the esterifying enzyme is situated towards the inner side of the cell[12]. The significance of these observations in relation to the mechanism of absorption of cholesterol is discussed later.

Synthesis of Retinyl and Cholesterol Esters

Earlier work had repeatedly shown that, during absorption from the intestine, the two alcohols, retinol and cholesterol, appear in the lymph mostly as their esters, thereby implying the presence of the esterifying enzymes in the intestinal mucosa. Though some work was reported in the earlier literature on the cholesterol esterase systems of the intestine and pancreas of rats, there was virtually no information on the enzymes synthesizing retinyl esters in these tissues.

Both intestine and pancreas of rats were shown to possess the two synthetic activities, and like the corresponding hydrolytic systems these enzymes also were associated with the microsomal fractions of the tissue homogenates. Other tissues, like liver, blood, spleen and kidneys, had negligible activities[10].

Like the hydrolytic systems of these tissues, the synthetic activities were not destroyed by acetone drying, and from the acetone powder they could be extracted (along with the hydrolytic enzymes) into aqueous solution. The enzymes, thus solubilized, showed some interesting differences in their properties regarding the esterification of cholesterol and retinol. Thus, while sodium taurocholate inhibited the esterification of retinol, it was a requirement for the synthesis of cholesterol esters[11]. The esterification of retinol by either tissue proceeded well with fatty acids containing more than 10 carbon atoms and no preference for the presence or absence of unsaturation in the fatty acid was exhibited. In contrast, the enzyme extracts from either tissue esterified cholesterol preferentially with unsaturated fatty acids; the pancreatic enzyme esterified the sterol with decreasing efficiency with an increase in the unsaturation of the fatty acid in the order oleic>linoleic>linolenic, while with the intestinal enzyme the order was linolenic>linoleic>oleic[13]. This led us to suggest that the intestinal and pancreatic enzymes are of independent origin. This view was confirmed by later experiments of Murthy *et al.*[14], where young growing rats were maintained for 6-8 weeks on a diet containing 1 per cent added cholesterol. In these trials, the hydrolytic enzymes of the pancreas and mucosa did not change significantly while, in contrast, the synthetic activities of the intestine, but not of the pancreas, for the esters of both the alcohols, were markedly elevated with the progress of time. Our suggestion that the pancreatic and mucosal cholesterol esterifying enzymes are of independent origin has recently been corroborated by other workers using different techniques. Thus, Lossow *et al.*[15] have reported that the small intestine of rats with nonfunctional exocrine pancreas can still manifest the cholesterol-esterifying activity, while Borja *et al.*[16] have reported that the mucosal homogenates from rats with a common duct fistula show definite cholesterol-esterifying activity.

During the course of this work it was consistently noticed that the pancreatic enzyme of rats synthesizing retinyl esters was generally more stable than the corresponding cholesterol-esterifying enzyme. Thus, preincubation rapidly destroyed the cholesterol ester synthetase, but not the retinyl ester synthetase. However, this rapid loss of the activity could completely be prevented by inclusion, before incubation, of small amounts of certain bile salts, in the medium. Of the several bile salts tested, only those with a hyroxyl group at position 7 of the phenanthrene ring were able to afford the protection even at very low concentrations; at the same time it was also shown that these bile salts irreversibly bind with the enzyme. Moreover, the same bile salts proved to be essential for optimal activity of both the hydrolytic and the synthetic enzymes of the cholesterol esterase system. These considerations led us to conclude that in these reactions the bile salts function not only as emulsifying agents but also probably as coenzymes[11].

Although there were indications and suggestions from the earlier work of Swell *et al.*[17] and of Korzenovsky *et al.*[18], that bile salts are necessary for the efficient hydrolysis and synthesis of cholesterol esters by the pancreatic enzymes, our extensive

study has shown that the detergent effects of various bile salts bear no relationship whatsoever to the rather specific requirements for the enzymic activities. Thus deoxycholate and dehydrocholate had little or no effect on the enzyme activities. It is, therefore, obvious that emulsification is not the only criterion for the proper functioning of the bile salts in the cholesterol esterase system. We have in fact reported that the synthetic activity of the cholesterol esterase system is inhibited by increasing the proportions of deoxytaurocholate in the assay system, which had led us to examine the possibility that the deoxy compound, being a structural analogue, might compete with the taurocholate. The experiments of Murthy and Ganguly[11] on the effects of taurocholate on this enzyme system, in the presence or absence of deoxycholate, have clearly demonstrated that the deoxycholate competes with the taurocholate for the active sites of the enzyme.

It is, therefore, clear that sodium taurocholate probably has two distinct functions in the cholesterol esterase system, viz. (i) it emulsifies the lipids finely, thereby exposing enormously larger surface areas; and (ii) it also activates the enzyme probably by binding with it and thereby functions as a co-enzyme. The activating effects of the bile salts on other lipolytic enzymes, like lipase, phospholipase, etc., have been known for a long time and it seems conceivable that in these systems also the bile salts have similar multiple functions. It would, therefore, appear that this natural detergent is chemically so constituted that it not only serves as a very powerful emulsifying agent, but also it can probably hook on to the lipolytic enzyme and at the same time combine with the lipid molecule, thereby serving as a bridge between the substrate and the enzyme in bringing them together. Our observations on the ability of the bile salts to stabilize the cholesterol esterase system have recently been confirmed by Vahouny *et al.*[19,20].

Separation of the enzymes hydrolysing and synthesizing retinyl and cholesterol esters — The effects of various inhibitors on the synthesis and hydrolysis of retinyl esters by the enzymes of rat intestine strongly suggested that the two reactions are catalysed by two independent enzymes. Thus, Tweens 20 and 80 strongly inhibited the synthetic activity, without having any effect on the hydrolytic system. It has already been stated that sodium taurocholate inhibits the esterification reaction, while at the same time it is essential for the hydrolysis of the higher esters of retinol. Di-isopropylphosphofluoridate (DFP), on the other hand, preferentially inhibited the hydrolysis, but not the synthesis, of retinyl esters[10]. The enzymes catalysing the synthesis and hydrolysis of cholesterol esters also displayed several differences in their properties.

From these data it became obvious that the hydrolysis and synthesis of retinyl and cholesterol esters are carried out by separate enzymes. Therefore, attempts were made to effect a separation of the two activities of vitamin A esterase and cholesterol esterase systems by subjecting the aqueous extracts of the acetone powder of rat pancreas to ammonium sulphate and calcium phosphate gel fractionation. While the former treatment failed to effect a separation, calcium phosphate gel adsorbed both the synthetic and hydrolytic activities of the cholesterol esterase system, but not of vitamin A esterase system. From the adsorbed state, 0·1*M* phosphate buffer (*p*H 7·4) readily eluted the cholesterol ester hydrolase, but not the synthetase, which in turn could be eluted with a 15 per cent ammonium sulphate solution[11] (Table 1). However, all attempts at effecting a similar separation of the two activities of vitamin A esterase system were unsuccessful. Nevertheless, the similarity in the behaviour of the two activities of cholesterol esterase and vitamin A esterase systems towards differential inhibition and inactivation strongly suggests that a separation of these activities should be possible.

TABLE 1 — SEPARATION OF VITAMIN A ESTERASES AND THE SYNTHETIC AND HYDROLYTIC ACTIVITIES FOR CHOLESTEROL OLEATE OF RAT PANCREAS

Treatment	Total activity			
	Cholesterol, μmoles		Retinol, μmoles	
	Liberated	Esterified	Liberated	Esterified
Water extract of acetone-dried powder	41·2	26·4	12·3	8·2
Supernatant of gel	2·5	1·1	6·5	3·9
25 m*M* phosphate buffer (*p*H 7·4)	4·6	3·0	4·8	3·6
0·1*M* phosphate buffer (*p*H 7·4)	24·1	nil	nil	nil
15% ammonium sulphate	2·0	15·5	nil	nil

Adapted from Murthy, S. K. & Ganguly, J., *Biochem. J.*, **83** (1962), 460.

Esterification of both the alcohols was consistently shown to be independent of any requirements for CoA and ATP[10,11]. The non-involvement of CoA and ATP for the esterification of cholesterol by rat pancreas and intestine has since been confirmed by Shah *et al.*[21]. Therefore, these seem to be unusual types of esterification reactions in not requiring the preliminary activation of the fatty acid, as is usually found in the synthesis of acetylcholine or triglycerides.

This extensive work, carried out over a period of several years and with various tissues of rats and chickens, has thus established that the three esterase systems, namely the common esterase, vitamin A esterases, and cholesterol esterases are independent entities. It has also shown that the cholesterol ester synthetase of rat pancreas is different from that of rat intestine. Finally, evidence has been obtained which strongly suggests that the hydrolytic and synthetic activities of vitamin A esterase system are probably due to independent enzymes. Also, the two activities of the cholesterol esterase system of rat pancreas have been successfully separated. We have, therefore, suggested that the hydrolytic enzyme of the cholesterol esterase system be named ' cholesterol esterase ', while the synthetic activity be called ' cholesterol ester synthetase '[11].

This point has recently been thoroughly discussed in an excellent review by Goodman[22].

It should be pointed out that this is the first time that a clear separation of the hydrolytic and synthetic activities of an esterase system has been demonstrated, thereby proving that the two reactions are actually carried out by two separate enzymes and are not due to the reversible nature of one reaction.

It may also be mentioned in passing that the International Commission on Enzyme Nomenclature has named vitamin A esterase as 'vitamin A acetate hydrolase' and has given the number 3.1.1.1.2. Similarly, 'cholesterol acetate hydrolase' has been allocated the number 3.1.1.1.3.

Absorption from Intestine

The phenomenon of absorption from the intestine is a complex process. In spite of its receiving continued attention of many workers for well over a century now, it still remains little understood. However, with the development of newer biophysical techniques like electronmicroscopy and with the application of radioactive isotopes, coupled with the rapid advances made in our knowledge of biochemistry at the cellular level, over the past decade or so, there has been a remarkable improvement in our understanding of this subject.

While in the past the entire process of absorption used to be considered as one entity, it is now possible to subdivide the whole process into several phases, like the luminar phase, the cellular phase and the post-cellular phase. During the luminar phase of absorption, which is called digestion in common terminology, the fragments and macromoles of food materials are broken down to smaller units, after which these units are picked up by the intestinal mucosa and transferred inside the cell. In some cases, like lipids in general, these units are reassembled inside the mucosal cell, which may be called the cellular phase of absorption. At the post-cellular phase the lipid materials are perhaps further assembled in the form of chylomicra for transport through the lymphatic system.

Triglycerides

Probably the continued interest in the field of absorption of triglycerides, sustained for over a century, can be cited as a typical example of what has been said in the preceding paragraphs. This particular area has witnessed rather extensive work spread over about a century, and yet the actual mechanism of absorption of triglycerides began to be understood only recently. From the work carried out in several laboratories in recent years it now appears that triglycerides (TG) are hydrolysed in the lumen of the small intestine by the pancreatic lipase to the 2-monoglyceride (2-MG), which then forms a micelle with the bile salts and free fatty acids. The micellar 2-MG eventually enters the mucosal cell, where it is re-esterified to give back TG. Although it has been shown that the mucosal cell contains an enzyme that can directly acylate the 2-MG with 2 moles of fatty acyl CoA to form TG, it is not yet clear as to how the fatty acids gain entry into the mucosal cells. Some time back it was thought that the mucosal phospholipids may participate in the transport of free fatty acids into the cell. But, from ^{32}P turnover studies it was concluded that the phospholipids may not be so important in this process. By feeding labelled free fatty acids to rats, we were able to demonstrate very rapid uptake of the fed labelled free fatty acid by the phospholipids of the mucosa of rat intestine. Thus, contrary to earlier views, our investigations have indicated that the mucosal phospholipids may participate in the transport of at least a part of the free fatty acids during absorption of triglycerides[23].

Carotenoids

Considerable species difference has been noted regarding the absorption and storage of different types of carotenoids. Thus, cows absorb mostly β-carotene, while birds prefer mostly the hydroxy carotenoids. Human beings, on the other hand, do not show any special preference for any particular type of carotenoid, while most mammals, like rats, goats, sheep, etc., reject all types of carotenoids. Attempts were made by Ganguly *et al.*[24] to find an explanation for this peculiar species difference, and for this purpose rats and chickens were dosed with β-carotene, lycopene and lutein, dissolved in groundnut oil, separately or together, after which the intracellular distribution, in the mucosal cell, of the different carotenoids, was studied 2-3 hr after the dose. While the mucosal cells of the rats contained practically none of the carotenoids fed, lutein was present in large concentrations in the mucosa of the chickens. In these birds the bulk of the lutein of the mucosal cell was associated with the particulate materials, especially the microsomal fraction of the cell homogenate. Lycopene, however, was not taken up by the chicken intestine. These experiments, therefore, showed that the structure of the carotenoid determines the ability of the animal to absorb it. Thus, though both lutein and lycopene are non-provitamin A carotenoids, the presence of the hydroxyl groups in lutein made it possible for the chicken to absorb it. It is equally important to note that even though lutein was given in solution in oil, only very small amounts of it were present in the supernatant fraction of the homogenate of chicken mucosa, which implied that the carotenoids are not mechanically absorbed in solution in oil. We have, therefore, suggested that during absorption the carotenoids are thrown out of fat solution and are then absorbed at the molecular level, probably attached to lipoproteins which are species specific[24].

Cholesterol

Similarly, although extensive work has been done on the absorption of various sterols, all this work has only revealed yet another aspect of the complexities of intestinal absorption. Thus, it has been known that the plant sterols are poorly absorbed by animals, while the animal sterols are more efficiently absorbed.

Regarding absorption of cholesterol itself, it used to be generally believed that the sterol esters fed are totally hydrolysed before they can be absorbed

by the mucosal cell; ultimately the absorbed sterol appears in the lymph mostly in the esterified form. These processes are naturally carried out by appropriate enzymes. Our initial experiments on the absorption of cholesterol showed that, irrespective of whether rats or chicks were fed free cholesterol, its acetate or oleate ester, or are not given any cholesterol, the net cholesterol contents of the mucosal cells can hardly be changed[24]. One of our most striking observations was that there is virtually no cholesterol ester in the mucosal cell of rats following a large dose of the sterol or its esters, and that the bulk of the sterol of the cell is associated with the microsomal fraction of the cell homogenate. It, therefore, became clear that the sterol is not absorbed in fat solution, but is thrown out of such solution during absorption, otherwise, it should have been present in the supernatant fraction of the mucosal cell homogenate. It was also evident that the sterol ester is de-esterified during absorption. We, therefore, suggested that cholesterol is absorbed by a rather unique process of displacement of the unesterified sterol of the lipoproteins of the membraneous structures of the mucosal cell, like the cell membrane and the membranes of the endoplasmic reticulum.

Proof for this hypothesis came from our later investigations with ^{14}C-labelled cholesterol[25,26], the results of which showed that following feeding of labelled cholesterol to rats, it is rapidly taken up by the mucosal cells and that it is bound to the microsomal particles to the extent of about 80 per cent of the cellular labelled sterol. In these experiments also the mucosal cells contained very little labelled sterol ester, while the specific activity of the cholesterol of the microsomal fraction was highest of that of all cell fractions.

As stated earlier, our recent work has established that the cholesterol ester hydrolase of the mucosal cell is located on the brush border facing the lumen of the small intestine, while the esterifying enzyme is oriented towards the inner side of the cell[12]. In the same report we have shown that when labelled cholesterol, free or esterified, was fed to rats, no traces of the labelled sterol ester could be found in the isolated brush border of the rat, while the brush border rapidly picked up, in both cases, the fed cholesterol in the unesterified form. There were, however, small amounts of the labelled sterol ester in the mucosal cell after a certain time. Therefore, we have proposed[12] that the process of absorption of cholesterol may be as follows. The dietary cholesterol esters are hydrolysed by the cholesterol ester hydrolase of pancreas or of the mucosal brush border or of by both, after which the brush border rapidly absorbs the de-esterified sterol and transfers it into the mucosal cell, by a mechanism of displacement, and it is then slowly re-esterified for transport through the lymph. Independent work of Shiratori and Goodman[27], which was published almost simultaneously with our report, has fully confirmed that complete hydrolysis of cholesterol esters occurs prior to absorption of cholesterol when the sterol is fed in the esterified form.

Retinol and Its Esters

It was widely believed, although without any compelling evidence, that during absorption the retinyl esters are hydrolysed in the lumen of the intestine, following which the alcohol is re-esterified in the intestinal wall. The only information available in this regard seemed to be the observations of Gray *et al.*[28] and of Eden and Sellers[29]. Thompson *et al.*[30] have demonstrated extensive hydrolysis in the intestine of the fed retinyl esters and have shown that retinol is absorbed exclusively through the lymphatic system, and in the esterified form. But these earlier observations did not fully establish that the esters have to be totally hydrolysed before they can enter the mucosal cell and that they are subsequently re-esterified before they are transported from these cells. Nor did they throw any light on the nature of the esters that are transported from the small intestine during absorption.

In our work on the absorption of vitamin A[24], cholesterol and carotenoids, we had shown that when retinol, its acetate or palmitate esters are fed to rats or chickens, extensive hydrolysis of the fed esters occurs in the lumen of the intestine. However, irrespective of whether the alcohol or its esters were fed, vitamin A of the intestinal mucosa was predominantly in the esterified form and the bulk of this ester was localized in the supernatant fraction of the mucosal cell homogenate[24].

In continuation of this work a reverse-phase paper chromatographic analysis of the retinyl esters of the mucosal cell, intestinal muscles, lymph, blood and liver of rats showed that, following feeding of retinyl acetate, only its higher esters can be found in the intestinal muscles, lymph, blood and liver[31]. Small amounts of the acetate, along with larger concentrations of the higher esters were, however, detected in the mucosal cells; it is possible that the acetate is held mechanically on the cell surface. These experiments, therefore, showed that during absorption retinyl acetate is hydrolysed and that the alcohol formed is re-esterified. However, the exact nature of the higher esters of rat tissues remained unknown till an improved reverse-phase chromatographic method for the rapid separation of the various higher esters of retinol was evolved[32].

When rats were fed retinol dissolved in carriers of widely different fatty acid composition and 2·5 to 3 hr later, the retinyl esters of their tissues were analysed employing the improved technique of chromatographic separation of the higher esters, in all cases the intestinal mucosa contained largely retinyl palmitate with appreciable amounts of other esters that reflected the fatty acid composition of the vehicle used. In such cases, as the aqueous colloidal dispersion, where no fatty acid was present in the carrier, the mucosal cells always contained considerable amounts of retinyl esters, the bulk of which was invariably palmitate. In contrast, the intestinal muscles and blood always contained the palmitate to the extent of about 90 per cent of the total esters present, while the liver, under all circumstances, appeared to contain only the palmitate[32].

As already discussed, the mucosal enzyme esterifies retinol non-specifically with fatty acids containing 10 or more carbon atoms; this readily explained the presence in the mucosa of a mixture of retinyl esters, when the carrier contained a variety of fatty acids. However, in the absence of any exogenous fatty acids an appreciable amount of the palmitate was always present. It is possible that this palmitic acid is derived from the glycerides or phospholipids of the cell by a mechanism of trans-esterification.

Though these results showed that during absorption the acetate is totally hydrolysed and that the retinol is re-esterified, they did not establish whether all other esters, especially the palmitate, are similarly de-esterified at the time of absorption. Further experiments[33] were, therefore, designed in order to obtain information in this regard. Acetate, stearate, laurate, palmitate and linoleate esters of retinol, separately dissolved in groundnut oil, were fed to rats, after which the retinyl esters of the tissues of the rats were analysed using similar procedures, as adopted in the previous experiments with retinol. Irrespective of the ester fed, palmitate was the most abundant of all the esters in the mucosa, while the picture in the rest of the tissues was almost the same, as was previously obtained with retinol. Just as it was observed in the earlier experiments with retinyl acetate, with the higher esters also it was not possible to detect more than traces of the fed esters in the tissues other than the mucosa. Therefore, it became clear that even the higher esters are totally hydrolysed at the time of absorption.

However, these experiments could not prove whether the palmitate also is hydrolysed or not, and *in vitro* experiments conducted with the everted intestinal sacs of rats furnished further evidence that all esters, including the palmitate, are hydrolysed before absorption[34].

It is clear from the data presented in Table 2 that, regardless of whether the incubation medium contained retinol, its acetate, stearate, laurate, linoleate or palmitate ester, the supernatant fraction of the mucosal cell homogenate invariably contained significant amounts of the palmitate. These data also show that, irrespective of the type of the higher esters of retinol introduced into the medium, there is virtually no difference in the amounts of retinol found in the incubation medium, as well as in the amounts of retinyl palmitate recovered from the supernatant and particulate fractions of the homogenate; this is true even when the palmitate is in the medium. Therefore, it appeared logical to assume that the palmitate follows the same fate as the other higher esters.

Although these experiments clearly indicated that all esters of retinol are hydrolysed during absorption, they failed to establish the exact mechanism by which the vitamin enters the cell. A study of the intracellular distribution of retinol and its esters, at the time of active absorption, *in vivo* and *in vitro*, threw considerable light on the possible mechanism. Thus, the results of the experiments, where rats were dosed with different esters or with the free alcohol and 2-3 hr later their mucosal cells were fractionated into the particulate and supernatant fractions, showed that the particulate fraction always contained more of the alcohol, while the supernatant fraction contained more of the esters. Resolution of the ester fraction revealed that the palmitate was the most abundant of all the esters in both fractions of the cell (Table 3).

These results thus substantiated our conclusions from the *in vitro* experiments with everted intestinal sacs (Table 2). Further, in separate experiments with the everted intestinal sacs, where compounds like TEPP and DFP that strongly inhibit the hydrolytic enzyme were introduced into the incubation medium along with the esters, negligible amounts of retinol or its palmitate ester could be found in either the particulate or supernatant fractions of the cell homogenate (Table 4). These findings also, therefore, showed that no absorption of vitamin A can take place without prior hydrolysis of the retinyl esters. In contrast, introduction into the incubation medium of inhibitors for the esterifying enzyme (sodium taurocholate or Tween 20) made no difference in the absorption in similar *in vitro* systems[34].

We have assumed that the supernatant fraction of the cell homogenate represents the cell sap and the microsomal particles the endoplasmic reticulum and the cell membrane. On the basis of these

TABLE 2 — UPTAKE OF RETINOL AND ITS ESTERS BY EVERTED INTESTINAL SACS OF RATS

(Values expressed as μg. retinol in the free or esterified state per whole of the medium and for the particulate and supernatant fractions obtained from the whole of one intestinal sac)

Vitamin A introduced	Medium vitamin A (μg.)			Vitamin A in mucosa (μg.)				
	ME	P	AL	Particulate			Supernatant	
				ME	P	AL	ME	P
Alcohol	—	10	2205	—	38	200	—	85
Acetate	1125	16	630	27	26	225	1·5	55
Laurate	2997	9	293	10	4	57	6·0	69
Stearate	3015	7	245	6	6	66	7·5	46
Linoleate	3125	4	277	5	6	45	7·0	52
Palmitate	—	2925	255	—	8	62	—	50

ME, ester of incubation medium; P, retinyl palmitate; and AL, retinol.
Adapted from Mahadevan, S., Seshadri Sastry, P. & Ganguly, J., *Biochem. J.*, **88** (1963), 534.

TABLE 3 — DISTRIBUTION OF RETINOL AND ITS ESTERS BETWEEN THE PARTICULATE AND SUPERNATANT FRACTIONS OF THE HOMOGENATES OF THE INTESTINAL MUCOSA OF RATS 2-3 HR AFTER FEEDING OF RETINOL OR ITS ESTERS IN GROUNDNUT OIL

(Values are for the whole intestine from one animal)

Form of vitamin A fed	Particulate fraction						Supernatant fraction					
	Ester (μg.)					Alcohol (μg.)	Ester (μg.)					Alcohol (μg.)
	A	S	P	M	L		A	S	P	M	L	
Alcohol	0	6	25	15	3	284	0	4	90	5	3	96
Acetate	24	2	22	5	1	252	14	1	73	2	0	87
Laurate	0	1	26	4	10	145	0	0	71	1	3	39
Stearate	0	9	17	2	3	135	0	4	62	2	1	39
Palmitate	0	3	19	1	2	142	0	3	74	2	1	41
Linoleate	0	2	28	8	2	129	0	2	68	3	0	59

A, acetate; S, stearate; P, palmitate; M, myristate; and L, laurate esters of retinol.
Adapted from Mahadevan, S., Seshadri Sastry, P. & Ganguly, J., *Biochem. J.*, **88** (1963), 531.

TABLE 4 — THE EFFECT OF TETRAETHYL PYROPHOSPHATE (TEPP) AND DI-ISOPROPYL FLUOROPHOSPHATE (DFP) ON THE UPTAKE OF RETINOL, RETINYL ACETATE OR RETINYL STEARATE BY EVERTED INTESTINAL SACS OF RATS

Form of vitamin A in medium	Incubation medium vitamin A (μg.)		Intestinal-mucosa vitamin A (μg.)					
			Particulate fraction			Supernatant fraction		
	ME	AL	ME	P	AL	ME	P	AL
Retinol								
Without TEPP	32	3300	0	40	214	0	78	37
With TEPP	20	3300	0	38	222	0	66	39
Retinyl acetate								
Without TEPP	2300	785	28	41	256	13	94	41
With TEPP	2100	30	150	2	18	53	10	13
Retinyl stearate								
Without TEPP	2800	253	0	9	63	7	48	4
With TEPP	3000	240	10	8	58	6	53	7
Without DFP	2900	260	3	10	65	7	57	7
With DFP	3100	25	93	2	6	13	6	2

ME, medium ester; P, retinyl palmitate; and AL, retinol.
Adapted from Mahadevan, S., Seshadri Sastry, P. & Ganguly, J., *Biochem. J.*, **88** (1963), 534.

assumptions we have interpreted the above results as follows. The fed retinyl esters are hydrolysed in the lumen of the small intestine either by the pancreatic retinyl ester hydrolase or by the hydrolase situated on the outer surface of the mucosal cell. Only the alcohol form then crosses the cell membrane to be re-esterified non-specifically by the esterifying enzyme situated inside the cell and with the fatty acids available there. Once the ester is formed, it is automatically released from the particulate materials of the cell into the cytoplasm, after which some specific lipoprotein, which can more easily recognize the palmitate ester of retinol, preferentially picks it up and transports it through the lymphatic system to the liver. The other esters, left in the mucosa, are probably de-esterified and eventually re-esterified with palmitic acid, whereupon they are readily removed.

All this work was based upon the resolution of retinyl esters of rat tissues by the technique of reverse-phase chromatography on silicone-impregnated filter papers. It now appears that this method is not sensitive enough to reveal the presence of minute amounts of retinyl esters. Using far superior techniques Huang and Goodman[35] have recently demonstrated that the retinyl esters found in the lymph of rats absorbing vitamin A are mainly composed of retinyl palmitate and stearate esters, with the palmitate being the most predominant, while smaller proportions of oleate and linoleate are also found.

Retinal

Retinal occupies a pivotal position in the metabolic map of vitamin A, not only because it is one of the intermediates in the conversion of β-carotene to vitamin A, but it is also an intermediate in the formation of retinoic acid from retinol. Equally important is its function in the visual system. Probably the only significant report available in the literature regarding absorption of retinal is that of Glover *et al.*[36], who claimed that, following oral administration of retinal, it could not be detected in the intestinal wall, blood and liver of rats, while the alcohol and its esters were found in these tissues. It was, therefore, suggested by these workers that the aldehyde is not absorbed unchanged, and that during absorption it is reduced to the alcohol form in the intestine.

In more detailed investigations on the absorption of retinal in normal rats, we have demonstrated[37]

TABLE 5 — INTESTINAL ABSORPTION OF RETINOIC ACID IN RATS

(Rats with very low reserves of vitamin A were starved for 24 hr and then given a single dose of 5 mg. of sodium retinoate mixed with 0·5 g. of the vitamin A free diet, and sacrificed at the given time intervals. Values are expressed as μg. of retinoic acid for the whole of stomach, intestinal contents, mucosa and muscles of small intestine and liver, and per 5 ml. of blood)

	1 hr	2 hr	3 hr	4 hr	6 hr	24 hr	48 hr
Stomach	1240	1100	1024	1074	488	186	5·5
Intestine							
Contents	333	118	98·7	58·8	22·5	5·0	4·5*
Mucosa	81·4	42·0	67·0	59·5	9·6	3·75	1·1*
Muscles	22·7	28·7	41·7	37·2	11·0	1·5*	1·5*
Blood	18·6	16·3	14·7	13·4	6·07	2·53*	2·81*
Liver	50·0	44·0	47·2	54·7	12·0	3·4*	2·70*

*Apparent values of retinoic acid as calculated from Carr-Price colour reaction.
Adapted from Deshmukh, D. S., Malathi, P., Subba Rao, K. & Ganguly, J., *Indian J. Biochem.*, **1** (1964), 164.

that, when rats are dosed with 5 mg. of retinal in oil, although significant amounts of the aldehyde are reduced to the alcohol in the intestine, considerable amounts of the unchanged aldehyde are found in the mucosa and muscles of the small intestine, and also in blood and liver of such rats. The identity of retinal isolated from the tissues of rats was established by several criteria, like reverse-phase and thin-layer chromatography, characteristic absorption spectra, etc. Thus, contrary to the well-accepted views, we have conclusively proved that significant amounts of retinal are absorbed by the rat in the unchanged state.

Retinoic Acid

Retinoic acid was first synthesized in 1946 by the Dutch workers, Arens and van Dorp[38], who found it to be as active as retinyl acetate in its ability to support growth of rats. But these workers detected no retinoic acid, retinol or retinyl esters in the liver of the rats that were given massive doses of the acid, which had suggested that retinoic acid is neither absorbed as such nor is reduced in the animal body. After this, work on retinoic acid was more or less relegated to the background, till Moore made the interesting suggestion in 1953 that if retinoic acid is not reduced in the animal body, it cannot meet the requirements of vision, where the aldehyde form of the vitamin functions as a prosthetic group of the visual pigments. Following up this remarkable suggestion of Moore, Dowling and Wald[39] soon showed that rats grow normally when supplemented with retinoic acid in place of retinol, and that such rats became blind. This spectacular demonstration of Wald immediately became the starting point for renewed interest in the metabolism of retinoic acid. Thus, as will be seen shortly, we showed retinoic acid to be far superior to retinyl acetate in stimulating growth of vitamin A deficient rats. Even more exciting has been the recent work on the failure of retinoic acid to meet the reproductive requirements of both male and female rats (these aspects will be discussed later).

The view that retinoic acid is neither well absorbed by animals, nor is stored in their tissues was further strengthened by several later observations: (i) that the acid-supplemented rats become vitamin A deficient soon after the withdrawal of the acid supplements; (ii) that no retinoic acid was ever detected in the tissues of normal animals; and (iii) that more than 60 per cent of the acid seemed to be excreted unabsorbed after an oral dose. In contrast to these observations, during our work on the biopotency of retinoic acid, we noticed that a single dose of 500 μg. of the acid was able to sustain the growth of vitamin A deficient rats for nearly 28 days. It, therefore, became obvious that not only is the acid absorbed by rats, but also its effects persist for a considerable length of time. These considerations, therefore, led us to the logical conclusion that the question of absorption of retinoic acid should be re-investigated, and the results presented in Table 5 show that appreciable amounts of it can be found in rat tissues soon after the administration of an oral dose[40]. It will also be seen from Table 5 that after absorption it very rapidly disappears from the tissues of the rat. That the compound isolated from the rat tissues is retinoic acid has been established from its characteristic spectral properties, UV absorption and Carr-Price reaction, and from its behaviour during adsorption and thin-layer chromatography on alumina.

Storage of Vitamin A in Liver

Type of Ester

It has already been discussed that regardless of the type of the retinyl ester fed to rats, the vitamin appears in the blood mostly as the palmitate and is ultimately stored in the liver mostly as the palmitate. While Mahadevan and Ganguly[32] were able to adduce sufficient evidence to prove that the retinyl ester of rat liver is palmitate, Subba Rao *et al.*[41] made more extensive investigations on the fatty acid component of the retinyl ester of the liver of yet another species, the sheep, which is a ruminant. Sheep liver was extracted with petroleum ether and from the total lipids the bulk of the phospholipids was removed by cold acetone precipitation. The concentrate, thus obtained, was treated with pancreatic lipase in the presence of mercuric chloride, which preferentially inhibits the retinyl ester hydrolase activity, and by this means most of the glycerides were removed. The retinyl ester fraction was then purified by repeated

chromatography on MgO: celite columns, and finally by reverse-phase paper chromatography.

Right from the beginning of purification reverse-phase paper chromatography showed only one ester band, which became more and more prominent with the progress of purification. But this band could correspond to either retinyl palmitate or oleate. At the final stage of purification, the ester band was eluted and saponified. The liberated fatty acid was then subjected to paper chromatographic analysis before and after exhaustive hydrogenation, which procedure should convert oleic acid to stearic acid. In both cases only one spot corresponding to that of palmitic acid was obtained, thereby ruling out the possible presence of oleic acid.

As already indicated, we had suggested that a certain degree of specificity of a lipoprotein that can preferentially bind with the palmitic acid ester of retinol might explain the preferential transport and storage of this ester. Attempts were made by Mahadevan *et al.*[42] to prove this hypothesis by injecting intracardially aqueous Tween dispersions of retinal, retinol, the acetate, laurate, myristate, stearate, palmitate or linoleate ester of retinol into rats having very low liver reserves of vitamin A. Blood, liver, lungs, spleen, kidneys and intestine of these rats were analysed 5 min. to 24 hr after the injection. Whenever the esters were injected, some hydrolysis took place in most of the tissues. Immediately after the injection, the injected ester was present in high concentrations in blood, but it fell very rapidly within a period of one hour. Irrespective of the ester injected, at all time intervals, the palmitate was found in considerable quantities in the liver, where its concentration showed progressive increase with time. However, after injecting other esters, small amounts of the injected ester could be detected in this tissue up to 6 hr after the injection, while after 6 hr till 24 hr, no other ester, but the palmitate, was detectable.

As in the case of our work on the absorption of vitamin A, the retinyl esters were separated by reverse-phase paper chromatography, which is apparently not sensitive enough to detect the presence of small amounts of retinyl esters. Using finer techniques, Goodman *et al.*[43] have since then shown that rat liver stores predominantly retinyl palmitate with traces of other higher esters, especially stearate. Similarly, Futterman and Andrews[44] have shown that in rat liver the major esters of retinol are 69 per cent palmitate, 15 per cent stearate and 6 per cent oleate.

Locale in the Liver

Fluorescence microscopic studies of Popper[45] had indicated that vitamin A is probably stored in the Kupffer cells of the liver, but this work did not establish how the alcohol and ester are stored. Further evidence in this regard was obtained from the experiments of Krishnamurthy and Ganguly[46], where rats, whose reticuloendothelial system was blocked by repeated injections of Indian ink, were given a large oral dose of retinol. Analysis of their livers, 24 hr after the dose, showed that the amount of retinyl ester deposited in the livers of the Indian ink-treated rats was considerably reduced, as compared to that found in the control rats. In contrast, the amount of retinol in the livers of the animals of either group remained unchanged (Table 6).

TABLE 6 — EFFECT OF INTRAPERITONEAL INJECTIONS OF INDIAN INK ON THE RETINYL ESTER AND RETINOL VALUES IN BLOOD AND LIVER OF RATS

Treatment	Blood μg./ml. plasma		Liver, μg./organ	
	Ester	Alcohol	Ester	Alcohol
Negative control (6)*	tr	tr	10·2	1·4
Saline + 3 mg. vitamin A (6)	1·9	1·2	2238	56·0
Indian ink + 3 mg. vitamin A (6)	5·1	1·9	1168	54·3

*Figures in parentheses refer to number of rats.

Adapted from Krishnamurthy, S. & Ganguly, J., *Nature, Lond.*, **177** (1956), 575.

Mode of Storage

The above experiments thus showed that retinol and its esters are probably stored in the parenchymal and Kupffer cells of the liver respectively. In later experiments, where rat and chicken liver cells were fractionated by differential centrifugation into the various cell components, it was noticed that while retinol is diffusely distributed among the various cell fractions, its esters are located predominantly in the supernatant fraction. As an explanation for this differential distribution we suggested that the ester and the alcohol are associated with different types of lipoproteins of the liver[7]. Considerable evidence was collected from our later experiments in support of such hypothesis[47]. Thus, while diethyl ether alone failed to effect the extraction of more than traces of vitamin A from the homogenates of rat liver, prior treatment of the homogenates with various protein denaturants, followed by shaking with ether, led to a total extraction of the liver vitamin A. However, in most of these cases a differential extractability of the two forms of the vitamin was noticed. Equilibrium dialysis of rat liver supernatant against buffers of various *p*H values resulted in the separation of two protein fractions that carried the ester and the alcohol separately. Similarly, ammonium sulphate fractionation of rat liver supernatant effected a separation of two protein fractions, which were associated with the two forms of the vitamin[47]. It was, therefore, deduced that retinol and its esters are stored in rat liver in association with separate lipoproteins of the parenchymal cells and Kupffer cells respectively[48].

Such mechanisms of storage in association with proteins is not confined to retinol alone, because in chicken liver, which stores hydroxy carotenoids also, these pigments were shown to be similarly associated with lipoproteins, mostly of the mitochondrial and microsomal particles of the cell homogenate. These particulate materials of the

cell, when treated with 0·1*M* NaOH, yielded a soluble carotenoid-protein complex, which could be precipitated by lowering the *p*H of the extract with dilute acid or by adding ammonium sulphate; the precipitate could then easily be redissolved[49].

Relationship between Blood and Liver Retinol

In 1947 Glover *et al.*[50] claimed to have demonstrated a direct relationship between the retinol concentrations of blood and liver of rats. Ganguly and Krinsky[51], however, were unable to confirm this claim of Glover *et al.*[50], and instead showed that in rats the plasma retinol maintains a rather steady level irrespective of the liver retinol contents. As an explanation a tentative hypothesis was put forward suggesting that the blood retinol level is maintained by the degree of association between retinol and a blood protein that carries it. These findings of Ganguly and Krinsky[51] were fully confirmed by High and Wilson[52], who also supported the concept of carrier protein for vitamin A. Later on Krinsky *et al.*[53] further extended this work and the entire position was exhaustively reviewed by Ganguly[18]. Since then it has been widely accepted that the blood vitamin A is maintained almost at a steady level by the carrier protein and this concept has assumed great significance in view of the frequently observed low levels of blood vitamin A leading to many symptoms of vitamin A deficiency in the kwashiorkor children, in spite of their occasionally having adequate liver reserves of the vitamin. This aspect has been thoroughly reviewed by Mahadevan *et al.*[54] and is further discussed later.

However, more recently John *et al.*[55] seem to believe that the correlation between the blood and liver retinol concentrations is not yet clear. In their attempts to obtain further information in this regard they gave 32 vitamin A depleted male rats supplements of 5 μg. of 3-dehydroretinal (vitamin A_2 aldehyde)/day for 2-3 weeks till they reached a body weight of 130-170 g., after which the rats were divided into eight groups of four each, and each rat was given a specified dose of 3-dehydroretinal for three consecutive days. From these trials the authors claimed that "a direct proportionality between the 3-dehydrovitamin A concentration in blood and the unesterified 3-dehydrovitamin A concentration in liver has now been established", and have finally concluded "it is likely that, although the blood vitamin A concentration may not vary between wide limits at various concentrations of their unesterified vitamin A, a close proportionality between the blood and liver unesterified vitamin A does exist". In their attempts to explain the disagreements between Glover *et al.*[50] and Ganguly and Krinsky[51], the same authors have stated that, while the former workers had used 6- to 9-month old rats, the latter group used mainly 3- to 5-month old rats. But it is not so. In fact, the latter workers, in addition to using 3- to 5-month old rats, had also used two batches of 24 and 26 normal adult male rats. What is more, individual values obtained from each of these rats were plotted, which gave a total of 50 points, while in the case of the younger animals 3-4 of them were pooled together for each point.

As against such large number of rats employed by Ganguly and Krinsky[51], John *et al.*[55] have used a total of 32 rats which were divided into eight groups of four each. Out of these, only seven points have been given, and, although it has not been clearly stated whether the rats were pooled within groups or not, presumably these are pooled data. It should be noted that the values have been expressed as μg. 3-dehydroretinol/g. liver and μg. 3-dehydrovitamin A (and not 3-dehydroretinol)/100 ml. blood. The latter values ranged between 2·67 and 7·52 μg. 3-dehydrovitamin A/100 ml. blood, while Ganguly and Krinsky's[51] values were 13·6 and 38·1 μg. retinol/100 ml. plasma and not 17·4 and 27·0 μg./100 ml., as stated by John *et al.*[55]. In view of the well-known wide variations in the plasma retinol levels in animals, it would appear that a large number of cases with proper statistical treatments would be essential, and, therefore, such limited number of points, as given by John *et al.*[55], cannot be considered convincing. It is important to note that very small amounts of the vitamin were estimated in the presence of overwhelming quantities of tissue lipids, in which case it is doubtful whether such small differences, as shown by these workers, are really meaningful so as to justify the claim that a direct correlation has now been established. It is even more important to remember that retinol, and not the dehydro compound, is the natural vitamin for rats, so that the relationship between blood and liver retinol contents of this species certainly cannot be established by using 3-dehydroretinol. Therefore, it should be quite clear that the claims of John *et al.*[55] that they have demonstrated "a close proportionality between the blood and liver unesterified vitamin A does exist" are totally unacceptable.

BIOLOGICAL FORMATION OF VITAMIN A ACTIVE COMPOUNDS

In vitro Conversion of β-Carotene to Vitamin A

Ever since Moore established, more than 30 years ago, that β-carotene gives rise to vitamin A inside the animal body, it used to be generally believed that the carotene is converted to vitamin A in the liver. Contrary to such well-accepted views, about two decades ago it was conclusively demonstrated from three different laboratories that the dietary carotene is efficiently converted to vitamin A in the small intestine of rats. Since then repeated attempts were made in several laboratories to demonstrate the *in vitro* conversion, with isolated enzyme systems prepared from animal tissues, and until recently, all these attempts have proved unsuccessful[56].

Very recently, making use of modern sensitive techniques, like thin-layer chromatography and use of ^{14}C-labelled β-carotene, Olson and Hayayishi[57] and Goodman and Huang[58] have independently claimed to have effected the *in vitro* conversion of β-carotene to vitamin A by isolated enzyme systems prepared from the intestine and liver of rats. The reaction seems to be aerobic and NADP dependent. It has been claimed that the carotene molecule is centrally cleaved by a di-oxygenation reaction

giving rise to retinal, which is ultimately reduced to retinol.

In collaboration with Dr George Hubscher of Birmingham University (UK), who spent a few months in our laboratories, we attempted to effect the *in vitro* conversion of chemically synthesized 15-15'-$^{14}C_2$-β-carotene with the homogenates of guinea-pig intestinal mucosa. Our results are in broad agreement with those of the other two groups, in that, following incubation and thin-layer chromatography, we did find sufficient radioactivity in the three fractions corresponding to those of retinal, retinol and retinoic acid. Our experience also has been that it is aerobic and NADP dependent. Addition of Fe^{2+} greatly enhanced the reaction. However, our repeated attempts to identify the reaction products have uniformly failed. None of the other two groups has produced convincing evidence establishing the identity of the fractions isolated. We, therefore, feel that till such convincing proof of identity of the products is produced it cannot be said that *in vitro* conversion of any significant extent has been conclusively demonstrated.

Retinal and Retinoic Acid

It has already been indicated that retinal is a pivotal intermediate in the metabolic map of vitamin A. When carotene is the source of vitamin A (and normally major source of the vitamin for animals is carotene), it is usually broken down to retinal in the intestine, after which the aldehyde is reduced to the alcohol, which in turn is ultimately stored in the liver in the esterified state. Moreover, as will be seen shortly, it gives rise to retinoic acid by an irreversible oxidative process. Therefore, even the stored retinyl ester has to be converted back to the aldehyde, for it to form the acid. Finally, it directly functions in the visual system.

As discussed before, when the Dutch workers[38] could not detect retinol or its esters in rat liver after massive doses of synthetic retinoic acid, it became quite apparent that the acid is neither absorbed nor reduced in the animal body to the aldehyde and the alcohol. When Wald[39] subsequently showed that retinoic acid can meet the requirements of growth, but not of vision of rats, it was not only conclusively proved that the acid cannot be converted back to the aldehyde inside the animal body, but at the same time it was also clearly established that the functions of vitamin A in meeting the requirements for vision and growth of rats are completely independent and separable processes.

However, although retinal has been found to be present in some animal tissues, e.g. the retina, no retinoic acid has ever been detected in nature. Also, the dietary retinal cannot be its source in the animal body, because, wherever retinal is present, it is present only in small amounts which would obviously be reduced to the alcohol form during the process of absorption.

Therefore, it becomes quite clear that suitable enzymes must be present in the animal body to hydrolyse the stored ester to the alcohol, which in turn should be successively oxidized to the aldehyde and the acid. Repeated attempts at effecting any significant hydrolysis of the stored ester by the liver enzyme consistently failed in the hands of several workers, and had led to the general belief that the liver is unable to hydrolyse its own ester[59]. But, recently Mahadevan *et al.*[60] have shown that the nuclear fraction of rat liver homogenate can slowly hydrolyse retinyl palmitate to retinol. Apparently, this enzyme shows considerable preference towards retinyl palmitate, and, as will be recalled, we have shown that rat liver preferentially stores retinyl palmitate.

Although earlier workers had demonstrated that the liver alcohol dehydrogenase can oxidize retinol to retinal and that it is an NAD-dependent reaction, we have shown that rat liver supernatant contains enzymes that can convert retinol to the aldehyde and the aldehyde to retinoic acid[61]. Ammonium sulphate fractionation of rat liver supernatant resulted in a separation of two enzyme fractions, one precipitating at 45 per cent saturation and the other between 45 and 70 per cent saturation. The more soluble fraction readily gave retinal from retinol in the presence of NAD. It is a sulphydryl enzyme and it seems to be identical with the liver alcohol dehydrogenase. The other fraction, which oxidizes retinal to retinoic acid, does not require any added cofactors. Like other aldehyde oxidases, this enzyme is a molybdo-flavoprotein.

Thus it was proved that the animal liver possesses all the enzymes that are necessary for the constant formation of retinoic acid from the stored retinyl ester. It has been well known that in animal liver the stored ester is always accompanied by considerable amounts of retinol. Therefore, the only intermediate now required to complete the whole sequence of reactions was the aldehyde. Indeed, we have clearly demonstrated the presence of retinal in normal rat liver in definite, but small, amounts[61]. It may be mentioned here that till recently it used to be widely believed that retinal occurs only in the retina. Not only have we shown its presence in rat liver, but several other workers also have shown it to occur in other biological materials like fish roes, hen's eggs, etc.

However, liver is not the only tissue in rats that can oxidize retinal to retinoic acid. We had earlier shown with tissue homogenates that rat intestine also can oxidize retinal. In later experiments, where everted intestinal sacs of rats were incubated with retinal in the medium, considerable amounts of retinoic acid together with some amounts of retinol and retinyl esters were formed both in the sac and in the medium (Deshmukh, D. S. & Ganguly, J., unpublished results). However, while both oxidation and reduction of retinal could be demonstrated with the everted sacs, after feeding retinal we were never able to detect retinoic acid in rat intestine, although considerable amounts of the unchanged aldehyde together with retinol and retinyl esters were isolated from the same tissue. In contrast to these observations with the intact mucosal cells, we were not able to effect any reduction of the aldehyde with the whole mucosal homogenates and the reaction apparently proceeded towards oxidation only.

This whole work, therefore, brings out a very pertinent point regarding the relationship between the demonstration of the presence of an enzyme in any tissue and its possible biochemical function there. Thus a powerful aldehyde oxidase has been shown to be present in rat liver and we have shown here that when abundant supplies of the substrate (retinal) required by the enzyme are made available, it can form considerable amounts of retinoic acid. And yet, in the living animal this enzyme remains apparently dormant, because no retinoic acid has ever been demonstrated in the liver of normal rats, in spite of the fact that the same tissue stores abundant amounts of retinyl esters and retinol and some retinal. In this case it is quite obviously due to the insufficient supply of its substrate, retinal, because we have shown this compound to be present only in traces in normal rat liver.

In contrast, the behaviour of the intestine with respect to the oxidation and reduction of retinol appears to be more complex. Thus we are confronted with the situation that, on the one hand, in the *in vitro* system with the everted intestinal sacs, the oxidative process is more powerful, while, on the other, in the living animal, when the aldehyde is fed, the reaction goes only towards reduction and no oxidation ever occurs. It is equally puzzling that when the *in vitro* systems are compared within themselves, the everted sacs can effect significant reduction of the aldehyde, but once the organization of the same cell is disrupted by homogenization, it at once loses its ability to reduce the aldehyde, while retaining its properties of oxidizing it.

However, work on retinoic acid appears destined not to have a smooth sailing. Just at the moment, where several aspects of the work on retinoic acid began to appear most promising, the question began to be raised that retinoic acid cannot be considered to be a natural metabolite of the vitamin, because all work on it was being carried out with the chemically synthesized compound, and that no retinoic acid was ever detected in normal animal tissues, nor was it conclusively shown to be absorbed by animals. These were quite legitimate criticisms. However, as will be recalled, we demonstrated that it is absorbed by rats, but that it rapidly disappears from its tissues[40]. Similarly, we also showed that considerable amounts of it can be found in the living rat, if sufficient amounts of its immediate precursor, which is retinal, are injected into the animal[62]. Thus, we showed that within 5 min. of an intravenous injection of an aqueous dispersion of 2 mg. of retinal, 35 μg. of retinoic acid can be isolated from the liver and 7·0 μg. from the intestine of rats. But here also, it rapidly disappeared from the rat tissues. At about the same time Dunagin *et al.*[63] demonstrated that retinoic acid is rapidly excreted through rat bile as a glucuronic acid conjugate.

Therefore, now it appears possible to find some explanation why retinoic acid has not been detected in normal animal body. First of all, the amount of retinal, its immediate precursor, available in the animal liver at any given time, is very small, so that it will permit the generation of only very limited amounts of the acid. Secondly, there are reasons to believe (as will be seen later on) that the acid might be converted into other metabolites, which might escape detection. Finally, if any excess of the acid is formed, it is immediately excreted through the bile as a glucuronide.

There are other ways of looking at the forces controlling the generation of retinoic acid. Vitamin A is unique among all the vitamins, in that no other vitamin is stored in such large amounts in the liver. This probably indicates that during the process of evolution there were alternate periods of abundance and scarcity, so that the animal had to evolve such efficient mechanisms of storing sufficient amounts of it to tide over the scarcity periods. At higher dosage levels retinyl esters are highly toxic and can kill the animal. Retinoic acid is toxic even at lower dosage levels. Therefore, the animal stores this potentially toxic material without killing itself and this it does by several mechanisms. Firstly, it stores it as the retinyl palmitate, whereby it makes it rather inert physiologically. Secondly, it tucks the ester away in the Kupffer cells of the liver, whereby it removes it from general circulation. Thirdly, it has evolved a very weak enzyme in the liver, which allows only very small amounts of it to be hydrolysed at any given time. An equally effective block has been placed at the next step, where the retinol is to be oxidized to the corresponding aldehyde. The equilibrium of the liver alcohol dehydrogenase that carries out this reaction is far towards the reduction of the aldehyde to the alcohol, so that even if larger amounts of the alcohol are available in the liver, very limited amounts of the aldehyde can be formed. By introducing so many controlling mechanisms the animal has not only prevented its own death by self-generated toxicity, but has also made sure that such a vital commodity as vitamin A is well preserved and is used up only sparingly. Now, even after all these controlling steps, if any excess of retinoic acid is generated, the animal immediately detoxifies it by forming the glucuronic acid derivative which is ultimately eliminated through the bile.

It should be mentioned here that although alcohol dehydrogenase and aldehyde oxidase have been known to occur in animal tissues, their physiological importance was never fully understood. Our work has now assigned a vital role to these enzymes in the metabolic regulation of retinol.

Other Possible Active Derivatives of Vitamin A

Incubation of an ethanolic solution of retinol with sheep kidney homogenates for 2 hr at 37°C. in phosphate buffer (*p*H 6·8, 0·1*M*) gave rise to a highly coloured compound, which could be purified on alumina columns and finally crystallized from methanol. This compound exhibited absorption maxima at 335 and 435 mμ in ethanol and did not answer the Carr-Price or TBA (thiobarbituric acid) reaction. The synthesis of this compound was proved to be enzymic and the enzyme activity was localized in the mitochondrial fraction of sheep kidney homogenates.

In other trials, rat liver homogenates were incubated with ethanolic solutions of sodium retinoate in phosphate buffer (*p*H 7·8, 0·25*M*) at 37°C.

for 3 hr (Malathi, P. & Ganguly, J., unpublished results). The reaction mixture was extracted with petroleum ether and ethanol and the extract chromatographed on alumina columns. Two distinct chromatographic bands were eluted from the column, one of which (eluted first and termed fraction 1) showed spectral properties very similar to those of retinoic acid ($\lambda_{max.}$ in ethanol, 351 mμ and $\lambda_{max.}$ of the colour developed with antimony trichloride, 575 mμ). But, while the acid was tenaciously adsorbed on the column, this band was eluted with relative ease. It is quite possible that it is constituted of retinoic acid esters. The other band (fraction 2), which moved less rapidly on the column, had a $\lambda_{max.}$ in ethanol at 338 mμ and showed an absorption maximum at 555 mμ in the antimony trichloride reaction. When 15-^{14}C-retinoic acid was diluted with unlabelled retinoic acid and similarly incubated with rat liver homogenates, the specific radioactivity of fraction 2 isolated compared quite favourably with that of the substrate retinoic acid, thereby proving that it is not formed by decarboxylation of retinoic acid. It is possible that it is a lactone formed by a preliminary introduction of a hydroxyl group in the polyene side chain of retinoic acid. When tested in vitamin A deficient rats, it showed slight superiority over retinoic acid in its growth-promoting activity. We do not claim it to be an active metabolite of retinol. But, if it is indeed formed by the introduction of a hydroxyl group in the side chain of retinoic acid, that by itself should prove quite interesting, because, in that event, the growth-promoting effect of retinoic acid would seem to be retained even after the polyene side chain is modified in this manner (Malathi, P. & Ganguly, J., unpublished results).

MODE OF ACTION OF VITAMIN A

Growth

Although earlier work had shown that synthetic retinoic acid can support growth of vitamin A deficient rats, there was practically no information available regarding the exact biological activity of retinoic acid, as compared to retinyl acetate. Bioassay experiments were, therefore, carried out with vitamin A deficient male rats and it was shown that retinoic acid is 49 and 140 per cent as active as retinyl acetate, when given in the form of its sodium salt by mouth or by intraperitoneal injections respectively[64].

During these bioassay experiments we consistently noticed that vitamin A depleted rats receiving 5 or 10 μg. of the acid show a pronounced growth response of about 8-10 g. within 24 hr of the first dose and at the same time drink an enormous amount of water, while the corresponding retinyl acetate fed rats registered a growth response of 1-2 g. and drank considerably less water. Water and nitrogen balance studies of such rats showed that, while nitrogen retention in both groups was comparable, the acid treated rats drink more water, but actually excrete less urine. It was thus proved that the spectacular increase in weight of the acid fed rats is due to retention of more water.

We had interpreted these results as follows. Several workers have repeatedly demonstrated that the adrenal glands of rats suffer histological damage during vitamin A deficiency. Since these glands synthesize and secrete hormones that regulate the mineral and water balance of animals, it was suggested that the loss of weight during the depletion of vitamin A is probably due to dehydration of the animals brought about by the unbalanced synthesis of these hormones by the damaged adrenal glands while, in contrast, the gain in weight following vitamin A administration probably reflects the rehydration of the animals through a correction of the hormone synthesis by the repaired adrenal cells. Retinoic acid, being several steps ahead of the ester, should be in a position to act faster and thereby effect such pronounced differences in growth response[61].

To obtain evidence in support of this theory, Juneja *et al.*[65] carried out experiments where the sodium and potassium levels of plasma of rats were followed with the progress of depletion of vitamin A. It was observed that while the sodium level shows a consistent increase, the potassium level actually goes down during the deficiency. Oral administration of retinoic acid to such rats at the weight-plateau stage led to an immediate correction of the plasma sodium and potassium levels, while retinyl acetate took a much longer time.

These observations on the plasma sodium and potassium levels of vitamin A deficient rats have been indirectly confirmed by independent and simultaneous work of Woelfel *et al.*[66] with vitamin A depleted dairy calves. It was shown by these workers that the concentrations and daily output of sodium and chloride in the urine of such calves were lower, as compared to the normal calves. Urine from the deficient animals had lower than normal specific activity, osmolality, total solids, urea nitrogen and creatinine. These workers have interpreted the results on the basis of the reasoning that, during vitamin A deficiency, structural and, possibly, functional changes take place in the kidneys, which may lead to defective renal clearance of the urinary constituents. This seems to be a more plausible explanation, because in such a situation also sodium can be concentrated in the animal body due to renal damage. There is some evidence in support of such a possibility, because it has been reported that blood uric acid concentrations markedly increase in vitamin A deficient chicks. We are, therefore, in broad agreement with such interpretations. However, as will be seen later, we have collected a large volume of evidence to show that normal steroidogenesis in both male and female rats depends upon the vitamin A nutritional status of the animal.

Sulphate Metabolism

In recent years the importance of sulphurylation, like that of phosphorylation and carboxylation, in biochemical reactions, is being increasingly appreciated. Numerous sulphated compounds have been isolated from living tissues of plants and animals, and probably the most important of them are the sulphated mucopolysaccharides. Fell and

her colleagues appear to be the first to point out that sulphate metabolism may be linked to vitamin A metabolism. Since then several groups of workers have tried to obtain evidence in support of such a possibility.

Sulphurylation consists of two enzymic steps, viz. activation of sulphate, or the formation of PAPS (3′-phosphoadenosine 5′-phosphosulphate), and the transfer of the 'active sulphate' to an acceptor. The acceptor can be mucopolysaccharides, phenols, or many other biological compounds. In 1960 Wolf and Varandani[67] provided evidence to suggest that in the rat colon sulphurylation of mucopolysaccharides is depressed in vitamin A deficiency, while Varandani *et al.*[68] claimed decreased synthesis of PAPS in the colon homogenates of the deficient rats, which could be restored by the addition of retinol *in vitro*. Since then we have attempted to investigate the possible effects of vitamin A deficiency on sulphurylation of phenols.

In our initial experiments[69], vitamin A deficient and corresponding pair-fed control rats were intraperitoneally injected with ^{35}S-labelled sulphate, following which their urine was collected separately from each individual rat for 24 hr (most of the injected labelled sulphate was excreted in the urine within this period). In the deficient rats the urinary excretion of ethereal sulphate was reduced with a concomitant increase in the amounts of inorganic sulphate, the neutral sulphate showing practically no change, as compared to those obtained with the pair-fed controls.

The enzymes, called 'arylsulphatases', hydrolyse phenolic sulphates. Therefore, two alternative explanations for the reduced excretion of ethereal sulphates in the deficient rats became apparent, viz. increase in the activities of the arylsulphatases or reduced synthesis of phenolic sulphates. A thorough investigation of the tissue distribution and kinetic properties of the arylsulphatases in normal rats was at first undertaken, after which changes, if any, in their activities in the deficient and pair-fed controls were examined. Practically no changes in the activities of these enzymes could be noticed during the mild deficiency stage, while in acute deficiency only arylsulphatases A and B showed some increase[69].

A study of the changes in the activities for sulphurylation of phenols by the livers of such rats clearly showed that even at the mild stage of the deficiency the enzyme activities were reduced by about 60 per cent, with no further change taking place with the progress of deficiency. *In vitro* addition of retinol or retinoic acid fully restored the lost activity[69].

In continuation of this work, Subba Rao and Ganguly[70] investigated the effect of vitamin A deficiency on the two enzymic steps involved in the sulphurylation of phenols and it was shown that both the enzyme activities are significantly depressed during the deficiency. Here, *in vitro* addition of retinol was more effective in restoring the lost activity of the first step, while retinoic acid was more effective in the second. This effect of vitamin A in restoring the loss of the enzyme activities appeared to be rather specific, because no significant restoration was possible by the addition of other fat-soluble compounds, like β-carotene or palmitic acid, or vitamins E, D or K. Oral administration of retinoic acid or retinyl acetate to vitamin A deficient rats completely restored the lost activities of both the enzymes.

At this stage the claims regarding the possible relationships between vitamin A and sulphate metabolism appeared to be heading for some serious controversy. Thus, while Varandani *et al.*[68] had earlier claimed a loss in the ATP-sulphurylase activity of the colon homogenates of vitamin A deficient rats, which could be restored by the addition of retinol *in vitro*, Sundaresan and Wolf[71] reported that the synthesis of PAPS is depressed in vitamin A deficient rat tissues, but they could not restore the lost activity by adding retinol or retinoic acid *in vitro*, and could do so with a metabolite derived from retinoic acid. This was followed by another communication by Sundaresan *et al.*[72] claiming the isolation of an enzyme-bound metabolite of vitamin A, which apparently functions in the reactivation of ATP-sulphurylase. In the meantime, Pasternak *et al.*[73] reported that PAPS synthesis remains unaffected in the cornea and colonic mucosa of vitamin A deficient rats and rabbits.

However, excepting for Pasternak *et al.*[73], there appeared to have been general agreement that PAPS synthesis suffers a loss in vitamin A deficiency and the point of disagreement appeared to be the ability of retinol to restore the lost activity. Subba Rao and Ganguly[74], therefore, re-investigated this question and again demonstrated that PAPS synthesis in the liver of the rat is significantly depressed even with the onset of vitamin A deficiency, further loss taking place at the acute deficiency stage. Only at the milder stages of the deficiency retinol, added *in vitro*, fully restored the lost activity, while retinoic acid was partially effective; no such restoration could, however, be effected at the acutely deficient stage. In our attempts to clarify the differences between our findings with those of Wolf and his associates, we have offered the following explanation. If in their earlier experiments, Wolf and his associates had used rats that were mildly vitamin A deficient, and in their later trials severely deficient animals were used, the failure to effect reactivation with added retinol would be quite obvious. Also, if any metabolite of retinol were to participate in this reaction, it is conceivable that at the earlier stages of the deficiency the enzyme systems responsible for the generation of such a metabolite could still be active, while it might have been totally lost at the acutely deficient stage. Similarly, the failure in effecting any reactivation by the addition of retinol or retinoic acid to the purified ATP sulphurylase was explained on the basis of the argument that the enzyme system(s) that forms the metabolite from these compounds might have been removed during the process of purification.

Almost simultaneously with the report of Subba Rao and Ganguly[74], several papers have appeared more or less substantiating their claims. Thus Sundaresan[75] has again shown that PAPS synthesis suffers loss during vitamin A deficiency in rats. In

his experiments, several methods, like molybdolysis of ATP, pyrophosphorolysis of APS and incorporation of ^{35}S-labelled sulphate into PAPS were employed in order to prove that ATP sulphurylase activity is depressed in the deficient rat liver. The loss could be restored by the *in vitro* addition of a lipid extract containing an acid metabolite of retinoic acid. More interesting has been the work of Carroll and Spencer[76], according to whom foetal rat liver preparations show a low rate of PAPS synthesis and a complete lack of sulphotransferase activity towards various receptors. Retinol and retinoic acid, when added to the foetal liver preparations, stimulated both sulphate activation and sulphotransferases. At about the same time, Mukherji and Bachhawat[77] and Perumal *et al.*[78] independently reported reduced uptake of ^{35}S-sulphate into the mucopolysaccharides isolated from tissues of rats made deficient of vitamin A. While the former workers have averaged values obtained from rats of both sexes and analysed several of their tissues, the latter group has separately presented data from the intestine of male and female rats. However, these observations of both groups have confirmed the earlier similar claims of Wolf and Varandani[67]. More significant is the fact that Perumal *et al.*[78] found no appreciable differences in the ^{35}S-sulphate incorporation into the mucopolysaccharides of the deficient female rats, while the difference was most marked in the males. It, therefore, appears possible that some of the disagreements reported in the literature could be due to the use of rats of unspecified sex. Finally, Guha and Roels[79] have confirmed the claims of Subba Rao *et al.*[69] that arylsulphatases A and B of rat liver are increased in their specific activities during vitamin A deficiency, while Hall and Straatsma[80] have confirmed a marked reduction in PAPS synthesis in the liver, but not in the retina, of vitamin A deficient rats.

Therefore, there now seems to be general agreement that sulphate metabolism is in some way connected to vitamin A metabolism.

Amino Acid Metabolism

Investigations into the metabolic role of vitamin A in yet another area, viz. protein metabolism, showed that vitamin A deficiency leads to a negative nitrogen balance in rats. Oral administration of retinoic acid or retinyl acetate corrected the defect in the urinary excretion of nitrogen, as caused by the deficiency (Subba Rao, K. & Malathi, P., unpublished results).

During the course of this work, it was noticed that the tissue levels of free phenylalanine and tyrosine are markedly increased, while the threonine concentration is reduced in the deficient rats as compared to those in the pair-fed controls[81]. These could be corrected by oral supplementation of retinol or retinoic acid. It has been known that the metabolism of phenylalanine and tyrosine is directly connected with that of ascorbic acid. It, therefore, appeared possible that the increase in the tissue levels of the free phenolic amino acids might be due to a reduction in the synthesis of ascorbate. Therefore, ascorbate was injected into the deficient rats, which led to a correction of the increased concentrations of the phenolic amino acids, leaving the threonine levels unchanged. Also, rats maintained on the depletion diet and given supplements of ascorbate lived much longer and maintained better health as compared to the corresponding controls receiving the depletion diet, but no ascorbate[82].

TABLE 7 — URINARY EXCRETION OF ASCORBIC ACID BY VITAMIN A DEFICIENT RATS FOLLOWING CHLORETONE TREATMENT

State of deficiency	No. of rats	Av. food consumption (g./day)	Av. ascorbate excreted (mg./day)
Pre-plateau	6	10·6	7·3±1·3
Pair-fed normal	6	10·6	7·5±1·8
Plateau	6	7	6·5±0·8
Pair-fed normal	6	7	6·9±0·2
Deficient	6	1·9	0·89±0·31
Pair-fed normal	6	1·9	5·8±2·1

Adapted from Malathi, P. & Ganguly, J., *Biochem. J.*, **92** (1964), 521.

Further investigations showed that the ascorbic acid contents of the livers of vitamin A deficient rats were markedly lower as compared to those in the corresponding pair-fed controls. Also, the urinary excretion of ascorbate under the influence of chloretone was significantly reduced in vitamin A deficient rats (Table 7). Finally, the *in vitro* synthesis of ascorbate from L-γ-gulonolactone by the microsomal enzymes of rat liver was found to be reduced by about 50 per cent at the onset of the deficiency and thereafter it gradually decreased to almost no synthesis at the acute deficiency state. *In vitro* addition of either retinol or retinoic acid to the reaction mixture had no effect[83].

A detailed study of the various enzymic steps connected with the metabolism of phenylalanine and tyrosine showed that the enzymes transaminating phenylalanine with pyruvic acid and tyrosine with α-ketoglutarate were not affected at all the stages of deficiency. *p*-Hydroxyphenylpyruvic oxidase was, however, reduced by about 50 per cent at the weight-plateau stage; the loss of the activity continued with the progress of deficiency and became most marked at the acute stage. Administration of ascorbate to the A deficient rats or its addition *in vitro* to the assay system partially restored the lost activity.

It was thus concluded that the ability of the rat to synthesize ascorbate is directly dependent upon its vitamin A nutritional status. Therefore, some of the symptoms observed in vitamin A deficient animals may be due to a secondary deficiency of ascorbate superimposed on the direct effect of vitamin A deficiency.

Lipid Metabolism

Wiss and Gloor[84] had shown that in the liver of vitamin A deficient rats more of the ^{14}C-labelled mevalonate is incorporated into the ubiquinones, with a corresponding reduction in the incorporation

into cholesterol as compared to those observed in normal livers. The phenolic ring of the ubiquinones is probably derived from phenylalanine. It, therefore, appeared to us that the enhanced incorporation of labelled mevalonate into the ubiquinones in vitamin A deficient rat liver might be due to the increased availability of the phenolic ring arising out of the raised free phenylalanine and tyrosine levels of the tissues of rats during the deficient state.

Since administration of ascorbate could correct the increased levels of the phenolic amino acids in the tissues of the A deficient rats, the effect of ascorbate on the incorporation of ^{14}C-labelled mevalonate into squalene, cholesterol and ubiquinone fractions of vitamin A deficient rat liver unsaponifiables was investigated. Rats kept on the depletion diet for two weeks were given daily injections of 500 μg. of neutralized ascorbic acid for a few weeks, while they were being maintained on the same diet. At the stage, where these rats failed to grow for over a fairly long period of time (15-20 days), they were given by intraperitoneal injection 10 μC. of 2-^{14}C-labelled mevalonate and sacrificed 4 hr later. Pair-fed rats receiving supplements of vitamin A or no vitamin A served as controls and received similar injections of labelled mevalonate.

In these experiments, about 94 per cent of the incorporated radioactivity was found in the sterols, 2·5 per cent in the ubiquinones, and the rest in the squalene fraction in the liver of the normal animals. In the livers of vitamin A deficient rats, the corresponding values were 53, 17 and 30 per cent respectively, while in the ascorbate treated vitamin A deficient rats these values were 70, 10·5 and 19 per cent respectively[85]. Thus the reported increased incorporation of labelled mevalonate into the ubiquinones of vitamin A deficient rat liver might at least partially be due to the reduced availability of ascorbate.

Reproduction

Effect of Retinoic Acid on the Reproduction of Male and Female Rats

Earlier nutritional work had repeatedly shown reproductive failure in vitamin A deficient rats of both sexes. In these studies the general pathological derangements, as caused by vitamin A deficiency, had obscured the real effect of vitamin A on reproduction. However, Wald's recent experiments, where he demonstrated the inability of retinoic acid to meet the visual requirements of rats, opened up the pertinent question as to whether retinoic acid can meet other important physiological requirements, like reproduction. A few years ago, work reported from Morton's laboratory indicated that retinoic acid cannot meet these requirements of both male and female rats. We have also been pursuing this line of work independently, and in a preliminary communication we have reported our findings[86]. Since then this work has been further extended with more concentrated efforts and our major observations can be summarized as follows.

Weanling male and female rats were maintained on a vitamin A deficient diet and were supplemented with 5 μg. of retinoic acid or 1 μg. of retinyl acetate per rat per day for 10-12 weeks. When the acid treated male and female rats were allowed to mate, conception took place, but it resulted in the production of still-born litters of reduced size and weight. Mating of the male and female rats that were given retinyl acetate instead of retinoic acid resulted in the littering of viable pups of normal size and weight, while mating of the retinoic acid treated females with the males given retinyl acetate again led to the birth of still-born litters of reduced size and weight. On the other hand, mating of the females given retinyl acetate with the males supplemented with retinoic acid resulted in normal conception and regular littering, but in such cases there was less number of pups per litter as compared to the corresponding controls receiving retinyl acetate.

It appeared from these experiments that retinoic acid may not be able to meet the reproductive requirements and that the partial functioning of reproduction of these animals was probably due to the presence of residual amounts of retinol or its esters in their body. The above groups of rats were, therefore, allowed to continue on their respective dietary regimen for a period of another 8-10 weeks, after which they were allowed to mate in groups as described in the above experiments. At this stage mating of normal females with the acid treated males resulted in no conception, while mating of the acid supplemented females with the retinyl acetate fed or normal males led to normal conception, but the pregnancy was terminated with gestation-resorption beginning on the 14th or 15th day of pregnancy. Here also, mating of the male and female rats that had received retinyl acetate throughout resulted in normal conception and littering.

The most significant points here are, the acid treated females show normal oestrous cycle, while the testes of the corresponding males are oedematous and are reduced in size.

Correction of the Reproductive Failure due to Retinoic Acid Treatment

Steroid hormone treatment — Weanling female rats (18-20 days old), placed on vitamin A deficient diet till they started losing weight, showed irregular oestrous cycle, xerophthalmia and other symptoms of vitamin A deficiency. They were then supplemented with retinoic acid (20 μg./rat/day) with simultaneous subcutaneous injections of estradiol benzoate, progesterone or pregnenolone (100 μg./rat) on every fourth day for 40 days, with the control groups receiving 0·2 ml. of groundnut oil. These female rats were then allowed to mate with normal adult males, after which the treatment was continued during the pregnancy. The rats receiving estradiol benzoate did not conceive, while those treated with progesterone showed gestation-resorption beginning on the 16th day. Pregnenolone treatment, on the other hand, led to normal conception, gestation and littering, but the pups died soon after birth.

Another batch of similar rats was treated with (i) progesterone plus oestrone (2 mg. plus 0·5 μg./rat/day) or (ii) pregnenolone (10 μg./rat/day), from the third day of pregnancy. Fifty-six per cent of rats receiving progesterone plus oestrone showed normal littering, while 100 per cent success was obtained with the pregnenolone treated group.

Treatment with protein hormones — In another set of experiments similar retinoic acid treated pregnant rats were given from the 8th day of pregnancy various pituitary hormones, like HCG (human chorionic gonadotrophin) plus LTH (luteinizing hormone), LTH plus oestradiol benzoate, HCG or PMS (pregnant mare's serum). Other similar rats were treated with macerates from fresh pituitaries of adult female rats, or water extracts from acetone powder of sheep pituitary or were given pituitary grafts under kidney capsules. None of the other treatments proved as successful as pituitary grafting under kidney capsules, in which case 80 per cent of the rats maintained full term pregnancy. But none of them littered and live pups could be obtained by caesarian operations on the 21st day of pregnancy.

Effect of retinoic acid treatment on compensatory hypertrophy of the ovaries of rats — In yet another set of experiments normal and retinoic acid treated adult female rats were unilaterally ovariectomized, and were then allowed to continue on their respective dietary regimen for one month. After this period, the ovaries of the retinoic acid treated rats showed a distinct reduction in weight and in the number of corpora lutea, compared to the corresponding controls.

Effect of vitamin A deficiency on the in vitro and in vivo synthesis of steroid hormones in male and female rats — Preliminary experiments regarding the incorporation of 4-^{14}C-cholesterol into the steroids of the adrenals, testes and ovaries of normal and vitamin A deficient rats had indicated a definite reduction in the incorporation of the radioactivity into the steroid fractions obtained from the deficient tissues. Earlier similar attempts from other laboratories also had yielded similar results. But, this information did not indicate which of the enzymic steps involved in the biosynthesis of the steroid hormones might be affected by the deficiency. It has been known that the Δ^5-3β-ol steroid dehydrogenase is a rate-limiting enzyme system in the biogenesis of steroid hormones and that it is present in almost all the steroid producing glands of many vertebrates. Therefore, in view of the importance of this particular enzyme system in steroidogenesis, the effect of vitamin A deficiency on its acitivity was studied in detail[87].

This enzyme system converts the Δ^5-3β-ol steroids into the corresponding Δ^4-3-oxo steroids. It was found by us that the enzymic conversion of the Δ^5-3β-ol steroids, like pregnenolone, 17α-hydroxypregnenolone and dehydroepiandrosterone to the corresponding Δ^4-3-oxo steroids, progesterone, 17α-hydroxyprogesterone and androstenedione is significantly reduced in the adrenals, testes and ovaries of rats, even at the mild deficiency stage, with further loss taking place on prolonging the deficiency. In all these tissues the loss in the enzymic production of androstenedione from dehydroepiandrosterone could be restored by the *in vitro* addition of retinol or retinoic acid, but not by retinal, and the restoration of the loss was possible only at the mild stage of the deficiency.

Further work with rat adrenal glands revealed that vitamin A deficiency markedly affects the synthesis of deoxycorticosterone and corticosterone from pregnenolone, even at the mild deficiency stage, with further loss taking place at more advanced stage of the deficiency. Retinol or retinoic acid could restore the loss *in vitro* at the mild but not at the acute deficiency stage. The deficiency had, however, no such effect on the synthesis of deoxycorticosterone from progesterone or of corticosterone from progesterone or deoxycorticosterone. It was, therefore, concluded from these results that vitamin A deficiency exerts its effects primarily on the enzyme system Δ^5-3β-ol steroid dehydrogenase[87].

Finally, an analysis of the steroid contents of the normal and deficient adrenals showed that, as compared to the normals, the deficient adrenals contained less amounts of deoxycorticosterone and corticosterone, but higher amounts of aldosterone.

Detection of vitamin A in the endocrine glands of rats — The results described above thus strongly indicated that proper functioning of the endocrine glands depends upon vitamin A nutritional status of the animal. It was, therefore, natural that attempts should be made to investigate the possible presence of vitamin A in these glands. Glands, like pituitary, adrenals, testes, ovaries and placentae of normal adult rats were extracted and the extracts were extensively purified by adsorption chromatography on alumina columns, followed by thin-layer chromatography on alumina plates. Retinol and its esters were identified not only by their behaviour during chromatography and by their characteristic fluorescence under ultraviolet light, but also from their characteristic absorption spectra, UV absorption and Carr-Price reaction. Making use of these techniques, it was possible for us to identify definite but variable amounts of retinol in the pituitaries, and of retinyl esters in the adrenals, testes, ovaries and placentae of rats.

METABOLISM OF VITAMIN A IN PROTEIN MALNUTRITION

In recent years protein malnutrition has been recognized to be a major deficiency disease of the human beings, especially of the growing children in most of the developing countries of the world. But what is more serious is, clinicians have repeatedly drawn pointed attention to the fact that very often children suffering from protein malnutrition show signs of vitamin A deficiency also[88]. Widespread occurrence and seriousness of this dual deficiency have been generally recognized by the health and nutrition workers, and yet no satisfactory explanation seems to have been made available for a proper understanding of the fundamental causes of this malady.

It appeared to us that it might be possible to find some explanation and we, therefore, undertook detailed investigations of the possible derangements that might take place, due to protein deficiency,

TABLE 8 — RELATIVE RATES OF INTESTINAL CONVERSION OF β-CAROTENE TO VITAMIN A IN RATS MAINTAINED ON DIETS CONTAINING 5, 10 AND 20 PER CENT CASEIN

(Values are expressed as μg. retinol or carotene for whole of the intestinal wall and liver and for 100 ml. blood. The initial blood levels of retinol in the three groups of rats were about 25-30 μg./100 ml. blood, while the livers contained only traces of vitamin A)

	Dietary protein %	1 hr		3 hr		6 hr		12 hr		24 hr	
		A	C	A	C	A	C	A	C	A	C
Intestinal wall	5	2·9	13	6·5	31	6·2	22	2·4	8	2·1	4
	10	10	12	15	26	8·5	19	5·2	5	4·1	3
	20	11	9	20	23	12	18	6·5	5	5·1	3
Blood	5	33	—	39	—	42	—	39	—	38	—
	10	43	—	71	—	45	—	43	—	45	—
	20	51	—	82	—	53	—	53	—	50	—
Liver	5	3·4	—	6·5	—	8·2	—	11	—	12	—
	10	4·5	—	12	—	23·4	—	30	—	31	—
	20	6·7	—	18	—	33·5	—	42	—	43	—

A, retinol plus retinyl esters; and C, carotene.
Adapted from Deshmukh, D. S. & Ganguly, J., *Indian J. Biochem.*, **1** (1964), 304.

at the various steps of absorption, transport, storage and metabolism of vitamin A, as well as in the intestinal conversion of β-carotene to vitamin A. The limitations in carrying out this type of investigations in this laboratory have been, there being no clinical facilities available for work on human beings, we had to confine the entire work on rats kept on synthetic diets. In spite of such limitations, our experiments have probably yielded some clearcut results under controlled experimental conditions. These results cannot, however, be extrapolated *in toto* to the human beings, because in the latter cases usually these deficiencies are precipitated not just by protein insufficiency alone, but by a deficiency of multiple nature, which are often accompanied by various infections also. Nevertheless, as will be seen below, our observations should lead to some broad conclusions, which in turn should lead to a more fundamental approach to this problem.

Intestinal Conversion of Carotenes to Vitamin A

Usually the provitamin A carotenoids are the most important sources of vitamin A for human beings, and are probably the only source of vitamin A for people suffering from protein malnutrition. The effect of protein malnutrition on the utilization of dietary carotenoids thus assumes great significance, and, therefore, we considered it necessary to investigate this aspect.

Growing rats, weighing about 50 g. and with little liver reserves of vitamin A, were kept on synthetic diets containing 5, 10 and 20 per cent casein for about 30 days, after which, following starvation for about 24 hr one rat from each group was given in one single dose 3 mg. of β-carotene dissolved in oil and mixed with 1 g. of fat-free diet. The β-carotene and total vitamin A values of the stomach, intestinal contents, intestinal wall, blood and liver of the rats were then determined at different time intervals ranging from 1 to 24 hr after

TABLE 9 — INFLUENCE OF DIETARY PROTEIN CONTENTS ON THE UTILIZATION OF β-CAROTENE IN RATS GIVEN 0·4 MG. OF CAROTENE FOR SEVEN CONSECUTIVE DAYS

(Values are expressed as μg. of total vitamin A or carotene for the whole of the intestinal wall and liver and per 100 ml. blood, and μg. carotene in the faeces voided over seven days)

		Dietary protein		
		5%	10%	20%
Intestinal contents	Carotene	18	10	5·5
	Vitamin A	—	—	—
Intestinal wall	Carotene	5·5	3·7	2·1
	Vitamin A	3·5	5·5	7·2
Blood	Carotene	—	—	—
	Vitamin A	32	41	54
Liver	Carotene	—	—	—
	Vitamin A	32	63	102
Total faecal excretion of carotene over seven days		2079	1787	1321

Adapted from Deshmukh, D. S. & Ganguly, J., *Indian J. Biochem.*, **1** (1964), 204.

the dose. It was consistently observed that the concentrations of total vitamin A in the intestinal wall, blood and liver were progressively reduced with a progressive lowering of the protein intake, while in contrast, the β-carotene values of the intestinal wall and intestinal contents were inversely related to the dietary protein levels (Table 8). Similarly, when other similar rats were given 4 mg. of β-carotene daily for seven consecutive days, vitamin A contents of the tissues of the rats receiving lower protein diets were less, while there was a corresponding increase in the excretion of the carotene in their faeces (Table 9). It was thus demonstrated that the conversion of β-carotene to vitamin A in the intestine of rats is drastically reduced in protein deficiency[89].

Absorption of Retinal

It has already been discussed that retinal is considered to be one of the intermediates in the conversion of β-carotene to vitamin A. It was, therefore, of interest to investigate the effect of protein malnutrition on the reduction of retinal to retinol in rat intestine during its absorption. Rats similarly raised on the diets of the three different protein levels were given 5 mg. of retinal in 0·2 ml. of groundnut oil, after which the retinol and retinyl ester contents of their tissues were analysed at various time intervals up to 24 hr after the dose. In these experiments also, at any given time interval, the blood and liver vitamin A values were always highest in the rats receiving the highest protein diets and lowest in those given the least protein. At 24 hr after the dose, there was a marked difference in the total vitamin A deposited in the livers of the three groups of rats, and these values were 260·5, 420·8 and 872·3 μg. per liver of rats belonging to 5, 10 and 20 per cent casein diets respectively[37]. Therefore, it appears clear that during protein malnutrition the step consisting of the reduction of retinal to retinol in the overall conversion of carotene to vitamin A suffers a serious loss and it may well be that this is the step that is most affected by protein deficiency.

Absorption of Retinyl Esters

In these experiments, similar young growing rats raised on the diets containing the three different protein levels were given in one single dose 10 mg. of retinyl acetate in 0·2 ml. of groundnut oil containing 1 per cent α-tocopherol. It will be seen from the data presented in Table 10 that within 1 hr of dosing, the concentrations of retinol in the intestine and blood were highest in the 20 per cent casein group and least in the 5 per cent casein fed rats. The concentrations of retinol and its esters in the intestinal mucosa and muscles of these rats increased with increasing protein intakes. There was a direct correlation between the dietary protein contents and the concentrations of blood and liver vitamin A also. However, the most important point to note here is that, while at the earlier time intervals the amounts of vitamin A deposited in the livers of the rats of the different groups showed pronounced differences directly reflecting the protein intake, later on this difference was nearly abolished.

It thus appears that, although, due to more efficient absorption, there was a sharper increase in the blood vitamin A concentrations leading to the deposition, at the earlier time intervals, of larger amounts of vitamin A in the livers of the animals receiving higher protein diets, ultimately over the longer periods of 24 and 120 hr all rats stored in their livers almost comparable amounts of vitamin A, irrespective of the protein level of their diets. This, therefore, clearly indicates that on reduced protein intake there is not so much reduction in the net amounts of vitamin A absorbed, but what actually happens is, the absorption process slows down considerably[90].

Storage and Mobilization

Groups of stock male rats, whose reserves had previously been built up by a single massive dose of 10 mg. of retinyl acetate, when kept for several weeks on diets containing 5, 10 and 20 per cent casein, showed that their blood vitamin A concentrations can directly be correlated with their blood albumin levels. Thus, in the rats receiving the 10 per cent casein diet, blood vitamin A and albumin levels progressively decreased with

TABLE 10 — RELATIVE RATES OF ABSORPTION AND STORAGE OF VITAMIN A IN RATS MAINTAINED ON THE 5, 10 AND 20 PER CENT CASEIN DIETS

(Values are expressed as μg. retinol for the whole of the contents, mucosa and muscles of the small intestine and the liver and per 100 ml. blood)

	Dietary protein %	1 hr		2 hr		3 hr		24 hr		120 hr	
		Ester	Alcohol	Ester	Alcohol	Ester	Alcohol	Ester	Alcohol	Ester	Alcohol
Intestinal contents	5	3882	460	3751	596	2015	582	39	17	—	—
	10	2579	849	1485	625	1332	406	130	81	—	—
	20	2452	1374	1368	773	1505	738	150	150	—	—
Intestinal mucosa	5	43	62	52	63	56	48	39	14	—	—
	10	51	124	90	108	78	90	30	19	—	—
	20	107	295	185	357	138	177	51	39	—	—
Intestinal muscles	5	39	35	89	80	75	75	44	10	—	—
	10	53	72	128	142	53	86	88	27	—	—
	20	130	213	270	279	211	213	64	43	—	—
Blood	5	26	41	98	104	230	116	30	62	14	58
	10	50	69	155	164	275	181	40	83	21	94
	20	168	107	390	184	400	260	130	160	27	120
Liver	5	39	2	66	7	112	13	2800	54	2700	43
	10	54	3	121	16	186	22	3200	140	3000	68
	20	135	12	240	27	407	37	3500	150	3400	120

Adapted from Deshmukh, D. S., Malathi, P. & Ganguly, J., *Biochem. J.*, **90** (1964), 98.

time, while in those receiving the 5 per cent casein diet the fall in blood vitamin A and albumin levels was much steeper. When these protein depleted rats were allowed to receive the 20 per cent casein diet, both albumin and vitamin A levels of blood steadily increased to the normal values. In contrast, the control rats receiving the 20 per cent casein diet throughout maintained their normal blood albumin and vitamin A levels[54,90].

At the termination of the experiment, the rats receiving the 20 per cent casein diet throughout had the least amounts of vitamin A in their livers, while those receiving the lowest protein diet had the highest liver stores.

Retinyl Ester Hydrolase and Synthetase and Retinal Oxidase

Homogenates prepared from the pancreas and intestinal mucosa of rats maintained on the diets of the three protein contents, when tested for their ability to catalyse the hydrolysis and synthesis of retinyl esters, showed that the specific activities of both the enzymes of both sources were markedly reduced in rats on the lowest protein diet, with an increase in the dietary protein contents leading to a progressive increase in the enzyme activities. The retinal oxidase of their liver showed a more pronounced fall on lower protein intake, the fall being almost proportional to the protein level of the diet[88].

Since publication of these results by us, Nir and Ascarelli[91], using chicks, have confirmed that reduced protein intake leads to a lowering of the plasma albumin and vitamin A levels, and to a drastic reduction in the mobilization of liver vitamin A. Rodel and Proll[92], on the other hand, in their first report on the relationship between protein intakes and vitamin A absorption in rats, while confirming that retinyl ester hydrolase and synthetase activities of intestinal contents and mucosa are markedly affected by protein deficiency, have claimed that lower protein intakes do not affect the absorption of vitamin A. However, their experimental conditions were completely different from those of ours and, therefore, the results of the two groups cannot be compared. Thus, while we had used weanling rats for protein depletion, rats used by Rodel and Proll were much older and bigger in size, in which case it would be doubtful if protein deficiency was satisfactory in their rats. Also, while we gave the dose of the vitamin in oil, Rodel and Proll gave it as a Tween dispersion in water. Since aqueous dispersions of vitamin A are very rapidly absorbed, such method of dosing may not reveal any significant differences. Finally, while our rats weighed between 80 and 120 g. and received 10 mg. of retinyl acetate, rats employed by Rodel and Proll weighed more than 200 g. and received 1·5-2·0 mg. of the vitamin. However, this was immediately followed up by a second communication by the same workers[93] confirming impaired absorption and storage of larger doses of vitamin A in protein deficient rats.

Rodel and Proll[92] have chosen to call the dose of 5100 6900 IU of vitamin A as unphysiological. It is not clear what is meant by unphysiological dose. When one considers in terms of the daily requirements of a rat, such a dose might be taken to be so. But, when absorption of vitamin A is being studied, such doses cannot be unphysiological, because compared to the net quantities of total lipids present in the daily diet of a rat, this amount of vitamin A is probably not too big for the rat to absorb. Nor will a single dose of such amounts of vitamin A cause any toxicity in rats. True, a rat is seldom called upon to absorb such quantities of vitamin A in one single meal, but, surely, that does not make such doses unphysiological. On the other hand, marginal doses may not reveal any marked differences in the blood vitamin A levels during absorption, as will be possible with a larger dose.

Summary

The work carried out by the author and his colleagues since 1955 under a research project sponsored by the Indian Council of Medical Research, on the various aspects of the metabolism of vitamin A, viz. (i) mechanism of absorption of glycerides, carotenoids, cholesterol and vitamin A; (ii) storage and transport of vitamin A; (iii) biological formation of vitamin A active compounds; (iv) mode of action of vitamin A; and (v) metabolism of vitamin A in protein malnutrition, has been reviewed. Reports of work on the metabolism of vitamin A from other laboratories, which have confirmed and extended the work of the author and his colleagues, have been discussed. Conflicting reports in a few areas have been critically analysed and attempts have been made to interpret them.

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Present-day Concepts of Soil Organic Matter

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IT has been conclusively established that soil organic matter improves the fertility of soils in the following ways: it provides plant nutrients, particularly nitrogen on decomposition; it improves the physical conditions of the soil; it acts as a source of energy for the growth of the microbial population which is responsible for a number of important biochemical processes in the soil; and it makes available plant nutrients from sources other than itself. In addition, there is growing evidence that free organic compounds and humic substances have beneficial effects on the plant's growth and that some organic substances obtained by the decomposition of organic matter in the soil check the incidence of plant diseases. Thus, in view of the great importance of soil organic matter in soil fertility, the study of the chemistry of soil organic matter has lately gained much importance and recently a number of highly significant papers[1-35], review and discussion articles[36-48] and monographs[49-51] have appeared on the various aspects of the subject. The present article gives a synoptic view of present-day concepts of the chemical nature of soil organic matter and its relation to soil fertility.

Chemical Nature of Soil Organic Matter

According to the majority of research workers in this field, soil organic matter can be classified into two groups[40,49,50]: (i) non-humic substances and (ii) humic substances.

Non-humic substances — Non-humic substances consist of plant and animal substances in various stages of decomposition, the decomposition products of plant and animal substances, the organic compounds excreted by soil microorganisms, and the bacterial plasma. These non-humic substances include all types of organic substances, namely proteins, lignins, carbohydrates, fats, waxes, tannins, microbial pigments and low molecular weight compounds.

Humic substances — A major fraction of soil organic matter consists of high molecular weight substances[40,49] which are comparatively resistant to decomposition and acidic in nature. They do not belong to any class of organic compounds. In view of the regularity of their occurrence in soils and certain special properties, it is very probable that they are *novel high polymeric substances*, produced under unique conditions prevalent in soil. These humic substances can be divided into humic acid, fulvic acid and humin fractions. These fractions differ from each other in their chain length and consequently in their molecular weights.

Chemical Nature of Soil Humic Polymer

The consensus is that soil humic polymer[40,49] is mainly derived from phenolic compounds, amino acids and possible sugar residues. Further, in the light of recent work, there is also a possibility that, at least in some soils, three types of polymers, based on phenolic compounds, amino acids and sugar residues respectively exist as separate series[40]. These seem to be linked to each other by very labile bonds.

The humic substances[52] are made of carbon, hydrogen, oxygen, nitrogen, phosphorus and sulphur; in addition, most of the elements of plant ash are also present in these substances, but they are not strictly a part of humus. The average molecular weight[52] of these substances is between 5000 and 50,000. Soil humic polymers contain[49,50,52-56] carboxyl, carbonyl, alcoholic, phenolic and quinoid groups and are complexed with 1-20 per cent inorganic matter.

Phenolic fraction[49,57-61] — It is generally believed that the highly condensed core of soil humus is based on the lignin and resorcinol phenolic compounds. On the oxidation of soil humic acids a number of phenolic substances, viz. syringaldehyde, *p*-hydroxybenzaldehyde, vanillin, vanillic acid, etc., are detected as the products. Besides, this core also contains nitrogen in heterocyclic rings.

Polysaccharides fraction[52,62-65] — Soil polysaccharides comprise at least 5 per cent of soil organic matter. On the basis of the studies on the hydrolysates of these substances, it has been established that the polysaccharides are composed of galactose, glucose, mannose, arabinose, xylose and rhamnose.

Nitrogen fraction[52,66-74] — On the acid hydrolysis of soil humus, 30-50 per cent of the nitrogen is liberated as amino acids. Therefore, it is presumed that amino acids in soils are present as proteinous matter. This presumption is also supported by the fact that proteins are present in substantial amounts in plants as well as in microbial protoplasma, which are the precursors of soil humus. However, it has not been possible to extract free proteins from soil humus and the evidence for their presence is only presumptive.

In addition, about one-third to one-half of the humic nitrogen is resistant to acid or alkali hydrolysis and is not polypeptide in nature. There is some evidence[38,70,75,76] that this nitrogen may be present in heterocyclic rings, but its exact chemical nature has not yet been elucidated.

The remainder of the soil nitrogen is present in nucleic acids[77-80] (about 10 per cent), amino sugars[81-84] (5-10 per cent), ammonia fixed with clay minerals[85,86] and in many other compounds in small quantities, especially free amino acids[87-89].

Soil Organic Matter in Relation to Soil Fertility

The importance of soil organic matter in relation to soil fertility need hardly be emphasized. During the last 15 years much significant work has been in progress on this problem and new concepts may emerge in this field. It is well established that soil organic matter provides plant nutrients upon its decomposition and that it improves the physical condition of the soil. So far the relative importance

of these two types of effects is not very clearly known. It is generally believed that under tropical conditions the contribution of soil organic matter to soil fertility is mainly due to its capacity to provide plant nutrients and that its effect on the physical condition of soil is very temporary because high temperatures result in its speedy decomposition. On the other hand, a recent study at the Indian Agricultural Research Institute, New Delhi, indicated that, on the application of organic matter to soils over the years, there can be a substantial increase in soil organic matter. However, there is no rigorous and scientific proof of either of these contentions.

Soil Organic Matter and Available Plant Nutrients

Significant advances have been made on the role of soil organic matter in making available plant nutrients from sources other than itself[47]. It is well known that in the majority of cases soil organic matter has a marked influence on the availability of soil phosphorus and trace elements. Despite the presence of a considerable amount of phosphorus in the soil, the supply to plants is limited owing to its fixation in insoluble forms, namely calcium phosphate, ferric phosphate, aluminium phosphate; or as adsorbed on clay minerals. Soil organic matter or organic materials make phosphorus available from these sources[47,90-92] and the mechanism of the phenomenon has been explained as follows. The organic acids, produced as a result of decomposition of soil organic matter, influence the *p*H and consequently make phosphorus available from calcium phosphates or these acids form complexes or chelates with the cations, and thus release phosphates. It is also likely that both these mechanisms occur simultaneously. It was also found that humus forms chelates or stable complexes with polyvalent cations, and there is a likelihood that in this way it makes phosphorus available from the inorganic phosphates[47].

Similarly, the supply of trace elements to plants in soil is also limited by their presence in the unavailable form[47]. It was found that synthetic chelates, e.g. EDTA, increase the absorption of these elements either by the absorption of chelates by the plants or by the liberation of the cations at the root surface. On the basis of this observation it is fair to assume that natural chelates influence the availability of trace elements. A number of workers[93-96] have reported that various humic fractions and other organic materials considerably increase the uptake of trace elements (thus preventing the iron chlorosis). However, it was also found that in a few cases the organic matter[97] fractions decrease the availability of some of these elements.

Soil organic matter also increases the uptake of potassium by the plant from sources other than itself[47,98,99]. The acids accelerate the weathering of potassium-containing minerals and consequently make it available. In addition, some microorganisms are capable of utilizing potassium-containing minerals, and on their death and decomposition potassium becomes available to plants.

Further, it has been suggested that soil organic matter plays a vital role in the fixation of nitrogen from the atmosphere. Dhar[100] has adduced evidence that on the decomposition of organic matter there is liberation of energy which is responsible for the fixation of nitrogen from the atmosphere. On the other hand, it has also been indicated that this energy[47] is utilized by the microorganisms which are responsible for non-symbiotic fixation[101]. The amount of nitrogen fixed by these microorganisms is considered to be at least 20 lb./acre/year and under suitable conditions it can be up to 60 lb./acre/year[102]. Russian workers have laid much emphasis on non-symbiotic fixation and reported that a large amount of organic matter is required for successful innoculation[47] with azotobacter.

Plant Growth vis-à-vis Absorption of Organic Compounds

Plant growth vis-à-vis the absorption of organic compounds is another important aspect on which useful and significant work has recently been carried out. It is well known that it is possible to have an optimum growth of plants in a solution[47] containing inorganic nutrients only. However, recent work has established[103] that plants can absorb large organic molecules through their roots (mol. wt 200-500). Therefore, it can be assumed that plants assimilate organic compounds from the soil and it is possible that they influence the metabolism of plants.

It is generally believed that sugars, amino acids, nucleotides, vitamins, antibiotics and phenolic compounds are present in small quantities in soils. Vitamins are produced by many bacteria in soils in the rhizosphere and it is reported that they are assimilated by the plants and stimulate their growth[104-106]. The Soviet workers in particular have produced evidence in support[106] of this contention. It has been found that indolyl-3-acetic acid (IAA), which is produced by a number of microorganisms[107] under suitable conditions, apparently exhibits auxin activity[108-110] and, under optimum dosage, stimulates the growth of root hair[47,111,112]. Similarly, interesting results have been obtained concerning the plant growth-promoting property of gibberellic acid[47].

It has been reported that other organic compounds also influence the metabolism of plants. In this connection phenolic compounds, which are believed to be the decomposition products of lignin in soil, are of special significance. These phenolic compounds possibly inhibit[113] the oxidation of IAA, may increase the formation of IAA from[114] tryptophane, act as a means of electron transfer[115] in plant respiration, and enable plants to resist the attack of pathogenic fungi. Various oxy-anthraquinones[116] and thymohydroquinone[117] have been reported to have a salutary effect on plant growth; in the case of vanillic acid conflicting results have been reported[118,119]. Humic acid has been found to stimulate root growth, increase absorption of plant nutrients and, in some cases, increase the crop yield[49,120-122]. In addition, phenolic compounds and humic acid affect the citric acid cycle, the accumulation of sugars in plants and consequently the osmotic pressure of cell sap[121].

Effect of Soil Organic Matter on the Incidence of Plant Diseases

A number of workers have indicated that soil organic matter has markedly beneficial effects on the

incidence of plant diseases. Its effect is twofold: to decrease the intensity of attack by pathogens[47,123-125], and to increase the resistance of plants to such attack[47,126-129].

It is well known that there exists in soils a balance between saprophytic and parasitic activity, and soil organic matter appears to favour the activity of the former[47]. Significantly, soil microorganisms produce fungitoxic substances, especially antibiotics[130]. In addition, the decomposition products of organic matter and the metabolic products of microorganisms may also have fungitoxic properties. A number of phenolic compounds, namely cinnamaldehyde, cinnamic acid, vanillin, ethyl vanillate, eugenol, and lignin-like fractions extracted from soils exhibit fungitoxic properties[47].

Moreover, organic compounds increase the resistance of plants to diseases[126-129]. It has been reported that phenolic compounds, obtained from the decomposition of organic matter, increase the resistance of plants to disease. In certain cases a correlation has been observed between the accumulation of phenolic compounds and the resistance of the plant to diseases[126]. So far it is not known to what extent a large number of organic compounds absorbed by plants are fungitoxic or induce metabolic reactions which are antagonistic to fungal growth.

Thus it is evident from this review that despite a large volume of work on soil organic matter during the past hundred years, its chemical nature is still obscure and some of its functions in soil fertility are not yet well defined. This poses a serious challenge to the ingenuity of workers in this field. However, in view of many refinements and advances in analytical techniques, and progress in polymer chemistry, it is very likely that in the near future there may be major breakthroughs in this field. It is generally believed that under tropical and subtropical climatic conditions soil organic matter contributes to plant growth mainly by supplying inorganic nutrients and that it has little part in improving the physical condition of the soil, in view of its speedy decomposition. On the other hand, it has been indicated that on the application of organic matter to soils over the years, there can be a substantial increase in the level of soil organic matter. The authors are of the opinion that this problem can be solved by studying the decomposition of ^{14}C-labelled organic matter over a period of many years and thereby establishing the rate of turnover and persistence of soil organic matter. The fundamental problem of the structure of soil humus should attract the attention of workers in our newly established agricultural universities. The claims, based on circumstantial or meagre evidence, concerning the contributions of soil organic matter to soil fertility (besides the supply of plant nutrients and improvement in physical conditions) also deserve objective and serious study.

Summary

The present-day concepts of the chemical nature of soil organic matter developed in the light of the recent work are surveyed. In particular, the modern view that humic substances are high molecular weight substances, produced under unique conditions prevalent in soils, is discussed. A brief account of physical, chemical and physico-chemical properties of humic substances is given. The recent advances on the role of soil organic matter in soil fertility are reviewed. A few profitable lines of investigations on soil organic matter obtained under tropical and subtropical climatic conditions are suggested.

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REVIEWS

THE MANY-ELECTRON PROBLEM by K. S. Viswanathan (Asia Publishing House, Bombay), 1966. Pp. viii+244. Price Rs 22.00

It is well known that only for a few selected systems, e.g. hydrogen atom and H_2^+, etc., exact quantum mechanical solutions are available. For systems consisting of many electrons one can, at best, hope for some approximate method of solutions. Early attempts in this direction were the Hartree and Hartree-Fock approximations which relied on the variational principle. These, however, fail to account for the correlated motion of electrons with antiparallel spins. In recent years, important theoretical techniques have been developed to deal with many-body problems.

The book under review is concerned with problems connected with the motion of electrons in atoms, molecules and to a certain extent metals. The author has given a reasonable account of the subject in the eleven chapters and a few appendices. While a self-contained description is available in a few chapters, others seem to be rather sketchy. The chapters on self-consistent field method, density matrices, Thomas-Fermi model and relativistic corrections are fairly adequate. However, Chapter 10, which deals with second quantization and some modern perturbation techniques, just touches the formal aspects briefly. An account of the application of these methods to a few specific systems which are now available would have improved the value of this chapter.

It would have been more profitable to discuss the problems of atoms and molecules in greater detail rather than add a chapter on plasma oscillations in metals.

The presentation is clear and the book will be of value to those who want to have an introduction for the many-electron problem in a single book of this size.

K. P. SINHA

QUANTUM MECHANICS by A. A. Sokolov, Y. M. Loskutov & I. M. Ternov; translated from the Russian by Scripta Technica (Holt, Rinehart & Winston Inc., New York; *Distributors in India:* India Book House, Bombay), 1966. Pp. xix +537. Price $ 11.00

The book attempts to give a broad survey of the formal structure of quantum mechanics with a few applications in the fields of atomic physics, solid state physics and nuclear physics. It has been divided into three parts. The first part deals with non-relativistic theory and the second with relativistic theory. The third part deals with the application of non-relativistic and relativistic theories to problems in nuclear physics. The authors have included all those topics where electron-spin is involved in the second part which deals with relativistic quantum mechanics. This is why, besides discussing the Klein-Gordon and Dirac relativistic Lamb shift and positron-electron system, they have also discussed, in this part, Pauli's theory of electron-spin, the fine and hyperfine structures in the spectra of atoms, and all subsequent details depending on spin like the theory of multielectron atoms, the periodic table and theory of molecules, including masers and lasers.

The third part is primarily devoted to a discussion of the usual scattering theory and second quantization with application of the latter to the theory of beta decay and the Lee-Yang theory of non-conservation of parity.

The book can be fruitfully used by advanced students and those who are conversant with the subject and would like to have a quick general survey of the field.

R. P. SINGH

CONTINUUM MECHANICS: I — THE MECHANICAL FOUNDATIONS OF ELASTICITY AND FLUID DYNAMICS by C. Truesdell (Gordon & Breach Science Publishers, New York), 1966. Pp. xvi+218. Price $ 7.50

This is a corrected reprint of papers which started the *Journal of rational mechanics and analysis* in 1951. The author wants it to be a direct intermediary to all the earlier sources.

The mechanical foundations of the continuum mechanics is now a recognized subject for study and research, and many universities give courses in it. While the physicists have shown little interest in it, the chemists and engineers have taken to it to explain a number of their experimental results. Its significance lies in its generality to achieve simplicity and clarity.

Chapter I gives a review of the classical theories of elasticity and fluid dynamics and the scope and plan of the work. Chapter II discusses isotropy and uses Reiner's analysis to show that two symmetric tensors of the second rank are isotropically related if and only if their matrices b and a satisfy the relation

$$b = A_0 I + A_1 a + A_2 a^2$$

A's being functions of invariants of a and I, the unit matrix. The classical theories can now be readily seen to be linear approximations to isotropic relationships.

Measures of deformation and their equivalence, conditions of compatibility, rates of deformation and strain, thermodynamics of deformations in solids and fluids are discussed in Chapter III. The next two chapters deal extensively with elasticity and fluid dynamics. Chapter VI gives a short account of superposition theories and Chapter VII, the progress and programme of general theories and future lines of research.

There are three appendices dealing with a programme of physical research and annotations to the various chapters. These contain critical remarks and point out areas for fruitful research. The bibliography starts with the year

1678 and ends with 1954, and contains over 800 items.

The book contains a wealth of information on various topics in continuum mechanics and a host of critical footnotes. As pointed out by the author, rapid advance has been made in the subject during the past decade. It now rests on the concepts of tensor-operators, algebraic set functions and memory functionals.

Mathematicians, physicists, chemists and engineers — all will find the reading of the book stimulating and rewarding.

B. R. SETH

LABORATORY PHYSICS: Parts A and B, Berkeley Physics Laboratory (McGraw-Hill Book Co. Inc., New York), Part A, 1964. Pp. x+115. Price $ 2.25. Part B, 1965. Pp. ix+117. Price $ 2.25

The two parts of the book under review are based on the experiments set up at the Physics Laboratory of the University of California, Berkeley, for the B.Sc. level students. The experiments are designed not necessarily to cover any specific field of physics, but, instead, to give the students an insight into various aspects of physics. There are, for example, experiments on electron motion (tied by illustrations to motion of particles under gravitational and other fields), resonance phenomena and oscillations, negative resistance, elementary transistor circuits, periodic structures, microwave and acoustic generation, propagation, polarization, diffraction and interference, concepts of phase and group velocity, etc. The approach is novel in the sense that the use of similar basic phenomena at work in various fields of physics is brought out. The experiments are designed to use electronic tools such as the CRT, electronic voltmeter, etc., almost exclusively in order to orient the student to these fast and versatile tools that are finding increasing use in present-day experimental work in almost all fields of science. The write-up of background material and instructions for experiments is lucid and makes special efforts at illustrating the similarity of phenomena underlying various fields of physics. The book is especially recommended to educators in physics to evaluate as to the extent that such unified laboratory courses can be developed in the country, particularly since most of the equipment required are now available indigenously.

O. P. GANDHI

APPLIED INFRARED SPECTROSCOPY edited by D. N. Kendall (Reinhold Publishing Corp., New York), 1966. Pp. xv+560. Price $ 23.00

Physical methods are now so much a part of the professional work of the chemist and advances in the field are so dramatically rapid that a comprehensive volume discussing the practical applications of any one such method would be most welcome to all. Infrared spectroscopy is one such physical tool on whose utility any comment will be just superfluous.

The volume under review is the outcome of the contributions of twenty-one specialists under the leadership of Kendall and they have concentrated primarily on the applications of infrared spectroscopy in specific problems in chemistry and allied sciences. The volume serves admirably well to acquaint the reader with all aspects of infrared spectroscopy, including techniques for preparing samples for spectral screening, principles of instrumentation and spectrophotometer operation as well as the applications of this technique in specific problems of structure determination, assay of chemical purity, identification of unknown materials, and other problems in diverse fields. The concluding three chapters on attenuated total reflectance, microsampling techniques, and use of computers in spectroscopy describe recent developments in the field. The text consists of a very broad but readable account of what can nowadays be achieved by means of infrared absorption spectroscopy.

The book is very well produced and indexed. Each section has an exhaustive list of references to relevant literature and review articles. The book will undoubtedly be found useful by all interested in this tool.

P.C.M.

APPLICATIONS OF MASS SPECTROMETRY TO ORGANIC CHEMISTRY by R. I. Reed (Academic Press Inc., New York), 1966. Pp. ix+256. Price 63s.

Mass spectrometers have been used for many years, particularly for the determination of stable isotopes. During the last two decades they have also been used in the petroleum industry for the quantitative analysis of complex mixtures of hydrocarbons, but it is only during the last five years that extensive use is being made of mass spectrometry for the elucidation of structure of complex organic molecules. This has been made possible by improvements in the design of mass spectrometers which enable the vapour of the organic substance, even relatively nonvolatile, to be introduced into the ion source with comparative ease. The author of this monograph has been one of the pioneers in the application of mass spectrometry to the structure elucidation of organic compounds.

The first chapter gives a brief account of the basic principles on which single and double focusing mass spectrometers are built. In Chapter 2, the essential features of mass spectra are introduced with definitions and explanations of terms special to this field. In Chapters 3 and 4, the fragmentation of hydrocarbons and compounds containing other hetero atoms like oxygen, nitrogen, sulphur, halogens, etc., is discussed with specific examples and broad generalizations are developed as to the possible modes of fission of organic molecules under electron impact. Chapter 5 deals with the application of mass spectrometry in the field of natural products in a wide variety of structures such as terpenoids, alkaloids, glycosides, amino acids and peptides. Chapter 6 deals with the principles employed in the analysis of mixtures. Chapter 7 deals with procedures which can be adopted with various classes of organic compounds in order that structural information can be obtained from the mass spectra. In Chapter 8 is shown how logical arguments based on mathematical principles can be employed in the interpretation of mass spectra.

The organic chemist faced with the task of interpreting mass spectra for the solution of complex organic structures will have to look elsewhere as, for example, Djerassi's monographs for guidance. The number of specific examples discussed in Dr Reed's monograph, particularly in the field of natural products, is small and the discussion of spectra meagre. The book can serve only as an introduction to the field from an author with rich experience of mass spectrometry and as such is to be welcomed.

T.R.G.

THE STATE AND MOVEMENT OF WATER IN LIVING ORGANISMS edited by G. E. Fogg (Cambridge University Press, London), 1965. Pp. vii+432. Price 75*s*.

Though water is a familiar liquid, its properties are anomalous compared to those of substances of the same electronic structure. H_2O is capable of entering into extensive hydrogen bonding with neighbour H_2O molecules. Consequently, water has a highly associated structure. Many of the unique properties of water and aqueous solutions follow from this and are of tremendous significance for biological systems which are composed mostly of water. The object of this symposium was "to bring together physicists and biologists to a critical examination of their concepts of the physical state of water in cells and of mechanisms by which it is transported".

The twenty-two papers presented at the symposium have been grouped into three parts: the Physical background, Water in the plant, and Water in the animal. The first part includes papers dealing with physical aspects, such as structure of water, properties of water in thin films, osmotic flow, etc. It also includes useful tables of physical and chemical properties of water. Apparently, the structure of liquid water is affected by the presence of biopolymers and, therefore, the results with aqueous solutions cannot be directly extrapolated to cellular systems. The second part contains papers dealing with water movement in soils and in various parts of a plant, such as root, stem and leaf. They are concerned with the force responsible for the movement of water and the mechanism(s) by which this occurs. The topics discussed are of both theoretical interest and practical importance. The papers in the third part cover a much wider area, such as water transport across membranes in different types of cells, water absorption and regulation, fluid exchange in the central nervous system, etc.

The papers are written in a clear and comprehensible style, although the material covered in the book spreads over diverse disciplines. One point which struck the reviewer as unusual was that the proceedings of the symposium do not include discussions on individual papers or groups of papers. The inclusion of discussions would have helped non-specialists in the field to have a critical appraisal of the material presented. The get-up of the book is excellent. Both biologists and physical scientists will find the book useful.

M. S. NARASINGA RAO

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

The book records the proceedings of the Plant Phenolic Group symposium held in 1964. In 1964, the group was renamed Phytochemical Group to include subjects other than phenolics and this change was foreseen by the organizing committee and a wider range of subjects were covered. There are 13 contributions written by well-known people in the field. The first chapter on 'Biological aspects of chemical reactions in higher plants' by Burges and Hurst gives a discussion on intracellular location, interaction of metabolites from different organelles, selective permeability of organelle members and biogenesis and evolution of organelles and makes for very interesting reading. The rest of the book is devoted to methods used in the study of biosynthesis (Swain), biosynthesis and regulation of carotenoids (Goodwin), biosynthesis of amino acids (Fowden), cellulose (Preston), lignin (Isherwood), piperidine alkaloids (Cromwell), anthocyanidins and flavonoids (Grisebach), chlorophyll (Lascelles), plant acids (Ranson), nucleosides and carbohydrate metabolism (Barber), and protein biosynthesis (Boulter).

Each field has been reviewed in a masterly fashion and the reader would find it rewarding to read the chapters by Goodwin, Fowden and Swain. In many other areas the contributors have done their best but were obviously handicapped by the slower growth of biochemical knowledge. This is especially so in the field of protein biosynthesis. It should also be pointed out that the reviewers have brought in appropriate information from the microbial field which should stimulate plant biochemists.

Compartmentalization is assuming greater significance in the regulation of metabolism. This is elegantly exemplified in the chapter on the regulation of terpenoids in plants which regulate the production of terpenoid material to suit its particular requirements at various stages of development by (i) segregation of enzymes and (ii) by the comparative impermeability of the plastid membrane to mevalonic acid. Biochemical unity is clearly seen in the review on amino acids by Fowden and also the versatility of plants to make a large number of amino acids not found even in the microbial field. It is unfortunate that adequate attention has not been paid to the biosynthesis of branched chain amino acids — valine, leucine and isoleucine — the pathway of which is now well established in plants by the work of several laboratories in the world.

Any drawbacks in either presentation or coverage may be overlooked in this maiden attempt of the Phytochemical Group and the reviewers deserve praise for the cogent discussions, especially in the biochemically underdeveloped areas. There are hardly any printing errors and the get-up is excellent, but the high price may limit the usefulness of the book. The book should be a valuable addition to any library for postgraduate and research students.

A. N. RADHAKRISHNAN

LABORATORY HANDBOOK OF TOXIC AGENTS edited by C. H. Gray (Royal Institute of Chemistry, London), Second Edition, 1966. Pp. ix+190. Price 24*s*.

The compilation of this handbook was undertaken at the instance of the Royal Institute of Chemistry, London, in 1958, with the idea of preparing a handbook on toxicology providing general information about the toxic hazards associated with the use of familiar chemicals in the laboratories.

It is absolutely essential that science students should be acquainted with the toxic influences of the laboratory chemicals. The need of such a book which could provide exclusive information to the students of undergraduate level about laboratory hazards and their baneful effects on the human life was long felt. This handbook fills this gap in an adequate way. The editor has managed to condense a good deal of information into this slim book. The handbook is divided into five sections. The first section contains the introduction and deals with general principles; the second section describes the precautions and the preventions; the third section dwells upon the first aid facilities in the laboratories; section four, occupying the major portion of the handbook, deals with the poisonous and corrosive gases, reagents and solvents, their harmful effects, and insidious dangers arising from such materials; the fifth section is exclusively devoted to the hazards of handling radioactive substances.

More than 240 poisonous substances are dealt with and their acute and chronic effects are briefly indicated. The first aid suggested in the event of any accident or emergency is restricted in the cases of inhalation, eye contact, skin contact, mouth contact and ingestion. The unique feature of this description is the inclusion of threshold limit value (TLV) for most of the chemicals. It is accepted by the American Conference of Government Hygienists and may act as a guide to planners in using volatile and dusty materials in laboratory operations.

A glossary of technical medicinal terms, used in the text, also appears at the end of the book. A bibliography appears only in the fifth section, where the dangers emanating from radioactive materials are discussed. The names of the toxic agents are arranged alphabetically which has probably obviated the necessity of a separate index. The printing and get-up are excellent.

Considering the size and purpose of the handbook, it makes a highly instructive and informative reading for workers in industrial, research and teaching laboratories. It is a welcome addition, particularly for the teaching profession. The specialized knowledge of toxicity, in addition to its immediate academic value, has also a far-reaching importance in the field of industrial toxicology, which is gaining recognition on account of widespread industrialization in the wake of rapid progress of science and technology. The handbook is bound to find a ready acceptance in the laboratories and the libraries alike.

S. H. ZAIDI

PUBLICATIONS RECEIVED

JUNCTION TRANSISTORS by John J. Sparkes (Pergamon Press Ltd, Oxford), 1966. Pp. viii +249. Price 25*s*.

CONCAST DICTIONARY: ENGLISH, FRANÇAIS, ESPAÑOL, DEUTSCH edited by Max Flick (Concast AG, Zürich), 1966. Pp. 248.

IONOSPHERIC RADIO PROPAGATION by Kenneth Davies (Dover Publications Inc., New York), 1966. Pp. xiv+470. Price $ 2.25

PRACTICAL RECOMMENDATIONS FOR THE DESIGN AND CONSTRUCTION OF PRESTRESSED CONCRETE STRUCTURES (Fédération Internationale de la Précontrainte, London), Provisional Edition, 1966. Pp. 81. Price £ 2

ORGANIC INSERTION REACTIONS OF GROUP IV ELEMENTS by Edmund Yanovich Lukevits & Mikhail Grigor'evich Voronkov (Consultants Bureau Enterprises Inc., New York), 1966. Pp. xii+413. Price $ 25.00

POLLEN GRAINS OF WESTERN HIMALAYAN PLANTS by P. K. K. Nair (Asia Publishing House, Bombay), 1965. Pp. viii+102. Price Rs 16.00

CNS DRUGS — A SYMPOSIUM (Council of Scientific & Industrial Research, New Delhi), 1966. Pp. xv +367. Price Rs 33.00; 66*s*.; $ 10.00

THE CHEMISTRY OF METALLIDES by Ivan Ivanovich Kornilov; translated from the Russian by J. W. Loweberg (Consultants Bureau Enterprises Inc., New York), 1966. Pp. xi+156

LASER CANCER RESEARCH by L. Goldman (Springer-Verlag, Berlin), 1966. Pp. vi+64. Price DM 16

THE DEVELOPMENT OF HIGH-ENERGY ACCELERATOR by M. Stanley Livingston (Dover Publications Inc., New York), 1966. Pp. xi+317. Price $ 2.50

BERYLLIUM TECHNOLOGY: Vols. 1 and 2, edited by L. McDonald Schetky & Henry A. Johnson (Gordon & Breach Science Publishers Inc., New York). Vol. 1, 1966. Pp. xii+678. Price $ 19.00 (paper); $ 35.00 (cloth). Vol. 2, 1966. Pp. xii+679-1255. Price $ 19.00 (paper); $ 35.00 (cloth)

HANDBOOK OF REFRIGERATING ENGINEERING: Vol. 2 — APPLICATIONS OF REFRIGERATION AND CRYOGENICS PROCESSING by W. R. Woolrich (AVI Publishing Co., Westport, Connecticut), 1966. Pp. xi+434. Price $ 13.50 (US); $ 14.50 (foreign)

CLAYS AND CLAY MINERALS edited by S. W. Bailey (Pergamon Press Ltd, Oxford), 1966. Pp. vi +447. Price £ 7

ACOUSTICS: DESIGN AND PRACTICE, Vol. 1, by R. L. Suri (Asia Publishing House, Bombay), 1966. Pp. xxviii+539. Price Rs 50.00

NOTES & NEWS

Anomalous reflectivity of shock waves

As a result of studies made at the Electronics Laboratory, General Electric Co., New York, on the reflection of light from shock waves, the anomalously high reflectivities of shock waves have been attributed to the formation of condensed water droplets (or ice particles) due to the adiabatic expansion caused in the progress of the shock waves. This explanation contradicts the earlier one for this phenomenon, viz. that these reflections are of Fresnel type and are caused by the separation of two regions of different densities by the wave front. Experimental observations showed that the intensity of the reflected light was three to four orders of magnitude greater than that which could be explained on the basis of a Fresnel-type reflection. Hence, it became necessary to find out whether a different phenomenon was responsible for such reflections.

Experimental study of the reflections was made using a set-up described by G. R. Cowan and D. F. Horning [*J. chem. Phys.*, **17** (1950), 1008]. Illumination was by means of a helium-neon gas laser and the shock tube was fitted with a means for controlling the humidity. No reflections were observed when the tube was operated in the normal manner. When the diaphragm was punctured, an expansion wave was initially propagated down the shock tube. Scattering was observed only when the propagation media contained moisture. Further, as the shock wave and expansion wave reverberated in the tube owing to reflections from the closed ends, the moisture alternately condensed and evaporated. Further observations showed that the periodic appearance of scattering could be directly correlated with the fall of pressure below ambient pressure. Hydrodynamic equations were used to calculate the lowest pressure and temperature reached during the expansion phase and the calculated temperature was below the dew point. The intensity of reflections was also consistent with the assumption that the phenomenon was caused by scattering from particles with radii of 1-5 μ. Anomalously high reflectivities have also been reported from regions of the atmosphere where there is air turbulence. It is possible that air turbulence may produce adiabatic expansions as a result of which the temperature is lowered below dew point. The condensed water droplets may be responsible for the observed phenomenon [*Nature, Lond.*, **211** (1966), 625].

A new phase in metal-semiconductor transition in VO_2

Recent observations of NMR and X-ray diffraction with 1°C. interval near T_C (68°C.) made at the Hitachi Central Research Laboratory, Kokubanji, Tokyo, have shown the existence of a new phase designated as the *T*-phase in the metal-semiconductor transition of VO_2, in addition to the already known *S*- and *M*-phases.

The observations were made on powder samples of VO_2 prepared by melt-reduction from V_2O_5 or by sintering V_2O_3 and V_2O_5. In all samples of VO_2, a new NMR pattern different from that of *S*- and *M*-phases appeared in the limited temperature regions near T_C. Also some extra X-ray diffraction peaks were observed in similar temperature regions, showing new lattice spacings of 3·23, 3·13, 1·57 and 2·43 A. The new NMR spectra showed similar features in the line shape to those of the *S*-phase and could be analysed in the same way as for *S*-phase. The *T*-phase seems to be semiconductive because (1) its temperature region lies below T_C, (2) values of ν_Q and η are close to those of *S*-phase, and (3) the sign and magnitude of the paramagnetic shift are in good agreement with those of *S*-phase. The crystal structure of *T*-phase has not yet been determined. The temperature region of the *T*-phase was different for different samples, though the positions of X-ray diffraction peaks and NMR results were the same for all the samples. These features suggest that the temperature region of *T*-phase may be sensitive to impurity concentration or perhaps to inner stress [*J. phys. Soc. Japan*, **21** (1966), 1461].

Promotion of activity of nickel catalysts by germanium support

The catalytic activity of nickel in the decomposition of formic acid is increased manifold when nickel deposited over carefully prepared germanium supports is used. It has been found by R. F. Baddour and M. C. Deibert of the MIT, Cambridge, Mass., that the electronic interaction between germanium and nickel has a strong promoting effect, increasing the catalytic activity by a factor of 1·33-3 for as few as one electron transferred per 10^5 nickel atoms in the catalyst layer [*J. phys. Chem., Ithaca*, **70** (1966), 2173].

In an attempt to demonstrate quantitatively the promoting effect of support-catalyst electronic interaction, a series of Ge-supported Ni catalysts with predictable variation in the magnitude of the electronic interaction were prepared from single crystal germanium samples with varying quantities and types of impurities, and their activities for formic acid dehydrogenation were compared. In view of the fact that any unwanted impurity in the germanium surface may lead to total insulation of the interface with nickel or considerably alter the electron transfer properties of the semiconductor, it is imperative that the preparation of the germanium support as well as the deposition of the thin nickel catalyst surface had to be carried out with utmost care by a procedure which yields clean and reproducible electrical contact between the two materials. Zn, As and Sb were used as dopes in the semiconductor support. Approximately 10 g. germanium chips obtained from single crystals, 1-3 mm. in size, were sealed in the side arm of a crushing apparatus and baked at 450°C. in a vacuum as high as 10^{-8} to 10^{-9} torr, and crushed by stainless steel hammer operated

by a solenoid coil working on pulsed direct current. The crushed powder was held in a quartz irradiating dish over which was decomposed rigorously purified nickel carbonyl vapour; the germanium powder itself was heated with IR radiation while the quartz dish was cooled by air streams. The desired amount of nickel could be deposited from a knowledge of the pressure increase and the known volume and recorded temperature of the system. Part of the prepared catalyst was taken for surface area measurement and the rest used for catalysing formic acid decomposition.

The activities of the catalysts were measured in a single pass continuous-flow reactor to which carefully purified formic acid vapour was fed. The reactor was a vertical glass tube 3 cm. in diameter with a glass frit to support the catalyst and thermo-well above and below to measure the reaction temperature. All kinetic runs were initiated at 240°C. and temperature cycles within 30° of that point were used to determine the activation energy. Following the reaction the unreacted formic acid was removed in cold stream and measured by gas chromatography.

The salient features of the Ge-supported Ni catalysts are: (1) though their activities decrease with time, the decrease is not as rapid as in the case of reduced nickel; (2) for reasons yet unexplained, there is a difference of an order of magnitude in the specific activities of the Ge-supported Ni and ordinary nickel catalyst; and (3) the Ge-Ni catalyst is more pressure sensitive.

Suck-back preventing device

A compact all glass no-return valve which obviates the sucking back of water from the pump into the apparatus, designed at the Central Drug Research Institute, Lucknow, is described. The valve incorporates a 12-15 mm. diam. ground glass disc resting on a 8-10 mm. diam. glass tube cut square, flared, flattened and ground at the edge. The tube leading in from the pump carrying two holes of about 2 mm. diam. near the edge (Fig. 1) is adjusted above the base tube, allowing for a 3-4 mm. clearing,

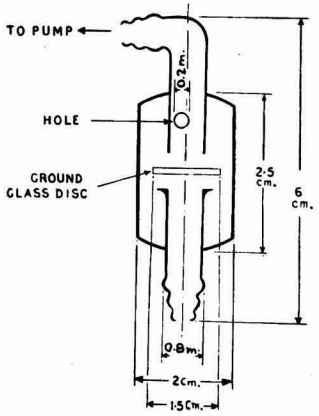

Fig. 1 — The suck-back preventing device

in which the glass disc moves freely. The whole unit may be housed within a 15-20 mm. diam. 2·5 cm. long glass tube which may form a ground-glass joint to permit ready dismantling and clearing. The unit can also be fused to a water vacuum pump. The valve has no corrosion problems.— SARAB DAMAN KUMAR & JASJIT S. BINDRA, *Central Drug Research Institute, Lucknow*

Electroslag refining method

Electroslag refining, a new and effective method of reducing nonmetallic inclusions in steel and producing ingots of uniform metallurgical structure, has been successfully developed up to commercial scale operation by AEI-Berlec Ltd, UK.

The material to be refined is cast into a consumable electrode. The lower end of the electrode is immersed in a pool of molten slag lying at the bottom of a water-cooled mould. The electrode is held vertically by a clamp which also carries the electric current providing the energy of the process, the circuit being completed by a conductor connected to the base of the mould.

The process could be started by introducing an exothermic mixture of divided aluminum and an oxidant at the bottom, lowering the electrode on to it, and switching on the current, when the exothermic mixture is sufficiently conductive to fire; the product of the exothermic mixture is Al_2O_3 which is a common constituent of the slag. Current flows between the electrode tip and the mould base, passing through the slag, which is a conductor with high resistivity. The slag gets superheated and melts the tip of the electrode. The molten metal begins to fall to the bottom of the mould where it is cooled and solidifies to form an ingot by the water-cooled mould base and the walls. The liquid slag, stirred electromagnetically by the current, washes the molten metal, thus effectively refining the metal before it solidifies. The slag pool is carried upward by the growing ingot, leaving a thin skin of solidified slag between the ingot and the wall. The slag is principally made of calcium fluoride, with additions of alumina and calcium oxide. Calcium oxide is especially useful in removing much of the sulphur.

The ESR technique reduces considerably the number of non-metallic inclusions and reduces the size and improves the distribution of any remaining impurities. As the ingot solidifies vertically, the inclusions are distributed more evenly over the entire cross-section, in contrast to normally cast ingots, where the impurity concentration will be more at the central axis owing to ' radial solidification '. Though superior reduction in gas content could be obtained by the vacuum arc method, plants based on ESR technique have the advantage of much lower costs [*AEI Engng*, **6** (1966), 184].

Platinum equipment for better two-ply glass tubing

A novel continuous glass melting furnace for the production of two-ply glass tubing used in sodium vapour lamps, constructed by Johnson, Matthey & Co., London, is claimed to be a major development in the glass industry. The platinum equipment consists of an all platinum-alloy valve placed inside a solidly constructed sheath of platinum-alloy-clad high temperature steel. The equipment is mounted in the base of the glass melting furnace, the outer sheath itself acting as a mandrel to form the outer skin of the two-ply tube. The sodium resistant glass is passed continuously into the

space between the valve and the outer sheath and is drawn through the apparatus to form the inner skin of the two-ply tube. The thickness of the sodium resistant layer is controlled by the valve setting. The equipment is operated continuously at the temperature of the molten glass, the high temperature strength and the high corrosion resistance of platinum ensuring the maintenance of high dimensional accuracy for the finished two-ply tube.

The new furnace has resulted in increased output and better quality than those obtainable by the hand pouring method [*Press Information from Johnson, Matthey & Co., London*].

Selective precipitation flotation process — A new analytical technique

Sebba has given the ' ion floatation process ' [*Ion floatation* (Elsevier Publishing Co., Amsterdam), 1962] in which the ion to be floated (colligand) is precipitated by a surfactant. The latter being in excess is adsorbed on the precipitate particles which are borne to the surface on a gas bubble. The disadvantage with this process is that comparatively more collector is required and often more time is required for complete flotation. Further, the higher solubility of the anionic collector than the cation-collector compound in some cases will result in the floating of the collector itself without removing the colligand. Of course this technique is more economical than the ' foam separation ' [Wallins, C., Ruff, E. E. & Thornton, J. E., *J. phys. Chem., Ithaca*, **61** (1957), 486]. However, attempts to make the process selective, by substituting long chain groups in the molecule of a specific analytical reagent, failed because of the difficulty in preparing such collectors, and the introduction of large groups changed the behaviour of the chelating part of the molecule.

To avoid all these disadvantages, this process has been developed into the precipitate flotation process [Malne, E. J. & Pinfold, T. A., *Chemy Ind.*, **23** (1966), 1299] in which small precipitate particles floating on the surface are collected by using a separate surfactant of charge opposite to that of the ions adsorbed on the surface of the particle, the mechanism being coulombic attraction between the adsorbed and surfactant ions. Separations of the hydroxides of elements like Fe, Cu, Zn can also be achieved by controlling the pH and this can be further extended to the flotation of other well-known inorganic precipitates (Sr can be floated with dodecylpyridinium chloride and separated from Cs) as well as organic precipitates (Pd and Ni dimethyl glyoxime can be floated with a cationic collector and separated from Co).

Another important development is that under certain conditions precipitate flotation can be achieved even without any surfactant at all (Pd nioximate). This process is powerful in that little surfactant is required and complete flotation and separation is done in a few minutes.— A. L. J. Rao, *Department of Chemistry, Punjabi University, Patiala*

Abstracts of Mycology

This new monthly journal being started on a 3-month trial run through a grant from the National Science Foundation is to serve as a prototype for computer-selected information tools. The journal will carry abstracts dealing with fungi, sorted out by a computer from the total content of *Biological Abstracts*.

Pertinent abstracts will be selected editorially as an integral part of the analysis that routinely produces the computer-composed Biosystematic Index to *Biological Abstracts*. From the abstracts appearing in each issue of *Biological Abstracts*, those dealing with fungi will be sorted out by the computer, to be repackaged as a separate publication. Annual subscription for the journal is $ 30.00.

Regional Research Laboratory, Hyderabad

Notable among the achievements of the laboratory reported in its annual report for 1965-66 are methods for the preparation of odd chain fatty amine N-oxides from fatty acids through a direct route, fatty oil hydrogenation catalyst from nickel formate, a catalyst for the conversion of benzene into cyclohexane at atmospheric pressure, and a yellow pigment of barium potassium chromate. A prototype plant with 2 tonnes a day capacity for producing various grades of active carbons was set up during the year.

Sulphate compounds have been prepared from castor oil via the compounds carrying primary and/or secondary hydroxy groups, and tested for surfactant properties. Compounds with both central and terminal sulphate groups have been found to show good surfactance with particularly good wetting power.

Thin layer chromatographic (TLC) methods have been developed for the detection of castor oil, and kusum oil in other oils and vegetable oils in ghee. During the course of periodate-permanganate oxidation studies on oleodisubstituted and monosaturated dioleoglycerides, methods have been developed and standardized to separate, on basic alumina TLC plates, the following compounds: di- and triglycerides from a mixture of fatty acids, monoazelao disaturated glycerides and monoglycerides, monoglycerides from the acidic compounds, and methyl oleate and triglycerides from a mixture of diglycerides, monoglycerides and acidic compounds.

The fatty acids of castor seed phospholipids, free of glycerides, have been isolated after saponification with 0·5N potassium hydroxide and converted into methyl esters. The examination of methyl esters had revealed the presence of palmitic, stearic, oleic, and linoleic esters as the major constituents.

In an attempt to find substitutes for tung oil, urethone oils have been prepared by reacting diglycerides corresponding to monomeric DCO with toluene diisocyanate. Varnishes based on these products compared favourably with tung oil varnishes. Stand oils of various viscosities have been prepared both from total refined sardine oil and from the liquid fraction of sardine oil. In drying properties these oils were found to be inferior to linseed stand oil.

A standard method has been developed for the quantitative estimation of the components in mixtures of synthetic glycerides

and glycerolysis products during alkyd resin manufacture by TLC techniques.

Resole type phenol-formaldehyde and Novolak type of resins have been prepared from styryl phenol and mixtures of *p*-cresol and 3-pentadecylphenol using basic catalysts (sodium hydroxide, ammonia, etc.) for the former and acid catalysts (oxalic and sulphuric acids) for the latter. The resins are being evaluated for their physical characteristics.

Air drying coatings with satisfactory resistance to water, dilute acid and alkali, and some organic solvents have been produced from cashewnut shell liquid residue by styrenation in the presence of sulphuric acid catalyst, followed by reaction with hexamine. A black enamel of satisfactory hardness, gloss and adhesion has been prepared from the shell liquid residue.

During studies on the mechanism of prevention of metallic corrosion of articles immersed in water, by paints prepared from linseed stand oil and alkyd media it has been observed that chromate pigments provide chemical inhibition in the beginning, but in due course barrier type of inhibition comes into play. Basic lead sulphate, iron oxide, barytes and carbon black are found to provide resistance inhibition, whereas white lead provides chemical resistance.

Different benzylamines, β-phenylethylamines, 1,2-diphenylethylamines, 4(3H)-quinazolones, *N*-aralkylanthranilic acids, β-phenylpropionamides, and 2-phenoxy acetyl and ethyl benzimidazoles have been synthesized and are being tested for pharmacological activity.

Eight 2,3-dihydro-1,4-benzoxazepin 5(4H)-ones, with substituents in the aromatic ring have been synthesized by Schmidt rearrangement. Based on the participation of the NH proton in spin-spin coupling, a method has been proposed for quickly distinguishing between the 1,4- and 1,5-benzoxazepin-ones.

Cracking of low temperature carbonization tar vapour at 600°C. has found to yield a gas with higher content of unsaturated hydrocarbons and calorific value.

Both methyl isobutyl ketone and butyl acetate have been observed to have the same efficiency for the extraction of tar acids from ammoniacal liquor, while di-isopropyl ether has been found to be a poor extractant.

A quantitative TLC method has been standardized to follow the progress of the chlorination of xylenols. Chlorination of 3,4-xylenol using trimolar proportion of *tert*-butyl hypochlorite has yielded three compounds, viz. tri-, di- and monochloro, 3,4-dimethyl cyclohexadien-ones. A mechanism based on the active participation of chlorinium ion was suggested for the chlorination.

Studies were continued on the mechanism, preparation and development of catalysts for cracking, reforming, ammonia synthesis, shift reaction, hydrogenation, desulphurization, polymerization of ethylene and direct conversion of ethanol to ethyl acetate. Bleaching earths from Mysore and Andhra Pradesh have been characterized and their utility is being tested for the bleaching of vegetable oils.

Pilot plant studies are in progress on a number of processes. These include benzyl alcohol and benzyl acetate from benzyl chloride; benzoyl chloride by direct hydrolysis of benzotrichloride and synthetic tanning agents based on naphthalene.

Announcements

▪ *A Symposium on Design Philosophy and Its Application to Precast Concrete Structures* will be held during 22-24 May 1967 in Church House, Westminster, under the auspices of the International Association for Bridge & Structural Engineering (IABSE). There will be four technical sessions: (i) Basic criteria concerning overall stability; (ii) Special design problems like loads and forces, joints, and effects of shrinkage and creep; (iii) Construction problems and the use of special materials; and (iv) Recommendations for codes of practice, tolerances and standardization. Further information can be had from the Cement & Concrete Association, 52 Grosvenor Gardens, London SW1.

▪ *The Fifth International Photobiology Congress* will be held during 26-31 August 1968 at Dartmouth College in Hanover, New Hampshire, USA. Sponsored by the Comite International de Photobiologie and organized by the Committee on Photobiology of the US National Academy of Sciences, this quadriennial meeting will discuss the following topics: photochemistry, photodynamic action, photosynthesis, bioluminescence, photochemistry of macromolecules, photodermatology, vision, instrumentation and action, and repair mechanisms. Further information can be had from the Secretary, Indian Photobiology Group, Chittaranjan National Cancer Research Centre, Calcutta 26.

▪ *At the Fourth Annual Meeting of the Indian Geophysical Union* held at Hyderabad on 5 January 1967, the following office-bearers were elected for the period 1967-1969: Dr S. Bhagavantam (President); Drs V. A. Sarabhai and N. K. Panikkar (Vice-Presidents); Dr S. Balakrishna (Secretary); and Drs M. S. Krishnan, K. R. Ramanathan, Hari Narain, V. Bhaskara Rao, S. N. Sen Gupta and M. Krishnamurthy (Members).

▪ *A Summer School in High Voltage Laboratory Techniques* is being conducted at the Department of High Voltage Engineering, Indian Institute of Science, Bangalore, from 8 to 20 May 1967. The course will cover basic principles of high voltage production measurement and testing techniques in 26 lecture hours supported by appropriate experiments and demonstrations in 31 hours of laboratory practice. The tuition fee for the course is Rs 500. Further particulars regarding admission, hostel accommodation, etc., can be had from the Department.

▪ *The Eleventh Indian Standards Convention* will be held during 25-30 Sept. 1967 at Chandigarh. Intending participants should write to Shri Kavaljit Singh, Organizing Secretary, Eleventh Indian Standards Convention, Manak Bhavan, New Delhi 1, for detailed particulars regarding the convention.

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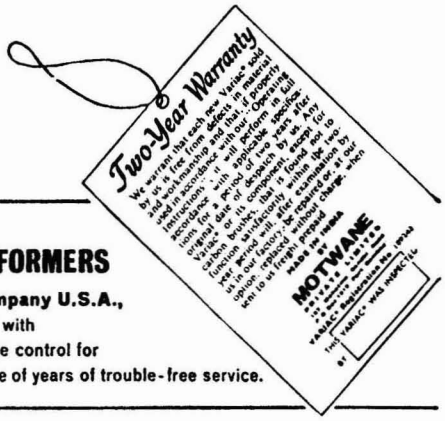

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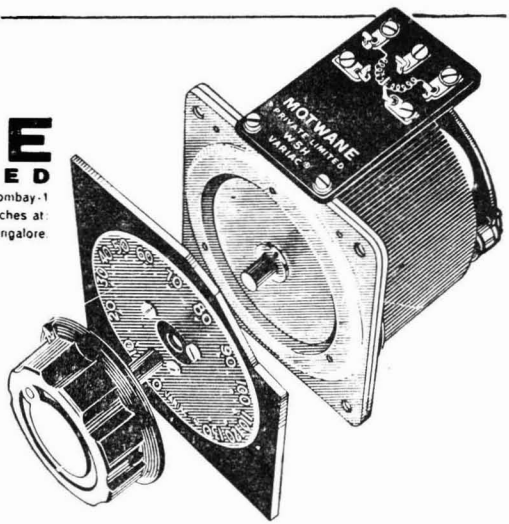

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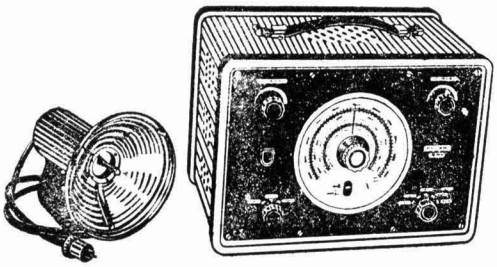

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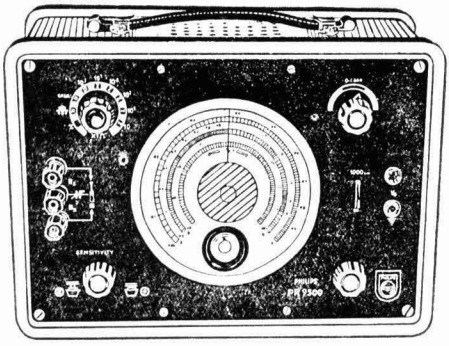

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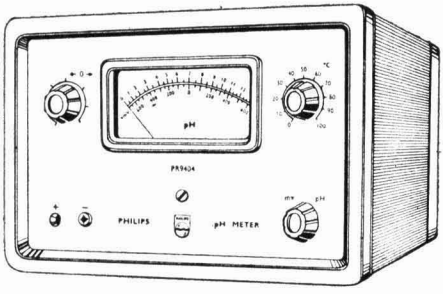

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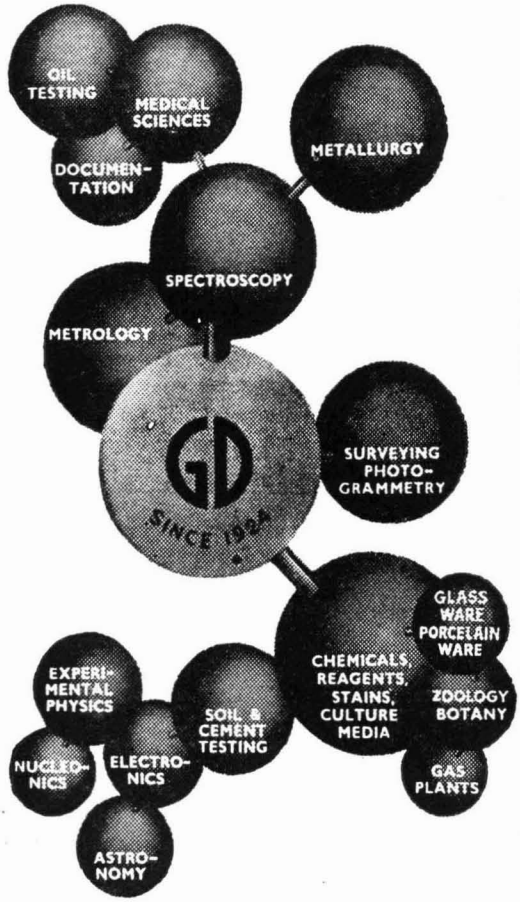

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