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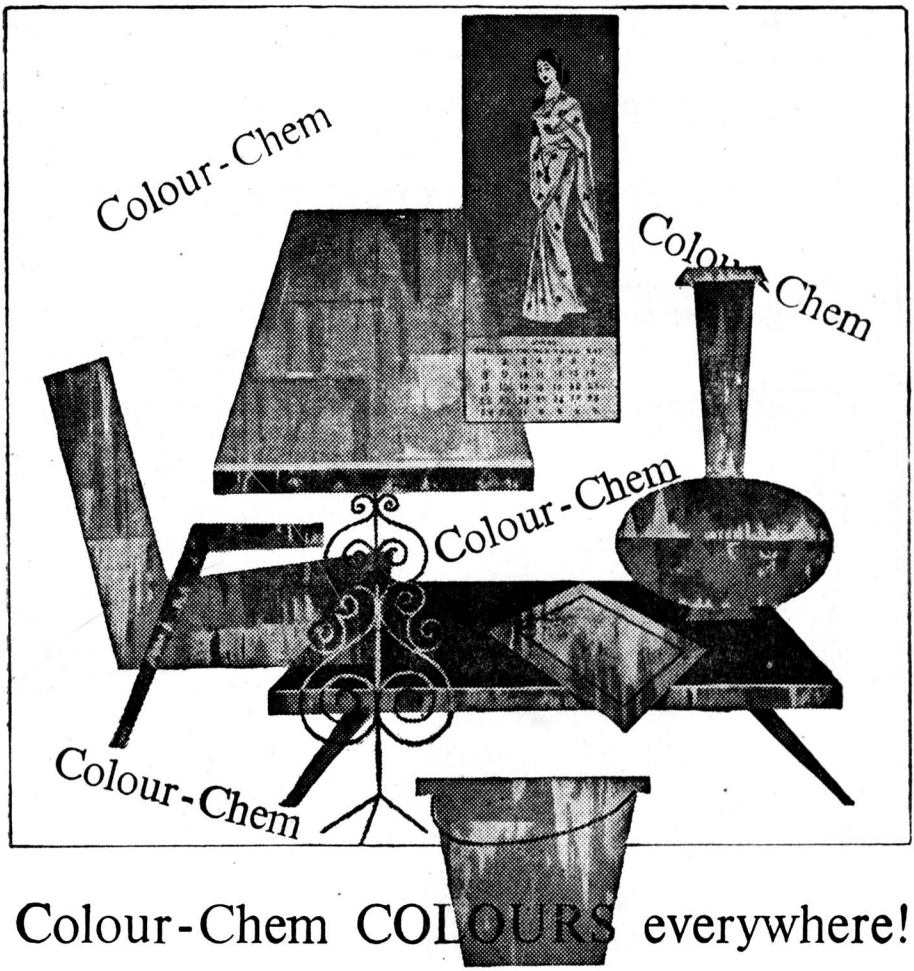

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INDEX TO ADVERTISERS

SUPPLEMENT

to

Glossary of Indian Medicinal Plants

by

R. N. Chopra, I. C. Chopra & B. S. Varma

In the year 1956, the Council of Scientific & Industrial Research, New Delhi published a Glossary of Indian Medicinal Plants with a view to presenting concise information regarding their properties, uses and important constituents. Over 2600 species, belonging to about 1350 plant genera, have been dealt with. The information is given under the botanical names of the plants, which are arranged in their alphabetical sequence; trade and vernacular names are also mentioned. The Glossary gives distribution of the plants, diseases for which the particular plant is used, and the active principles. Adequate literature references to the sources of information are also provided. The book ends with two comprehensive indexes: one pertaining to the vernacular and trade names, and the other to the chemical constituents.

In order to bring the Glossary up to date, this Supplement has been brought out. It follows the style of the Glossary and covers all relevant information published during the period 1955-64. The Supplement provides additional information on over 700 species already mentioned in the Glossary, and includes about 380 new species. Indexes covering additional vernacular and trade names and chemical constituents have been provided. The Supplement, like the original Glossary, will be useful not only to the practitioners of indigenous system of medicine, but also to all others who are interested in drugs of vegetable origin and common bazaar medicines.

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Man on the Moon

V. G. BHIDE
National Physical Laboratory, New Delhi

FOR millions of years, the airless, soundless satellite we call the moon has coursed an elliptical path around the earth — mysterious, exciting, always presenting the same face to the mother planet. The moon has excited the curiosity of man since the birth of human civilization and has been alluded to in the literatures of the world. The age-old dream of reaching the moon has been dramatically realized on 21 July 1969 when two American astronauts, Commander Neil A. Armstrong and Edwin A. Aldrin, set their feet on the moon's surface. The insatiable urge of the human being to explore the unknown and search the truth has reflected itself several times and in several forms in the history of human civilization. We have had Columbus sailing the uncharted seas discovering America in the hope of discovering India, Wright brothers flying for the first time, Admiral Peary's exploration of the North Pole, Sir Edmund Hillary and Tensing's conquest of the Mount Everest, Admiral Byrd's exploration of the Antarctic, and so on. However, the trip to the moon is without parallel — no other voyage of discovery, no previous travel of any kind is comparable to the Apollo 11 mission which landed man in flesh and blood on the surface of the moon. It was a journey unequalled in complexity and sophistication. Armstrong very significantly said while putting the first step on the moon, " That is one small step for a man, one giant leap for mankind ". Indeed, the story of exploration of space starting with the first flight of Wright brothers and ending, at least for the present, with the landing on the moon has ever been so fascinating and typifies the growth of science and technology over two centuries. During the meandering course it has taken over the years, it has crossed several milestones, such as the discovery of propellant engines, V_2 rockets, launching of the Sputnik by the Russians in 1957, subsequent Luna flights, series of Gemini and Mariner flights and then the Apollo series. The adventures of Columbus and Wright brothers were essentially individual efforts. In contrast, the Apollo mission is the culmination of the activity of literally innumerable men and women from different walks of life and typifies the change science and technology has undergone over the years.

In its execution, Project Apollo 11 drew heavily from earlier experiments. Several lunar probes were made and nearly a million photographs of the moon's surface were taken. All this helped in planning the mission, but there were no precedents for a man landing on the moon and this lent thrill and excitement to the mission. Throughout the Apollo 11 mission there were a number of breath-taking events and critical stages. Some of these are liftoff, parking orbit, translunar injection, orbiting round the moon, separation of the lunar module from the command module, soft landing of the lunar module at the desired spot on the lunar surface, getting off of the astronauts on to the surface of the moon and collecting valuable scientific data and precious lunar material, liftoff from the moon's surface, rendezvous of the lunar module with the command module, the return flight and finally the splashdown at the desired place in the Pacific. All these were executed with remarkable precision and perfection. Every stage followed the other at the appointed time, so much so that one could predict the stage of the mission with as much accuracy as one could predict the time of the sunrise and sunset here on earth. A voyage of such epic dimensions required every iota of astronaut's attention and skill. The process of checking, rechecking and monitoring the spacecraft was endless. Communication with earth was continuous. Although this achievement did not involve any epoch-making scientific discovery, it did imply remarkable technological perfection and collective activity. The level of equipment reliability has been pushed to a phenomenal degree. In the past, any process which achieved 99·9% reliability was considered perfect. But this percentage is too low for the exacting demands of man's space flight. The Apollo moon rocket that stands on the launch pad contains some five million parts. With the conventional reliability factor of 99·9%, on the average some 5000 parts would fail during the flight and this would be intolerable and even catastrophic. Space planners called for 99·999% reliability. As it turned out, in the Apollo 8 mission round the moon last Christmas, only five parts of non-critical nature failed to function. The reliability factor in this venture was 99·9999%. This near perfection of technology is among the tangible benefits of space programme. Since the space environment is so foreign to previous human experience, the questions as well as answers were new. The needs were great: better sensors, better computers, better sources of power, better knowledge of extreme heat and cold, better knowledge of high and low pressures, better understanding of man's physiology, better and perfect materials and alloys, etc. The honeycomb aluminium used in Apollo's inner critical

compartment, for example, is 40% stronger and 40% lighter than ordinary aluminium. The miniaturization and subminiaturization of almost perfect reliability has been developed. Indeed President Nixon very significantly said, "In probing wide horizons of space, we have discovered new horizons on the earth". This has an added dimension in the sense that it involved not only perfection in one process and industry, but accuracy, perfection and reliability were expected in all the industrial units which participated in the programme.

This was indeed a national programme and a national achievement. President John F. Kennedy during his address to the joint session of the US Congress on the urgent national needs said, "I believe that this nation should commit itself to achieving the goal, before this decade is out, of landing a man on the moon and returning him safely to earth". The goal set forth by President Kennedy did not imply one man going to the moon. He predicted with remarkable accuracy when he said, "If we make this judgment affirmatively, it will be an entire nation. All of us must work to put him there." Since that time, the Apollo mission has been spread across the length and breadth of United States covering every state and reaching into remote towns and hamlets. It is America's greatest national endeavour and outstanding example of cooperation between public sector, private sector, universities and government laboratories. The development of Saturn 5 rocket, the most powerful ever built, capable of generating 3,400,000 kg of thrust of power, so enormous as to be inconceivable, involved contracts with several gigantic industrial firms including Boeing, Douglas Aircraft, North American, International Business Machines, etc. Since human astronauts are an integral part of the control system, it was necessary to know how far they can reach, how much endurance they have and what jobs they can do better than machines. Much of this vital information was supplied by the universities. "Without the help of universities", NASA officials said, "this programme would be severely handicapped." From the universities came most of the ideas for experiments to be conducted in space and many of the instruments were developed in the university laboratories. The success of Apollo 11 mission is accounted for by the unique collaboration between government, industry and the universities. Perhaps a similar national goal in India would stimulate collective activity and bring forth the best that we are capable of.

Apart from the spin-off of the space flight in terms of the technological boost, the mission has some direct benefits. The moon's chief immediate resource is knowledge. Written upon the face of the moon is much of the history of the solar system — a record steadily erased from the surface of the earth by erosion, the laying down of sediments, etc. On the moon are craters, some small and some very big. They seem to be relics of an early period when the solar system was a dangerous place teaming with bodies in erratic orbits that, from time to time, crashed on the earth, moon, Mars and probably inner planets. By dating these craters it should be possible to estimate the rate of such impacts. Alternatively, these craters may be due to volcanic eruptions in the moon. The seismograph placed by the astronauts on the moon surface will make a record of the volcanic activity.

Similarly, on-the-spot study of the rocks of the moon would indicate whether moon itself is a giant asteroid that came close enough to the earth to be captured in orbit around this planet. The moon seems a stranger to this part of the solar system. The average density of the material of which it is made is considerably lighter than that comprising the inner planets, Mercury, Venus, Earth, Mars. The outer planets, Jupiter and others contain far more of the lighter elements. Does this mean that moon is intermediate — a child of the asteroid belt ? If so, we would have an asteroid close at hand for scientific study.

There are other theories for the origin of the moon. It has been proposed that moon was torn from the upper layer of the earth, or it is said the moon may have accumulated material left in orbit around the earth after earth was formed. However, scientific debates have cast doubts on both these views. This problem will be solved from an analysis of moon's rock. This study will throw light on the origin of the moon and the earth.

While the moon will obviously be used for a wide variety of scientific observations which cannot be made on the earth, none will be probably more important than those with radio telescope. Radio astronomy is one of the fastest growing sciences. The spectrum of radio waves is enormously broad and much of it is denied to antennas on earth because of the shielding effects of the atmosphere. Nevertheless, in recent years, the radio whispering of the universe has told us of the wondrous things — of violent events taking place within our milky way and other galaxies and regarding giant quasars which are astronomical objects of unknown nature emitting vast amounts of energy in the form of radio waves. Not only does our atmosphere obscure much that is going on in space, but man-made radio signals are making it more and more difficult to listen to the faint emission from afar. Observations on the far side of the moon would be shielded from such interference and their protection would be permanent. Furthermore, the moon's orbital flight would enable such antennas to scan the entire sky during one month. The giant antennas used in radio astronomy could be built far more lightly on the moon because there is less gravity and no wind.

Further, the environment of the moon is so different from that of the earth that minerals unknown on the earth should be found there. The moon has virtually no atmosphere; in fact, its vacuum is more nearly complete than any achievable in most laboratories. Its surface materials have, therefore, not oxidized as some of the earth rocks have. They have been subjected to millions — or even billions — of years of intense radiation from the sun unshielded by any air blanket. All these factors must have produced substances with properties foreign to our landscape. The moon may thus open new vistas in chemistry and mineralogy. As our knowledge of chemistry, metallurgy

and solid state electronic devices becomes more advanced, it is likely that many industrial processes will emerge that must be carried out in deep vacuum. On the moon that would mean out of doors and some have proposed that moon may become the home of specialized industries. As has been mentioned, the moon may have minerals unknown to earth. Even preliminary examination of lunar material brought by the astronauts has shown that lunar rocks contain a considerable proportion of titanium. Similarly, it may have raw materials whose store is fast depleting on earth.

What if the lunar rocks prove rich in material useful for technology? Can it be shipped to earth to augment our depleting resources? Some of those who look far beyond believe such shipments will be feasible and economical. They note that the difficulty of lifting heavy payloads from the earth to the moon is no indication of what it would cost to reverse the process. It takes about 50 tons of fuel to drive one ton of cargo from the earth into the orbit. This is partly because the gravity of earth is strong and partly because the spacecraft has to fight its way through the atmosphere. The moon, on the other hand, has much less gravity and no air. Whereas the velocity needed to escape from earth's gravity is 25,000 miles/hr, the escape velocity from the moon is only one-sixth of that. The earth's atmosphere places an additional constraint on determining the most economical launch procedure. It demands that one should get through the atmosphere as fast as possible. In other words, one must go straight up. On moon, it would be possible to launch spacecraft almost horizontally using a catapult system on rails. Such a vehicle would not need to carry its own propellant any more than a railway freight car.

It has been estimated that nearly 33 million dollars were spent in putting man on the moon. Naturally, the question arises whether the meagre scientific information one hopes to get and the possible exploitation of lunar resources justify such an enormous expenditure. Such questions are always asked and they were asked when the Russians launched the first Sputnik. No one hardly realized then that those Sputniks will within a few years serve to give weather forecasts, enable communication over distant parts of the earth via telstar, etc. Maybe the landing on the moon will in the years to come unfold benefits which we presently cannot foresee. Some have argued that one could have used this money to solve some of our immediate problems on earth. It may be realized that in advanced countries, the economy is intimately tied to defence. If this huge amount of money was not spent on sending man on the moon, it would perhaps have gone in for the arms race. Indeed after the space programme started in right earnest the feverish arms race between the two super powers has cooled off to some extent. In spite of all the arguments which may justify colossal outlay on such prestigious programmes, the fact remains that we on earth face far more challenging and acute problems, which need greater priority and immediate attention, such as removal of poverty, hunger, want and disease for millions of inhabitants on this planet. It is only through international cooperation and understanding we may face these challenges and make the inhabitants of this planet happy and satisfied.

Nuclear Physics & Solid State Physics Symposium

A Symposium on Nuclear Physics and Solid State Physics, organized by the Department of Atomic Energy, will be held at the University of Roorkee, Roorkee, during 26-29 December 1969. The last date for submitting abstracts of papers intended for presentation at the symposium is 20 October 1969. Further details may be obtained from the convener, Dr N. S. Satya Murthy, Nuclear Physics Division, 2-130H, Modular Laboratories, Bhabha Atomic Research Centre, Trombay, Bombay 85 AS.

Prof. D. N. Wadia—Obituary

IN the passing away of Darashaw Nosherwan Wadia on 15 June 1969, India has lost a geologist of eminence and the Geological Survey of India, one of its oldest and ablest representatives.

Born on 23 October 1883 at Surat in Gujarat, Dr Wadia had his education at Surat and Baroda and he obtained B.Sc. and M.A. degrees from the Bombay University. As there were no courses in geology at the Bombay University in those days, Wadia was essentially a self-taught geologist, who since independence of India became a legendary personality receiving such distinctions and honours which no other Indian geologist ever got. In the words of Dr W. D. West, former Director of the Geological Survey of India, Wadia " by his scientific achievements and by the authority of his counsel, stood like a giant, respected and revered, dominating the geological scene in India ".

Dr Wadia began his career in 1907 as a teacher in geology at the Prince of Wales College at Jammu in Kashmir where the surrounding mountains provided materials for his geological researches, while he drew inspiration from C. S. Middlemiss, formerly of the Geological Survey of India and a doyen among Indian geologists. It was during his tenure as a teacher at the Prince of Wales College that he wrote his *Geology of India for Students* which won him international reputation.

In 1921 Dr Wadia was appointed as an Assistant Superintendent (now designated as Geologist) in the Geological Survey of India and in the same year he presided over the Geology Section of the Indian Science Congress. In the Geological Survey of India, he continued his work on the north-west Himalayas in Kashmir, Hazara and adjoining areas. Here he carried out his most distinguished and monumental work by unravelling the syntaxis, i.e. the acute knee-bend of the Himalayas around the Nanga Parbat, which displayed his brilliant original thinking and great physical stamina. For this work Wadia was awarded the Back Award of the Royal Geographical Society and the Lyell Medal of the Geological Society of London.

Among Wadia's contributions to economic geology, mention may be made of his discovery of the Joya Mair structure in the Jhelum district, now in West Pakistan. The structure was described as suitable for commercial oil accumulation and when drilled it proved to be an oilfield as anticipated by Dr Wadia.

On retirement from the Geological Survey of India in 1938, Dr Wadia was appointed Government Mineralogist in Ceylon and he contributed to the knowledge of the physical, structural and economic geology of the island. His paper " The three superposed peneplains of Ceylon " published in 1943 in the Records of the Department of Mineralogy, Ceylon, is a classic.

On his return from Ceylon in 1945, Dr Wadia was appointed Mineral Adviser to the Member in Charge of Planning and Development in the Viceroy's Executive Council. He was instrumental in the setting up of the Indian Bureau of Mines, of which he became the first Director. In 1948, he was appointed Geological Adviser to the Department of Atomic Energy, which post he held till his last day. As Geological Adviser he was responsible for the establishment of the Atomic Minerals Division of the Department of Atomic Energy. The success this Division achieved in discovering a workable deposit of uranium in Singhbhum, Bihar, which is now being commercially exploited, was mainly due to his guidance and untiring efforts.

Besides general, structural and economic geology, Dr Wadia made significant contributions to geomorphology, soil geology, Pleistocene geology and vertebrate palaeontology. He had the rarest combination of maturity of judgement coupled with " an ever youthful mind looking ever forward ". As pointed out by L. Dudley Stamp, "it was he who noted the neglect of soil science in India and by his own writings pointed the way; it was he who realized how geography bridged the gap between the physical sciences, specially geology, and the humanities, and regretted its slow progress in India ".

Dr Wadia also influenced the progress of science in India through his addresses and writings. Thus, in his address to the National Institute of Sciences on the eve of Independence in 1947, he urged the importance of the utilization of scientific knowledge " in a dynamic, scientifically directed effort to uplift the whole structure of Indian social, industrial and economic life ". If this were done, then, according to him, India's " magnificent manpower, so far latent, or utilized to a bare fraction of its potential, applied to its great agricultural, water, forest and mineral resources, will lift the country out of its present abnormal economic and industrial depression and put it on the high road to progress and the welfare of its millions ". To this end, he advocated close cooperation between the scientists of India and those of the developed countries for mutual benefit.

It was only after his retirement from the Geological Survey of India that Dr Wadia held the distinguished offices of the President of the Indian Science Congress (twice), President of the National Institute of Sciences of India, of which he was a founder member, President of the Geological, Mining and Metallurgical Society of India, President of the Mining, Geological and Metallurgical Institute of India, and President of the Geological Society of India. He was elected a Fellow of the Royal Society, London, in 1957 and a much coveted honour was conferred upon him in 1963 when he was made the first National Professor in Geology. He presided over the Twenty-second Session of the International Geological Congress held in India in 1964.

Apart from the Back Award and the Lyell Medal, Wadia was the recipient of the Padma Bhushan from the Government of India, the Meghnad Saha

Medal of the National Institute of Sciences of India, and the P. N. Bose Memorial Medal of the Asiatic Society of Bengal.

The Council of Scientific & Industrial Research (CSIR), since its inception, had the benefit of his valuable advice and assistance on matters relating to the development of earth sciences and research in this field. He was closely associated with CSIR in several capacities: as Member, Governing Body and Board of Scientific & Industrial Research for several years; Chairman, Geological and Mineralogical Research Committee; Chairman, Indian National Committee on Oceanic Research; Chairman, Executive Council, National Institute of Oceanography; Member, Executive Council, National Geophysical Research Institute, Hyderabad; Member, Judging Committee for S. S. Bhatnagar Memorial Award; and Founder Member, *Journal of Scientific & Industrial Research.*

Dr Wadia was held in high esteem by geologists all over the world. His long and devoted service to the furtherance of earth sciences in India should be an example and a source of inspiration to future generations of Indian geologists.

Dr Wadia is survived by his second wife, Mrs Meher Wadia. Mrs Meher Wadia had been an ideal wife, whose care and devotion contributed much to his long and active life. Mrs Wadia acquired knowledge in geology from her husband and wrote *Minerals of India,* a popular book published by the National Book Trust, India, in 1966.

A. K. DEY

The Total Synthesis of an Enzyme

P. N. RANGACHARI
National Chemical Laboratory, Poona 8

THE rapid advances in the techniques of isolation, purification and sequence determination of proteins and polypeptides have stimulated organic chemists to devise new ways for the syntheses of these biologically important macromolecular compounds. The continuous development and refinement of classical methods of peptide synthesis has, in the last decade or two, resulted in the synthesis of a large number of pure natural peptides of complex structures. Mention may be made here of the synthesis of the peptide hormones, oxytocin, vasopressin, angiotensin, bradykinin, eledoisin, gastrin, ACTH, secretin and insulin. But these are all peptides with a molecular weight less than 10,000. The year 1969 has witnessed the synthesis of the enzyme ribonuclease with a molecular weight of 13,700 and this is the first 'true' protein ever synthesized. This achievement in the synthesis of an enzyme has opened the path for studies of how enzymes function as catalysts; it is now possible to modify the composition and structure of an enzyme and thus determine how it affects the catalytic properties.

The well-known structure of pancreatic ribonuclease A is given[1-5] in Fig. 1 to give an idea of the complexities involved in the ultimate synthesis of a long polypeptide chain of 124 amino acids and containing 4 disulphide bonds. It is already known from the work of Richards and his collaborators[6-9] that RNase A can be cleaved at a single bond without loss in enzymic activity to produce RNase S; this can be fractionated to give two fragments called the S-peptide (having the 1-20 amino acid sequence of the original RNase) and the S-protein (having the 21-124 sequence). Both these fragments do not individually show any enzymic activity, but when combined together in equimolar ratio exhibit full enzymic activity (RNase S'). This offers now the possibility of synthesis of the individual fragments or the entire polypeptide of 124 amino acids.

The synthesis has been simultaneously reported by two teams, Gutte and Merrifield[10] at the Rockefeller University and Denkewalter and his collaborators of Merck, Sharp and Dohme[11], but they have adopted entirely different techniques to arrive at the same result. The strategy employed by the two groups is given below in outline and the details can be obtained from the original papers.

Merrifield has employed the revolutionary process for peptide synthesis[12,13]— the solid phase method — which he has been developing and perfecting for the last ten years for the assembly of long chains of amino acids into protein molecules. This new approach to polypeptide synthesis is based on the idea of growing a peptide chain in a stepwise manner while it is anchored to an insoluble solid support. The peptide chain is at all times during the synthesis firmly and covalently linked to a solid particle and is, therefore, completely insoluble in all the reaction solvents. The reactions are all heterogeneous ones in which the insoluble peptide chain reacts with the soluble reagent. The main advantages in this new technique are: it is possible to separate efficiently the peptide chain from the starting materials and byproducts by simple filtration and thorough washing; the time required can be considerably decreased; high yields can be obtained because excess of soluble reagents are employed to force the reaction to completion and losses due to solubilization of intermediate products is negligible. Racemization is not detectable by very sensitive tracer methods. The general reaction scheme for solid phase peptide synthesis is given below. For a supporting solid, a copolymer of 98% styrene and 2% vinyl benzene in the form of small

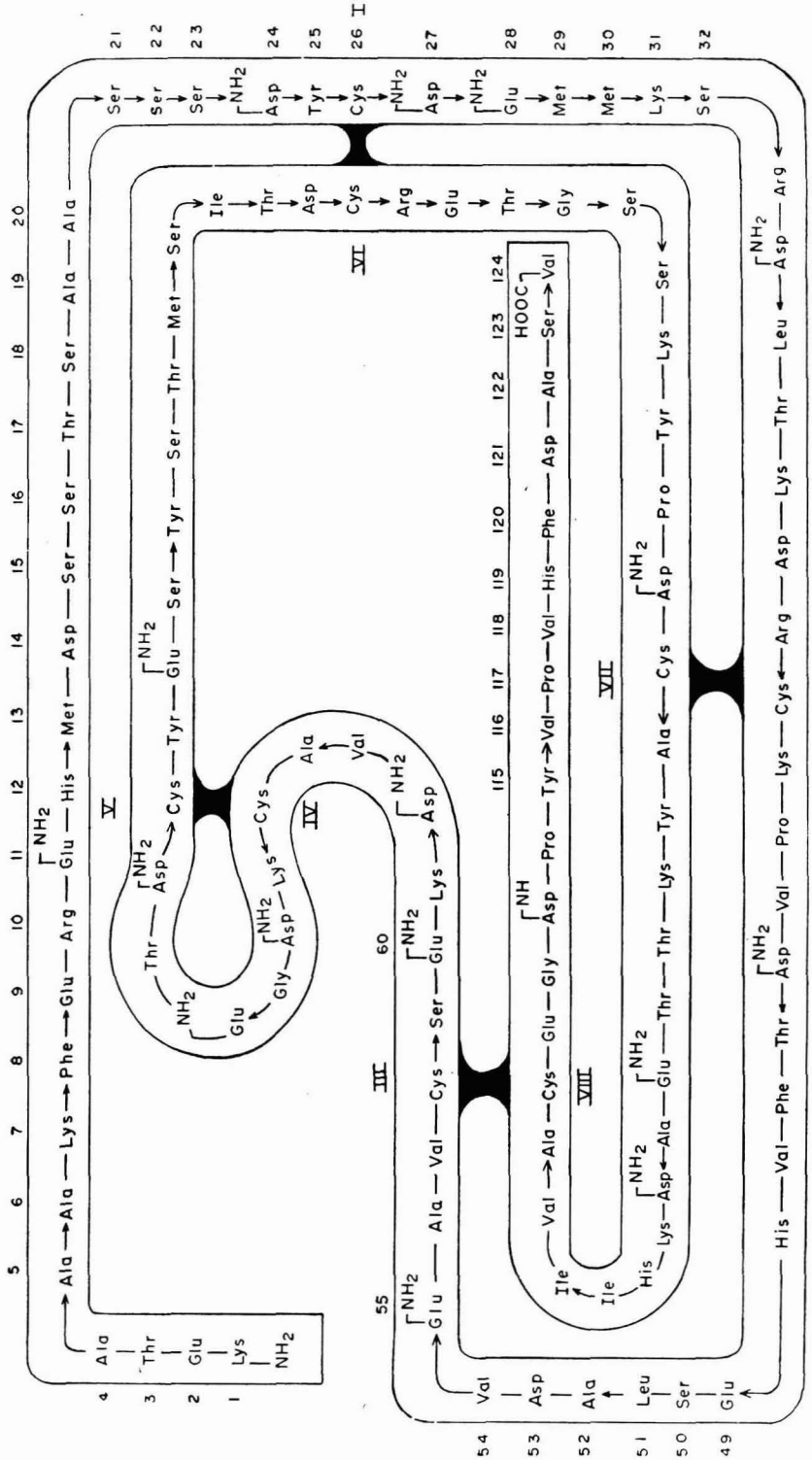
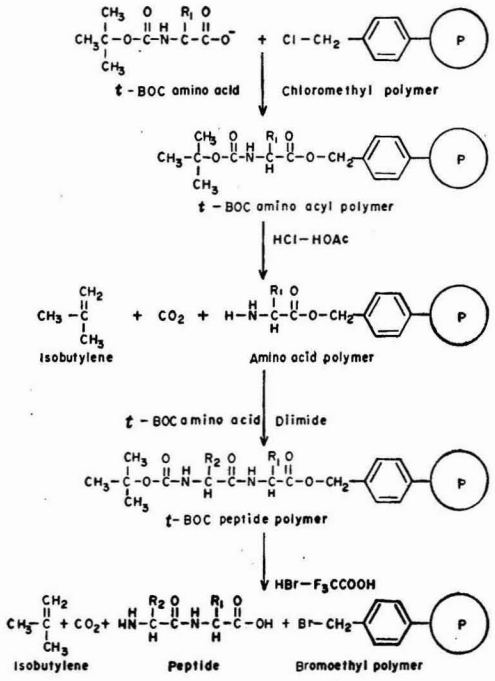


Fig. 1 — The covalent structure of ribonuclease A

beads (20-80 μ) is used. The aromatic rings are chloromethylated to provide functional groups on the resin, but only 10-15% of the rings are substituted to prevent overcrowding of the resin with peptide chains. The groups are not limited to the surface but occur throughout the resin matrix. The N-acylated amino acid corresponding to the C-terminal residue of the proposed peptide is then attached through the chloromethyl group to give a substituted benzyl ester derivative. Different amino protecting groups have been successfully employed. After removal of the protecting group and neutralization of the resulting hydrochloride with triethylamine, the next protected amino acid is coupled to the first. This and the succeeding amino acid derivatives can be activated in several ways, the most useful and common of which are the carbodiimide and the active ester methods. Coupling with carbodiimide is very rapid and convenient and is suitable with different solvents. But the important requirements of the solvent are higher dielectric constant and a capacity to promote the swelling of the resin. Methylene chloride and dimethyl formamide have proved to be the most effective solvents. However, the most important requirement is the necessity for a quantitative coupling reaction and it must essentially be a 100% reaction to achieve a successful synthesis of the peptide. By using an excess of activated reagent, an excess of time, a low crosslinked, high swelling resin and a good solvent the reactions have resulted in high yields. The peptide is finally released by passing a stream of HBr through a suspension of the peptide containing resin in a 1 to 1 mixture of trifluoroacetic acid and methylene chloride. The peptide thus released into solution is then subjected to a suitable purification procedure. This is the first time during the synthesis that the peptide is purified by any procedure other than simple washing (Scheme 1).



Scheme 1 — General reaction scheme for solid phase peptide synthesis

Employing essentially a similar procedure, the synthesis of bovine pancreatic ribonuclease A (RNase A)[12] is carried out in a stepwise manner starting from the amino group protected C-terminal amino acid, *t*-butyloxycarbonyl L-valine, esterified to 1% crosslinked polystyrene resin support. The automated solid phase method is followed in all its details. Besides the *t*-butyloxycarbonyl groups (BOC) as the amino protecting group[14-16], the benzyl ether (BzL) and the benzyl ester (OBzL) derivatives were used respectively to protect the hydroxyl groups of serine, threonine and tyrosine and the β- and α-carboxy groups of aspartic and glutamic acids. The same benzyl ether group is used to protect the sulphydryl group of cysteine while arginine is used as its nitro derivative. Methionine is used as the sulphone. Lysine is used as its benzyloxy carbonyl derivative. Histidine is unprotected. A threefold excess of N,N′-dicyclohexyl carbodiimide is used to mediate the coupling. BOC groups are removed with 50% (vol./vol.) trifluoroacetic acid in methylene chloride. The yield of the fully protected peptide-resin based on amino acid analysis and on the amount of valine originally taken is 17%. The polypeptide is cleaved off its solid support by treatment with anhydrous hydrofluoric acid[17,18] in the presence of anisole and trifluoroacetic acid for 90 min at 0-15°; removal of all the protecting groups is also simultaneously achieved. The product is then converted to its S-sulphonate RNase $(SSO_3^-)_8$ with Na_2SO_3 and $Na_2S_4O_6$ in 8*M* urea at *p*H 7·5[19,20]. The synthetic derivative, RNase $(SSO_3^-)_8$, is purified on Dowex 1-X2 resin followed by a gel filtration on Sephadex G-50. The faster moving Sephadex fraction is eluted at the same volume as natural RNase $(SSO_3^-)_8$ and is homogeneous in the paper electrophoresis system. The RNase $(SSO_3^-)_8$ is reduced to RNase $(SH)_8$ with mercaptoethanol in 8*M* urea at *p*H 8·5 (20 hr). The reduced product is then oxidized in air at 25° for 20 hr to form the four disulphide bridges of RNase[21-23]. The crude product is then fractionated over a column of IRC-50 in 0·2*M* sodium phosphate buffer (*p*H 6·47)[24]. The largest fraction is eluted at the same volume as reduced and reoxidized natural RNase. The synthetic ribonuclease thus prepared is indistinguishable from the natural enzyme by paper electrophoresis at *p*H 2·5. Performic acid oxidized samples, digested with trypsin, show practically an identical map of ninhydrin positive spots (14 expected)[25]; amino acid analyses of acid hydrolysates compare well with those of natural RNase. The synthetic enzymes show a specific activity of 13% by two methods[26-28] with yeast RNA as substrate and 24% with 2′,3′-cyclic cytidine phosphate[29] as substrate as compared with pure natural bovine pancreatic ribonuclease. The synthetic enzyme is completely

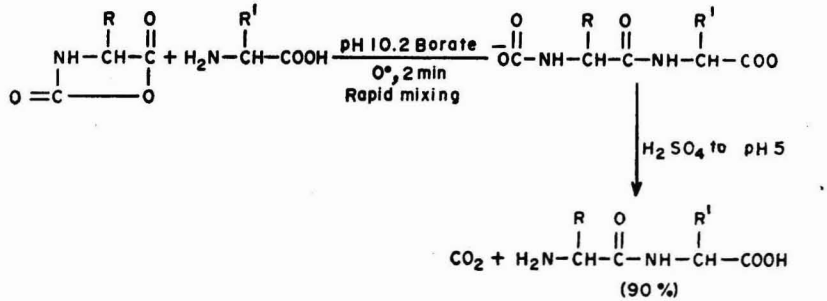
inactive towards DNA, 2′,3′-cyclic guanosine phosphate, 5′(3′-guanyl)cytidylic acid[30] (G_pC_p) and 5′(3′-adenyl)adenylic acid (A_pA_p). This demonstrates the high specificity of the synthetic material and is expected of RNase A. A further proof that the synthetic material contains an active RNase comes from a determination of the Michaelis constant and this turns out to be 2·4 mg/ml for natural RNase and 2·5 mg/ml for the synthetic product. These results provide direct evidence for the hypothesis[21,31-33] that the linear amino acid sequence contains all the requirements for chain folding and the formation of the active enzyme. The failure to obtain a fully active enzyme means only that the product is not yet pure. This assembly of 124 amino acid residues requires 369 chemical reactions and 11,931 steps of the automated peptide synthesis machine without any intermediate isolations.

Denkewalter and his associates[11] have attempted the synthesis in a different manner, namely homogeneous reaction condition in solution as opposed to Merrifield's heterogeneous reactions with a solid support. Richards and his collaborators[6-9] have already shown that RNase A can be cleaved at a single bond to produce RNase S without impairment of enzymatic activity; RNase S can be separated into two enzymatically inactive peptides, the eicosapeptide (S-peptide) and the tetrahectapeptide (S-protein). A combination of the two enzymically inactive fragments in equimolar ratio produced full enzymic activity (RNase S′). Since the complete primary structure of RNase A is known and since the S-peptide has already been synthesized by Hofmann *et al.*[34], Denkewalter and his group have proceeded with the synthesis of the S-protein, a tetrahectapeptide. They have gone about the synthesis of the S-protein for another important reason, that an oxidation of the reduced S-protein regenerates enzymic activity when assayed in the presence of the S-peptide; this is an observation already made by Haber and Anfinsen[35] and it leads one to infer that " information determining secondary and tertiary structure of RNase " is contained in the amino acid sequence of S-protein.

The synthesis of the S-protein is based on the fragment condensation method. Denkewalter and his associates have synthesized two large fragments, the tetracontapeptide[36] corresponding to the 21-64 sequence of RNase A and the hexacontapeptide[37] corresponding to the 65-124 sequence. These two large fragments have been assembled together to yield the protected tetrahectapeptide[38] in which ε-amino functions of lysine, the ω-amino group on serine-21 and all the eight cysteine sulphydryls are protected. To achieve this a total of 19 fragments have been prepared and about 40% of the bonds are formed through the use of the N-carboxy anhydrides (NCA)[39] and N-thiocarboxy anhydrides (NTA)[40], while the remainder are formed with the BOC-hydroxy succinimide esters of Anderson *et al.*[41]. The sulphydryls of the eight cysteine residues have been blocked by a new blocking agent, acetamido methyl group which is stable to trifluoroacetic acid at 25°, to anhydrous HF at 0° and to hydrazine and which can be selectively removed under mild conditions with Hg(II)[42]. In the fragment condensation method employed here, the intermediate fragments are isolated and purified by the conventional procedures like dry column chromatography on silica gel[39], thin layer chromatography in several solvent systems, amino acid analyses, etc. The acetamido methyl groups from the cysteine residues are removed in the end from the protected tetrahectapeptide and the free peptide is allowed to remain with 50% aqueous solution of mercaptoethanol at 25° for 17 hr. The disulphide bridges are formed and a desalting with Sephadex G-25 results in a protein fraction containing the S-protein. This synthetic S-protein is enzymically inactive against RNA or polycytidylic acid as substrate but shows activity on admixture with S-peptide. Denkewalter and his associates[43] have also acetamido methylated reduced natural S-protein and then regenerated the S-protein by exactly the same procedure which has been employed in the synthetic method. The S-protein so regenerated does not also show any enzymic activity alone but has activity on admixture with the S-peptide. Thus this group has been able to synthesize a fairly large enough protein which shows enzymic activity under the appropriate conditions.

The general scheme of sequential peptide synthesis with N-carboxy anhydrides as developed by Denkewalter *et al.*[44] is given in outline in Scheme 2.

The method consists essentially in a rapid mixing of the solid anhydride with an aqueous solution of an amino acid or peptide with a very close control of temperature and *p*H. Denkewalter *et al.* are able to carry out a sequential synthesis where others have failed. The condensation to form a peptide



Scheme 2 — Sequential synthesis with N-carboxyanhydrides according to Denkewalter *et al.*[44]

carbamate is done at *p*H 10·2 buffer at 0° and is complete in 2 min. Lowering the *p*H to 3-5 results in decarboxylation and liberation of the free peptide which is ready again for extension of the chain by a repetition of the process with a new carboxy anhydride amino acid. However, the peptide can be isolated and purified before proceeding with the synthesis.

Summary

The very recent report of the synthesis of ribonuclease by two different groups is given in outline. The basic methods of approach employed by these authors have been described to enable a clearer understanding of the synthetic procedure which has resulted in the preparation of the first 'true' protein having enzymic activity. The new path opened up by this achievement for studies on the function of enzymes is indicated.

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Irreversible Thermodynamics of Electro-osmotic Effects*

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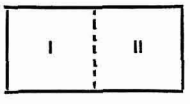
THE electro-osmotic phenomena are of considerable interest from the standpoint of biological transport[1-5], which has been stimulated by the need to understand permeability phenomena and secretion processes. Electro-osmotic transport in ion-exchange[6] has received attention because of its technical importance in desalination. A number of applications of electro-osmotic effects which include the use of these effects to pump fluids, generate electricity, measure pressure or flow have been proposed and investigated during recent years. Experimental studies of devices for underwater ultrasonic signal generators, receivers[7] and pressure transducers[8] employing the electro-osmotic principle have also been made.

Electro-osmotic effects are of intrinsic interest, since they give rise to a number of steady state phenomena which can be conveniently studied experimentally. These afford an example where the power of non-equilibrium thermodynamics[9-11] can be easily demonstrated. This is a unique phenomenon where non-linear transport processes have been experimentally observed[12,13] and studied.

Electro-osmotic effects are reviewed in the present communication with greater emphasis on the thermodynamic aspects. The thermodynamics of this phenomenon is discussed first. To understand the implications of the thermodynamic theory the theories based on the specific model of the barrier or the membrane are briefly discussed in the next section. Experimental studies of the phenomenon from the point of view of irreversible thermodynamics are discussed next, while non-linear effects are reviewed in the last section.

Non-equilibrium Thermodynamics of Electro-osmotic Phenomena

We shall first outline the theory of electro-osmosis and streaming potential based on thermodynamics of irreversible processes[9,14]. We consider two chambers separated by a very thin membrane, so that it merely serves as a dividing surface which we need not consider as a separate phase.



electric current

Chambers I and II contain a fluid with n components carrying electrical charges e_k per unit of mass. We assume that both the chambers are at the same temperature T and the concentrations are uniform in each chamber. M_k^{I} and M_k^{II} are the masses of component k in chambers I and II respectively. Similarly Φ^{I} and Φ^{II} are the potentials in the two chambers. In the system under discussion, we consider the transport of matter and electricity from one chamber to the other. In the process, both mass and charge would be conserved. Thus, we must have

$$dM_k^{\mathrm{I}}+dM_k^{\mathrm{II}}=0 \qquad (k=1,\ 2,\ \ldots\ n) \qquad \ldots(1)$$

and

$$\sum_{k=1}^{n} e_k dM_k^{\mathrm{I}}+\sum_{k=1}^{n} e_k dM_k^{\mathrm{II}}=0 \qquad \ldots \qquad \ldots(2)$$

Superscripts I and II refer to chambers I and II.

Now, our object would be to evaluate entropy production d_iS due to irreversible processes inside the system, which are simply the transport of matter and electricity. The total entropy production dS due to internal as well as external factors would be given by

$$TdS=Td\mathrm{S}^{\mathrm{I}}+Td\mathrm{S}^{\mathrm{II}} \qquad \ldots \qquad \ldots \qquad \ldots(3)$$

or

$$TdS=(dU^{\mathrm{I}}+P^{\mathrm{I}}dV^{\mathrm{I}}-\sum_{k=1}^{n}\mu_k^{\mathrm{I}}dM_k^{\mathrm{I}})$$
$$+(dU^{\mathrm{II}}+P^{\mathrm{II}}dV^{\mathrm{II}}-\sum_{k=1}^{n}\mu_k^{\mathrm{II}}dM_k^{\mathrm{II}}) \qquad \ldots(4)$$

where we have assumed the validity of the Gibbs equation for entropy production even outside equilibrium. This assumption is always used in non-equilibrium thermodynamics and it has been shown that this is justified for situations near equilibrium. The term U represents the internal energy; P, the pressure; V, the volume; and μ_k, the chemical potential of species k. From the first law of thermodynamics, we have

$$dU=dU^{\mathrm{I}}+dU^{\mathrm{II}}$$
$$=dQ-P^{\mathrm{I}}dV-P^{\mathrm{II}}dV+(\phi^{\mathrm{I}}-\phi^{\mathrm{II}})Idt \qquad \ldots(5)$$

where I is the current‡ given by

$$I=-\sum_{k=1}^{n} e_k dM_k^{\mathrm{I}}/dt=\sum_{k=1}^{n} e_k dM_k^{\mathrm{II}}/dt \qquad \ldots(6)$$

dQ is the heat absorbed from the surroundings.

Substituting the value of $(dU^{\mathrm{I}}+dU^{\mathrm{II}})$ from Eq. (5) into Eq. (4), we get

$$TdS=dQ-\sum_{k=1}^{n}(\mu_k^{\mathrm{I}}+e_k\phi^{\mathrm{I}}-\mu_k^{\mathrm{II}}-e_k\phi^{\mathrm{II}})dM_k^{\mathrm{I}} \qquad \ldots(7)$$

Now $dS=d_eS+d_iS$, where d_eS is the reversible entropy change due to interaction with the

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‡I is taken positive when the flow is from chamber I to II. $\Delta\phi$ is taken as positive when $\phi^{\mathrm{I}}\to\phi^{\mathrm{II}}$. Similar convention is used for volume flow J and $\Delta P=P^{\mathrm{I}}-P^{\mathrm{II}}$.

surroundings, and hence σ, the rate of entropy production, is given by

$$\sigma = \frac{d_i S}{dt} = -\sum_{k=1}^{n} \Delta(\mu_k + e_k\phi)\frac{dM_k^{\mathrm{I}}}{dt} \quad \ldots \quad \ldots(8)$$

where Δ represents the difference of a quantity in chambers I and II.

When the temperature and concentration remain fixed

$$\Delta\mu_k = v_k \Delta P \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(9)$$

where v_k is the specific volume of component k. Hence

$$\sigma = -\sum_k v_k \frac{dM_k^{\mathrm{I}}}{dt}\cdot\frac{\Delta P}{T} - \sum_k -e_k \frac{dM_k^{\mathrm{I}}}{dt}\cdot\frac{\Delta\phi}{T} \quad \ldots(10)$$

Eq. (10) enables us to spot out the fluxes and forces in the system by remembering that entropy production is the sum of the product of fluxes J_i and forces X_i, i.e.

$$\sigma = \Sigma J_i X_i \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(11)$$

Thus, it follows that the volume flow J and the current I are given by

$$J = -\sum v_k \frac{dM_k^{\mathrm{I}}}{dt} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(12)$$

and

$$I = -\sum_k e_k \frac{dM_k^{\mathrm{I}}}{dt} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(13)$$

The corresponding forces are $\Delta P/T$ and $\Delta\phi/T$. The explicit transport equations in this linear range would be written as

$$J = L_{11}\left(\frac{\Delta P}{T}\right) + L_{12}\left(\frac{\Delta\phi}{T}\right) \quad \ldots \quad \ldots \quad \ldots(14)$$

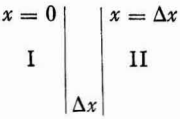
$$I = L_{21}\left(\frac{\Delta P}{T}\right) + L_{22}\left(\frac{\Delta\phi}{T}\right) \quad \ldots \quad \ldots \quad \ldots(15)$$

where L's are the phenomenological coefficients, governed by the following conditions:

(i) $L_{12} = L_{21}$ (Onsager reciprocity relation) ...(16)

(ii) $L_{11}L_{22} > L_{12}L_{21}$ on account of positive definite character of entropy production.

In the above discussion we have assumed that the barrier or membrane thickness is infinitesimally small. It can be seen that Eq. (10) can be deduced[11] even when the membrane has a finite thickness Δx



The flows passing through the membrane would be perpendicular to the surface and will have the same value at all points on the surface, provided the membrane is homogeneous. Since the internal entropy production occurs on passage to the membrane, we can also evaluate the net entropy production by integration over the thickness of the membrane by using the following expression:

$$T_\sigma = \int_0^{\Delta x} T\theta\, dx \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(17)$$

where θ is the entropy production within a volume element of unit area and of thickness dt. It can be shown that

$$\theta = J(-\text{grad } P) + I(-\text{grad } \phi)$$

so that

$$T_\sigma = J\int_0^{\Delta x} -\frac{\partial P}{\partial x}\,dx + I\int_0^{\Delta x} -\frac{\partial\phi}{\partial x}\,dx = J\Delta P + I\Delta\phi \quad \ldots(18)^*$$

For simplicity we have considered that all gradients are along the x-coordinate. The above formulation has been extended to the cases of composite membranes[15] and ion-exchange membranes[16,17].

Eqs. (14) and (15) easily yield the following relations for various steady states:

Electro-osmosis:

$$(J)_{\Delta P=0} = L_{12}\cdot\frac{\Delta\phi}{T};\ (J/I)_{\Delta P=0} = \frac{L_{12}}{L_{22}} \quad \ldots(19)$$

Electro-osmotic pressure:

$$(\Delta P)_{J=0} = -\frac{L_{12}}{L_{11}}\Delta\phi \quad \ldots \quad \ldots \quad \ldots \quad \ldots(20)$$

Streaming potential:

$$(\Delta\phi)_{I=0} = -\frac{L_{21}}{L_{22}}\cdot\Delta P \quad \ldots \quad \ldots \quad \ldots \quad \ldots(21)$$

Streaming current:

$$(I)_{\Delta\phi=0} = \frac{L_{21}}{T}\cdot\Delta P;\ (I/J)_{\Delta\phi=0} = \frac{L_{21}}{L_{11}} \quad \ldots(22)$$

These phenomena are interrelated on account of Onsager relation. Thus

$$\left(\frac{\Delta P}{\Delta\phi}\right)_{J=0} = -\left(\frac{I}{J}\right)_{\Delta\phi=0} \quad \ldots \quad \ldots \quad \ldots(23)$$

$$\left(\frac{\Delta\phi}{\Delta P}\right)_{I=0} = -\left(\frac{J}{I}\right)_{\Delta P=0} \quad \ldots \quad \ldots \quad \ldots(24)$$

It would be interesting to compare the magnitudes of σ for each of the steady states[18]. On substituting the values of J and I from Eqs. (14) and (15) in Eq. (10), we have

$$\sigma = L_{11}\left(\frac{\Delta P}{T}\right)^2 + (L_{12}+L_{21})\frac{\Delta P\Delta\phi}{T^2} + L_{22}\left(\frac{\Delta\phi}{T}\right)^2 \quad \ldots(25)$$

We can plot σ as a function of $\Delta P/T$ and $\Delta\phi/T$ on a three-dimensional diagram to yield an elliptical paraboloid with vertex at $\sigma = 0$, which corresponds to the equilibrium state (Fig. 1). The major and minor axes would be inclined to the axis representing $\Delta P/T$ and $\Delta\phi/T$ by an angle θ' given by

$$\theta' = \frac{3\pi}{2} + \tfrac{1}{2}\tan^{-1}\frac{2L_{12}}{L_{11}-L_{12}} \quad \ldots \quad \ldots \quad \ldots(26)$$

For a steady state when $J = 0$ and $\Delta\phi$ = constant, the entropy production would be represented by a section of the paraboloid, which would be a parabola

*Eq. (18) can be derived in a more rigorous way by using local differential equations (ref. 9, p. 407).

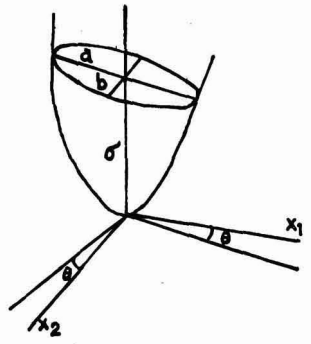


Fig. 1 — Geometrical interpretation of entropy production

TABLE 1 — VALUES OF σ FOR VARIOUS STEADY STATES

Steady state	
Streaming potential	$\frac{L_{11}L_{22}-L_{12}L_{21}}{L_{22}}\left(\frac{\Delta P}{T}\right)^2$
Electro-osmotic pressure	$\frac{L_{11}L_{22}-L_{12}L_{21}}{L_{11}}\left(\frac{\Delta\phi}{T}\right)^2$
Electro-osmosis	$\frac{L_{21}^2+L_{11}L_{22}-L_{21}(L_{12}+L_{21})}{L_{22}[L_{11}L_{22}-L_{12}L_{21}]}\cdot J^2 \sim L_{22}\cdot\left(\frac{\Delta\phi}{T}\right)^2$ when $L_{11}L_{22} \gg L_{12}L_{21}$
Streaming current	$\frac{L_{21}^2+L_{11}L_{22}-L_{21}(L_{12}+L_{21})}{L_{11}[L_{11}L_{22}-L_{12}L_{21}]}\cdot J^2 \sim L_{11}\left(\frac{\Delta P}{T}\right)^2$ when $L_{11}L_{22} \gg L_{12}L_{21}$

with coordinates of vertex given by

$$\sigma = \left(\frac{\Delta\phi}{T}\right)^2\left(\frac{L_{11}L_{22}-L_{12}L_{21}}{L_{11}}\right);\ \frac{\Delta P}{T} = -\frac{L_{12}}{L_{11}}\cdot\frac{\Delta\phi}{T} \quad ...(27)$$

This value of σ would correspond to steady state value, since it is minimum according to Prigogine's theorem. The values of σ_{min} for different steady states are summarized in Table 1.

It can be seen that the entropy production values for various steady states are not the same. The value of σ for streaming potential and electro-osmotic pressure would be the same when $(\Delta P)^2/L_{22} = (\Delta\phi)^2/L_{11}$. It also follows that since the entropy production is positive, $L_{11}L_{22}$ must be greater than $L_{12}L_{21}$.

Theories of Electro-osmotic Phenomena Based on Models of Membranes

In the development of the thermodynamic theory of the phenomena, it is not necessary to examine how electro-osmosis or streaming potential occurs. The theory is independent of the nature of the membrane or its character. It yields results in terms of phenomenological coefficients. It should be noted that these coefficients do depend on membrane characteristics. The Onsager reciprocal relation would be valid for the same membrane. In order to get the complete picture, including the reason for the occurrence of this phenomenon and the factors on which the phenomenological coefficients depend, we shall discuss the theories based on the specific models of the membrane.

Membranes are essentially of two types so far as their electrical nature is concerned: (i) uncharged type like pyrex sinter, and (ii) charged type like ion-exchange membranes. In the former, mobile charges come from the diffuse double layer at the solid/liquid interface, while in the latter, counter-ions are the mobile species. For example, in zeolites or cation-exchange resins, the cations are mobile, whereas the anionic body is not. Such membranes in contact with electrolytic solutions are preferentially permeable to cations. The ion-exchange membranes may be either homogeneous or heterogeneous. The former are coherent ion-exchanger gels, while the latter consist of colloidal ion-exchanger particles embedded in a binder, such as polystyrene or polyethylene.

In terms of mechanical structure, the membrane may be thought of as a bundle of capillaries or channels having a characteristic pore size and length. These may be arranged as (i) a parallel array, i.e. parallel to each other along the x-axis, or (ii) a series array. Membranes having the parallel array are the simplest and the average number of channels and pore size can be estimated from electro-osmotic measurements[19]. In the former case, the net flow would be the sum of flows from individual channels, while in the latter, the fluxes must be the same throughout the constituent layers. Thus, for a parallel array, having n capillaries,

$$J = \sum^{n} a_i j_i$$

where j_i is the flow through a single capillary; and a_i, the fractional area given by $A_i/\Sigma A_i$, where A_i is the area of cross-section of the ith capillary. The phenomenological coefficients L_{ik} would be given by

$$L_{ik} = \sum^{n} a_i l_{ik}$$

Similarly, for a series array having membranes α, β, γ, ..., we must have

$$J^\alpha = J^\beta = J^\gamma = \ldots.$$

A typical example of a double membrane system is the epithelial cell bounded on either side with liquid of different permeability properties[20].

If directional characteristics are considered, the membrane may be either isotropic or anisotropic. Examples of anisotropic membranes are cellular membranes including axonal membranes composed of different layers[21]. It has been pointed out[22] that sandstones and other rocks, sintered metals, sintered glass and unglazed ceramic bodies may be anisotropic with respect to permeability. In the following sub-section we shall consider the case of uncharged membrane or capillary.

Theory of electro-osmosis in uncharged membranes— It is believed that in uncharged membranes, electro-osmotic flow occurs on account of the existence of electrical double layer at the solid-liquid interface as shown in Fig. 2, where the solid is supposed to be negatively charged. The positive charges move towards the negative electrode when an electric field is applied. The potential near the

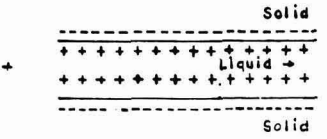


Fig. 2 — Mechanism of electro-osmosis

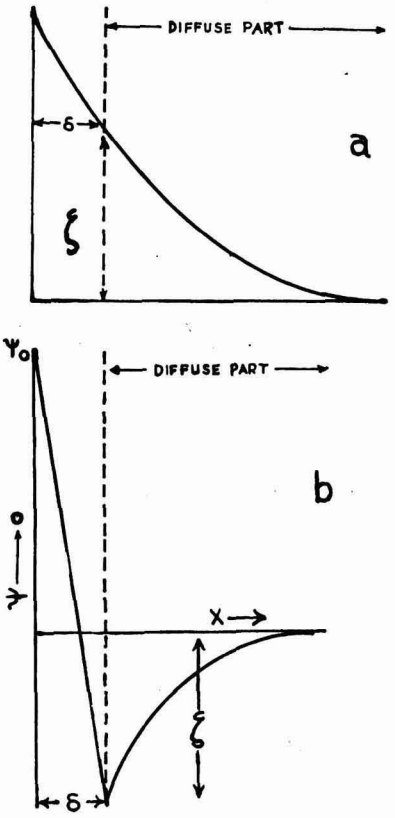


Fig. 3 — Potential distribution (a) when the adsorbed ion is of the same sign as the charge of the outer phase, and (b) when a counter-ion is adsorbed

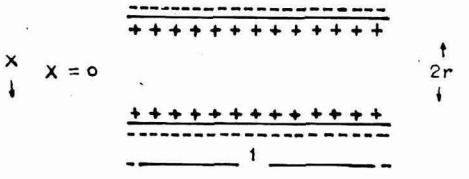
wall would have a fixed value (φ_0). The potential would drop as we move away from the wall in the manner shown in Fig. 3, on account of the diffuse nature of the double layer.

The term δ represents the thickness of the so-called 'Helmholtz double layer', which is fixed and the potential at the slip-plane is called the ζ-potential. The origin of charges in the double layer is not clearly known, but it is believed to arise on account of preferential adsorption of ions in many cases. The ions in the double layer are relatively immobile. The electrolytic transport in the diffuse part of the double layer gives rise to flow of ions in one direction which is responsible for unidirectional flow of solvent.

Theories of electro-kinetic phenomena have been advanced on the basis of Helmholtz double layer model of a parallel plate condenser[23]. This model has been improved by Gouy and Chapman[24], which yields an explanation of (i) the qualitative difference between φ_0 and ζ-potential, and (ii) the sensitivity of ζ-potential to the concentration of the non-potential determining ions. However, the refinements were unable to explain this frequently observed inversion of sign of ζ with increasing concentration of certain electrolytes. Further, the theory of Gouy and Chapman leads to capacitance values of this double layer which in concentrated solutions are too high by one order of magnitude. These drawbacks are removed when Stern's correction is applied[25]. But Stern's theory is basically qualitative in character, which is primarily due to the schematic manner in which the charge is divided into adsorption and diffusion components. The use of Langmuir adsorption isotherm introduces considerable limitations in the theory. Thus, the problem of true distribution of potential in the double layer is still an open question.

In spite of the above difficulties, a simple theory[26-28] based on Helmholtz model yields a microscopic picture which is useful in understanding the role of pore size and channel length along with the electrical characteristics of this interface in electro-kinetic phenomena. Whereas the macroscopic theory based on irreversible thermodynamics does not depend on any model, the theory discussed below would be valid provided the situation conforms to the model. Both approaches are complementary in understanding the phenomenon.

We shall consider a capillary of radius r and length l, filled with a fluid. We assume the existence of double layer similar to a parallel plate condenser. The radius of the capillary is considerably greater than δ, the thickness of the electrical double layer, so that at $x = r$ (at the axis of the capillary) $\varphi = 0$ and $d\varphi/dx = 0$



The application of potential gradient causes the fluid to move. In the steady state, the electrical force would just counterbalance the viscous force. At a distance dx from the wall,

$$\text{Electrical force} = \frac{\Delta\phi}{l}\cdot\rho\cdot dx \qquad \ldots \quad \ldots \quad \ldots(28)$$

and

$$\text{Viscous force} = \eta\left(\frac{dV}{dx}\right)_{x+dx} - \eta\left(\frac{dV}{dx}\right)_{x} = \eta\left(\frac{d^2V}{dx^2}\right)dx \qquad \ldots(29)$$

where η is the viscosity, dV/dx is the velocity

gradient, and ρ is the charge density. From Poisson equation

$$\rho = -\frac{\epsilon}{4\pi} \cdot \frac{d^2\varphi}{dx^2} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(30)$$

where ϵ is the dielectric constant. On equating the electrical force and the viscous force and using Eq. (30), we have

$$\frac{d^2V}{dx^2} = -\frac{\Delta\phi}{l} \cdot \frac{\epsilon}{4\pi\eta} \cdot \frac{d^2\varphi}{dx^2} \quad \ldots \quad \ldots \quad \ldots(31)$$

which on integration yields

$$\frac{dV}{dx} = -\frac{\Delta\phi}{l} \cdot \frac{\epsilon}{4\pi\eta}\left(\frac{d\varphi}{dx}\right) + \text{constant} \quad \ldots \quad \ldots(32)$$

Since the velocity gradient is zero along the axis of the capillary,

$$\frac{dV}{dx} = 0, \text{ when } x = r$$

Further, according to our model $d\varphi/dx = 0$, when $x = r$, so that using these boundary conditions we find that the constant of integration is zero, so that

$$\frac{dV}{dx} = -\frac{\Delta\phi}{l} \cdot \frac{\epsilon}{4\pi\eta}\left(\frac{d\varphi}{dx}\right) \quad \ldots \quad \ldots \quad \ldots(33)$$

On further integration we have

$$V = -\frac{\Delta\phi}{l} \cdot \frac{\epsilon}{4\pi\eta} \cdot \varphi + \text{constant} \quad \ldots \quad \ldots(34)$$

At the 'plane of shear', we have $\varphi = \zeta$. Since at this plane no slippage occurs, $V = 0$. Using these boundary conditions we get

$$V = -\frac{\Delta\phi}{l} \cdot \frac{\epsilon\zeta}{4\pi\eta} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(35)$$

so that

$$J = \pi r^2 V = -\frac{\Delta\phi}{l} \cdot \frac{r^2\epsilon\zeta}{4\eta l} \quad \ldots \quad \ldots \quad \ldots \quad \ldots(36)$$

Comparing Eqs. (19) and (36) we obtain

$$\frac{L_{12}}{T} = \frac{\epsilon r^2\zeta}{4\eta l} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(37)$$

In the integration of Eq. (31) it has been assumed that η and ϵ are independent of $d\varphi/dx$, the local field strength at any point in the double layer. This assumption may be incorrect, since the local electrical field in the double layer would be very high (of the order of 10^5 V cm^{-1}), if there is a fall of 100 mV in a distance of 100 Å. Such a high field would tend to reduce the dielectric constant. More rigorously, we should write

$$\frac{L_{12}}{T} = \frac{r^2}{4l}\int^{\varphi_0} \frac{\epsilon}{\eta} d\varphi$$

It should be noted that when $r \gg \delta$, the charge separation* in the capillary occurs only in a thin layer near the wall, so that the bulk of the fluid is electrically neutral. This would not be the case when $r \sim \delta$, since the radial distribution of the field charge would be strongly non-uniform[29]. Further, the above theory has the following additional limitations: (i) the exact location of the slip-plane is not known; (ii) the exact magnitudes of η and ϵ in the double layer are uncertain; and (iii) the true distribution of the potential in the double layer is not known.

A theory of streaming current produced on account of fluid flow through the capillary can also be developed on the above lines. Let us consider the region between the capillary and the slip-plane. The excess charge e per cm^2 within the distance δ will be transported along the wall with the moving fluid. The speed of the transport of the charge will be just equal to the velocity of the fluid u_δ. The streaming current I would be given by

$$I = 2\pi r.u_\delta.e \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(38)$$

The classical equation for laminar flow for a cylindrical capillary yields

$$u_\delta = \frac{1}{4\eta} \cdot \frac{\Delta P}{l}[r^2 - (r-\delta)^2] \sim \frac{1}{2\eta} \cdot \frac{\Delta P}{l} \cdot r\delta, \text{ since } \delta \ll r \quad \ldots(39)$$

For Helmholtz double layer, $\delta = \epsilon\zeta/4\pi e$. On making proper substitutions,

$$I = \frac{r^2\epsilon\zeta}{4\eta l} \cdot \Delta P \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(40)$$

On comparing Eqs. (22), (37) and (40), we find that

$$\frac{L_{12}}{T} = \frac{L_{21}}{T} = \frac{\epsilon r^2\zeta}{4\eta l} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(41)$$

so that the above theory is consistent with Onsager's reciprocal relations.

In order to complete the macroscopic description we shall evaluate L_{11}/T and L_{22}/T. If the pore size is much greater than the mean free path, this flow of the fluid through a circular pore of radius r would be governed by Poiseulle's equation†

$$J = \frac{\pi r^4}{8\eta l} \cdot \Delta P \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(42)$$

so that

$$\frac{L_{11}}{T} = \frac{\pi r^4}{8\eta l} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(43)$$

Further if $\varkappa$ is the specific conductance of the fluid, the conductance of the fluid in the capillary is given by

$$\frac{L_{22}}{T} = \frac{\pi r^2\varkappa}{l} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots(44)$$

When surface conductance $\varkappa_\delta$ of the solid is comparable to the specific conductance of the liquid

$$\frac{L_{22}}{T} = \pi r^2\left(k + \frac{2\varkappa_\delta}{r}\right)\Big/ l \quad \ldots \quad \ldots \quad \ldots \quad \ldots(45)$$

If we consider a composite membrane having a parallel array of n capillaries, the right-hand side of Eqs. (40), (42), (43) and (44) would have to be multiplied by n. The phenomenology for ultrafine capillaries would be slightly different and has been worked out by a number of workers[31-33].

*$\delta \sim 3{\cdot}06 \times 10^{-8} s^{-1/2}$ [cm], where s is the ionic strength.

†For elliptical pore L_{11}/T would be $\frac{a^3b^3}{a^2+b^2} \cdot \frac{1}{4\eta l}$, where a and b are the semi-major and semi-minor axes. For an equilateral pore this would be $\frac{27R^4}{20\sqrt{3}} \cdot \frac{1}{\eta l}$, where R is the side of the equilateral triangle[30].

Theory of charged membranes — The phenomenon of electro-osmosis has also been observed in ion-exchange membranes[6], which are characterized by a high concentration of fixed charges and a correspondingly high concentration of counter-ions. Pore radii are much smaller, approaching molecular dimensions, so that $r \ll \delta$. A large percentage of counter-ions is mobile. During electro-osmosis, these counter-ions move, carrying solvent along with them. The slip-plane occurs at the surface of each fixed ion, or, if it is hydrated, at the surface of primary hydration sheath. In general, ion-exchange membranes are 'perm-selective', permitting migration of the ions of the same type, i.e. the co-ions cannot be easily transferred. However, several membranes have been reported where one ion possesses a significantly higher mobility than the other permeable ion species in the membrane phase[34].

A theory of electro-osmotic phenomena suited to charged membranes has been developed by Schmid and coworkers[35-37]. It is assumed that an identical composition is maintained on the two sides of the membrane, so that there is no concentration gradient. The distribution of mobile species within the pores is also assumed to be uniform. The ionic fluxes J_i are given by

$$J_i = -Z_i\bar{C}_i\bar{U}_i\frac{\Delta\phi}{l}+\bar{C}_i\left\{-\frac{\Delta P}{\rho_0 l}+\omega\bar{U}_0\frac{\Delta\phi}{l}\right\} \qquad ...(46)$$

where Z_i is the valency of the ion; $\bar{C}_i$, the concentration of the ith ion; $\bar{U}_i$, mobility of the ion in the membrane; ρ_0, specific flow resistance; and ω, sign of fixed charges. The quantity inside the brackets on the right-hand side represents r_c, the rate of motion of the centre of gravity of the pore liquid. If $\bar{x}$ is the concentration of charges per unit volume, we have the following relation on account of the electro-neutrality condition:

$$\Sigma_i Z_i\bar{C}_i+\omega\bar{x}=0 \qquad ...(47)$$

The current I would be given by

$$I = F\Sigma Z_i J_i \qquad ...(48)$$

where F is the Faraday.

It follows that the streaming current and the streaming potential would be given by

$$(I)_{\Delta\phi=0} = \frac{\omega\bar{x}F}{\rho_0 l}\cdot\Delta P \qquad ...(49)$$

$$(\Delta\phi)_{I=0} = \frac{\omega\bar{x}F}{\rho_0\bar{\varkappa}}\cdot\Delta P \qquad ...(50)$$

where $\bar{\varkappa}$ is the specific electrical conductivity of the membrane.

The volume flow J is the product of r_c and the fractional pore area $\bar{\epsilon}$. Hence

$$(J)_{\Delta P=0} = \omega\bar{U}_0\frac{\Delta\phi}{l}=\frac{\omega F\bar{x}}{\rho_0 l}\cdot\Delta\phi \qquad ...(51)$$

where $\bar{U}_0 = FX/\rho_0\epsilon$.

It follows that

$$(I)_{\Delta\phi=0} = (J)_{\Delta P=0}$$

showing that the theory is in agreement with thermodynamic theory. If the pore radius is much greater than the mean free path, $l/\rho_0 = r^4/8\eta e$; we can then compare the results for charged and uncharged membranes, showing that $e/\frac{1}{2}r^2$ would be equivalent to ωFX. In the theory of charged membranes, no effort is made to correlate ωFX with the dielectric constant or other microscopic parameters.

Experimental Studies of Electro-osmotic Effects

The four electro-kinetic effects described by Eqs. (19)-(22) can be experimentally realized. Much of the earlier data[38-40] are of doubtful validity, since it is not known whether the pore geometry remained intact during the experiments of different kinds, since from Eqs. (40)-(44) it follows that the phenomenological coefficients are sensitive functions of pore geometry. Usually experiments are performed with a porous plug which may be considered as a bundle of capillaries. Blocking of capillaries is a serious experimental difficulty. There is a possibility of incomplete wetting of the capillaries due to various factors, including the presence of greasy material. If the blocking and incomplete wetting of the capillaries occurs during the experiment, obviously the character of the plug would change during the process and the validity of the results would be doubtful. Complications may also arise on account of (i) the action of the permeant on the capillary interface, and (ii) heating of the fluid in the pores. When solutions of electrolytes are used as permeant, it is doubtful whether ζ-potential would remain invariant, in spite of the accumulation of products of electrolysis, unless very small current is passed. In recent experiments[13,41], electronically regulated power supplies have been used for maintaining high potential differences with minimum possible passage of current. To avoid heating near the pores, magnetic stirring near the electrodes has been resorted to. The use of sinusoidally varying quantities in measuring electro-kinetic properties has been described by Cooke. The technique with the help of alternating current offers the possibility of eliminating electrode difficulties when using electrolyte solutions. However, the technique is not easy. The earlier measurements[8,42,43] were made at low $\Delta\phi$ and low ΔP. In a recent work[12], ΔP has been extended to a few centimetres of mercury and $\Delta\phi$ has been pushed further to 600 V.

For studying electro-osmosis described by Eq. (19), either the bubble technique or the capillary method[44,45] has been employed. In the former, difficulties due to evaporation of the permeant are avoided. However, the capillary along which the bubble moves has to be perfectly horizontal. The latter method is relatively simple. In principle, a definite potential gradient is applied along the membrane or the capillary, and the movement of the fluid in an external capillary is noted. Instead of a fixed potential gradient, one may apply a fixed current. The electro-osmotic pressure is measured by the pressure difference method. In order to measure the streaming potential, the fluid is allowed to flow through the membrane under a definite pressure gradient, which is maintained constant during the experiment with specially designed pressure heads. The streaming potential is measured with an electrometer, since the resistance of the

plug is usually high. The streaming current is measured in a similar fashion.

Considerable amount of work has been done on both charged[46-53] and uncharged membranes. We shall discuss some of the recent data in the context of the conclusions of the thermodynamic theory developed in an earlier section. It may be noted that Eqs. (14) and (15) are used as axioms in the development of the phenomenological theory. The only justification for these is that these are consistent with the theory developed in the subsequent section. A direct test of the validity of the linear phenomenological equation has been performed recently. For pyrex sinter with water as permeant, $(J)_{\text{total}}$, $(J)_{\Delta\phi=0}$ and $(J)_{\Delta P=0}$ have been measured individually. It is found that up to 60 V, the relationship $(J)_{\text{total}} = (J)_{\Delta\phi=0} + (J)_{\Delta P=0}$ is satisfied within the limits of experimental error[41]. The linear phenomenological equation for mass transport in the electro-osmosis of liquids has also been verified in a similar manner[54]. The data for volume flow for polystyrene sulphonic acid ion-exchange membrane also satisfy the linear phenomenological equation[55].

Previous data on electro-osmotic measurements in support of Onsager relations have been reviewed recently[56]. Only the experimental data of Rutgers and de Smet[42], Lorenz[43] and Cooke[8] offer reasonable support. However, Rutger's data refer to capillaries only and no idea can be formed regarding the range of ΔP and $\Delta\phi$ up to which Onsager relation is valid. Moreover, in the case of electrolyte solutions, some complications would arise in making a comparison on account of osmotic effects[57]. Lorenz used a composite membrane made up of quartz powder and pyrex sinter for support. Slight changes in packing of the plug are bound to vitiate the results. Further, the observed streaming current and streaming potential versus ΔP curves do not pass through the origin as expected. Lorenz has tested Onsager reciprocal relation for a very limited range, since ΔP has been varied up to approximately 30 cm of acetone and $\Delta\phi$ up to 8·5 V only. On the other hand, Cooke used pyrex-water system for study. In the absence of hydrodynamic permeability data for the system, it is difficult to comment on the results, since it is known that such a system normally has a poor reproducibility[41]. Clear confirmation of Onsager relation is obtained for pyrex-acetone system[13] since the experimental difficulties are minimum for this system. Typical values of the phenomenological coefficients for a few membranes are given in Table 2.

On substituting appropriate values for pyrex-acetone system[13], $L_{11}L_{22}$ is found to be of the order of 2×10^{-6} cm$^6A^2J^{-2}$, whereas $L_{12}L_{21}$ is equal to $6{\cdot}5\times10^{-8}$ cm$^6A^2J^{-1}$. Thus, $L_{11}L_{22} \gg L_{12}L_{21}$ and the inequality is proved. The current required for a flow of 1 cm^3/sec of acetone would be of the order of 25·6 mA, since $I = J(L_{22}/L_{11})$. The entropy production for various steady states can be estimated by using the relations given in Table 1. For streaming potential and streaming current $\sigma = 10^{-11}J$/deg/sec, when ΔP is of the order of 10^4 dynes. For electro-osmosis and electro-osmotic pressure, σ is of the order of 3×10^{-4} J/deg/sec, when $\Delta\phi = 100$ V.

TABLE 2 — VERIFICATION OF ONSAGER RECIPROCAL RELATION

Membrane	L_{11}/T cm^{3}sec^{-1} dyne^{-1}	L_{12}/T cm$^3A\,J^{-1}$	L_{21}/T cm$^3A\,J^{-1}$	L_{22}/T	Ref.
I	$3{\cdot}33\times10^{-6}$	$2{\cdot}5\times10^{-4}$	$2{\cdot}61\times10^{-4}$	$6{\cdot}3\times10^{-6}$	13
II	$0{\cdot}96\times10^{-6}$	$1{\cdot}13\times10^{-4}$	$1{\cdot}10\times10^{-4}$	—	12
III	$1{\cdot}27\times10^{-6}$	$0{\cdot}96\times10^{-4}$	$0{\cdot}92\times10^{-4}$	—	12
IV	$0{\cdot}92\times10^{-6}$	$0{\cdot}72\times10^{-4}$	$0{\cdot}70\times10^{-4}$	—	12
V	$1{\cdot}10\times10^{-6}$	$1{\cdot}07\times10^{-4}$	$1{\cdot}03\times10^{-4}$	—	12
VI	$1{\cdot}06\times10^{-6}$	$0{\cdot}97\times10^{-4}$	$0{\cdot}90\times10^{-4}$	—	12

The efficiency of energy conversion may also be estimated in view of the interest in the engineering applications of electrokinetic phenomena during recent years[58-60]. One may define the efficiencies of energy conversion E_e and E_s, which are related to coupling in electro-osmosis and streaming potential, as follows:

$$E_e = -\frac{J\Delta P}{I\Delta\phi}\,;\; E_s = -\frac{I\Delta\phi}{J\Delta P} \quad \ldots \quad \ldots \quad \ldots(52)$$

Calculations show that $E_s \sim 10^{-7}$, while $E_e \ll 1$ for pyrex-acetone sinter. In view of the extremely low efficiency, these cannot be utilized for energy conversion.

In the linear region where the fluxes are linearly related to forces, σ has a minimum value in the stationary state. However, according to Prigogine and Glansdorff, $-(d\sigma)_J = -\Sigma J_i \dot{X}_i \geqslant 0$, the equality holding for the stationary state[62]. The term $\dot{X}_i$ is the time derivative of force X_i. In the case of electro-osmosis

$$(d\sigma)_J = \left(L_{11}\frac{\Delta P}{T} + L_{12}\frac{\Delta\phi}{T}\right)\left(\frac{\Delta P}{T}\right) \quad \ldots \quad \ldots \quad \ldots(53)$$

For pyrex-water system, it turns out that $(d\sigma/dt)_J$ is of the order of -4×10^{-5} J/deg/sec/sec, when $\Delta\phi = 60$ V, which confirms the above generalization.

Water transference number t_ω is an important parameter in the study of ion-exchange membranes. The value of t_ω is given by

$$t_\omega = \left(\frac{J}{I}\right)_{\Delta P=0}\cdot\frac{F}{18} = \frac{L_{12}}{L_{22}}\cdot\frac{F}{18} \quad \ldots \quad \ldots \quad \ldots(54)$$

It follows that within the domain of validity of linear thermodynamics of irreversible processes, t_ω should be independent of current density. Actually such a behaviour was observed by earlier workers[29,63]. However, abnormalities at very low current densities have been noted, the reason for which is obscure[45]. Marked dependence of electro-osmotic water transport on current has also been observed at higher current densities[48], which may be due to non-linear effects discussed below.

Non-linear Effects

Both the thermodynamic theory and the macroscopic theory lead to the conclusions that (i) $(J)_{\Delta P=0}$ is proportional to $\Delta\phi$, and (ii) when $J = 0$, ΔP is proportional to $\Delta\phi$. Whereas the thermodynamics clearly shows that this may be true when

departures from equilibrium are not significant, the theories based on specific model do not set any limit to their validity. The non-linear region has been studied only recently for the first time by Rastogi and coworkers[12,64]. Some typical results presented in Figs. 4-6 show that the departure from linearity exceeds the experimental uncertainty beyond $\Delta\phi > 150$ V and $\Delta P > 20$ cm. However, the streaming potential varies linearly with pressure difference within the range studied (Fig. 7). The electro-osmotic data in the experimentally studied non-linear range are found to satisfy the followiig non-linear transport equation[12,64]:

$$J = L_{11}X_1 + L_{12}X_2 + L_{112}X_1X_2 + \tfrac{1}{2}L_{122}X_2^2 + \tfrac{1}{2}L_{1112}X_1^2X_2 + \tfrac{1}{2}L_{1122}X_1X_2^2 \quad ...(55)$$

It would be worth while to discuss the reason for departure from non-linearity at this stage[65]. Change in structural factors r, n and l, and the non-constancy of L_{ik} may cause deviation from linear behaviour. Since L_{ik} refers to mean temperature, pressure and potential prevailing in the membrane, these can vary if the gradients across the membrane

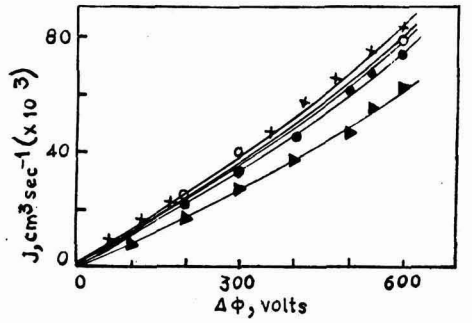


Fig. 4 — Dependence of volume flow (J) on potential difference ($\Delta\phi$) [○, membrane I; ●, membrane II; △, membrane III; ×, membrane IV; and ▲, membrane V]

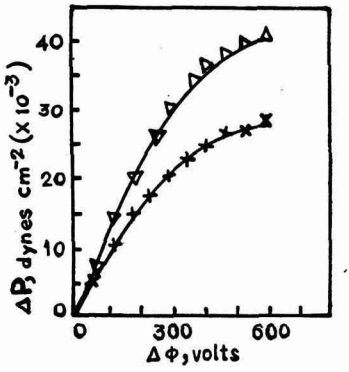


Fig. 5 — Dependence of electro-osmotic pressure (ΔP) on potential difference ($\Delta\phi$) [×, membrane IV; and △, membrane V]

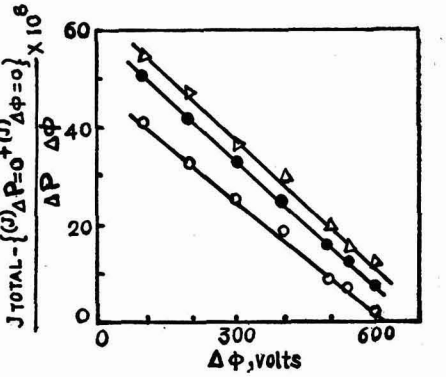


Fig. 6 — Estimation of L_{1122} and L_{112} using Eq. (11) [○, membrane I; ●, membrane II; and △, membrane III]

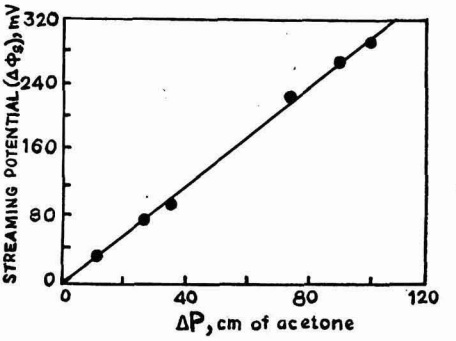


Fig. 7 — Estimation of L_{21} from streaming potential measurements

are non-linear. In electro-osmotic phenomena, there is interaction between flow due to a pressure gradient and the flow due to potential gradient. The former gives rise to high velocity gradient near the axis of the capillary and very low velocity gradient near the wall. In the case of electro-osmotic flow, it primarily takes place near the wall. The two flows would be independent, so long as there is no interaction near the wall and the net flow would be the sum of the two. The moment interaction starts, the flow may be expected to be non-linear. In physical terms, this may mean that the charge distribution near the interface would be disturbed when the flow exceeds a certain limit, thus probably affecting the position of the slip-plane.

Further work is needed to develop a macroscopic theory for the non-linear case. Refinements in the already existing theories based on models to include the non-linear case are needed. Experimental studies on isotropic, anisotropic and composite membranes are expected to yield greater insight into membrane processes, the understanding of which would help corresponding studies on biological membranes.

Summary

The thermodynamic theories of electro-osmotic effects in membranes have been reviewed. Theories of charged and uncharged membranes based on specific models have been discussed. Recent studies of electro-osmotic effects have been reviewed and discussed in the light of these theories, with special reference to non-linear effects.

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Sulphonyl Chloride—Chemistry & Application in Tanning Industry

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THIS review is concerned mainly with the manufacture of sulphonyl chlorides and their application in the tanning industry. The derivatives of sulphonyl chlorides, e.g. sulphonamides and aminosulphonamides, have been investigated intensively because of their value as chemotherapeutic agents. The use of sulphochlorides for tanning was not developed until World War II. During this war, Germany was confronted with the shortage of natural oils and was forced to develop synthetic oils for leather dressing[1,2]. Immendorfer[3] working in the I.G. Farbenindustrie was responsible for the development of sulphonyl chloride for leather tanning; it proved a good substitute for cod oil. It was later marketed as Immergan and was used widely in the leather industry. Several reviews and papers have since appeared on the use of sulphochlorinated mineral oils in leather tanning[4-8].

Preparation of Aliphatic Sulphonyl Chlorides

The various methods of preparing aliphatic sulphonyl chlorides can be broadly subdivided as follows: (1) sulphochlorination of hydrocarbons using ultraviolet light or in the presence of a catalyst (e.g. Reed's process); (2) formation of $-SO_2Cl$ radical using sulphuryl chloride and pyridine which is used to sulphochlorinate the hydrocarbons (e.g. Kharasch's process); and (3) aqueous chlorination of mercaptans, disulphides, etc.

Reed's process — Aliphatic sulphonyl chlorides can be prepared from various petroleum fractions having different carbon chain lengths. According to Balfe *et al.*[9], Immergan, the German synthetic oil, a C_{12}-C_{18} olefin fraction (known as Kogasin) from the Fischer-Tropsch process is hydrogenated to the *n*-paraffin (known as Mepasin). This is converted to the corresponding primary sulphonic chloride by a process described by Reed[10], namely running the paraffin down a column (packed with Raschig rings) up which sulphur dioxide and chlorine are passing and which is exposed to ultraviolet radiations. Reed's reaction is widely used commercially for the preparation of various aliphatic sulphonyl chlorides[11].

A complete review on the preparation of sulphonyl chlorides by Reed's process appeared[12] in 1958 and several others[13-16] in the period 1950-52, wherein the authors reviewed the German war-time work, the use of catalysts in the sulphochlorination reaction, and the purification and stability of the compounds prepared. Recently, Asinger[17] has reviewed the work done in his laboratory on the chlorination, sulphonation and sulphochlorination of C_6-C_{30} straight chain alkanes.

The general methods of preparation of aliphatic sulphonyl chlorides have been described in several patents[18-20]. Various linear aliphatic sulphochlorides using methane[21], propane[22,23], butane[24], hexane[25], octane[26-28], and other hydrocarbons[29-31], diesel and paraffin oils[32,33], aliphatic alcohol and fatty acid sulphonyl chlorides[34-36] were prepared by different workers.

Use of catalysts in Reed's process — In order to speed up the reaction between sulphur dioxide and chlorine during the sulphochlorination of hydrocarbons, various catalysts have been used in the presence or absence of actinic light[13,14]. Pyridines[37,38], amines[38,39] and azo compounds[40-42] were used widely as catalysts in such sulphochlorination reactions. In addition to these, azonitriles[43], organic peroxides[44-47], camphor[48], phosphates[49], organometallics[50,51], such as Et_4Pb, Et_4Sn, etc., and sulphur and sulphur chlorides[52] have also been used as catalysts. The main function of the catalyst in Reed's process is to avoid the side reaction leading to the formation of chlorinated oils. Also, the catalysts help to increase the yield.

Kharasch's process — In this process, instead of using chlorine and sulphur dioxide, sulphuryl chloride is used as the sulphonating agent for the aliphatic hydrocarbons[53]. Pyridine and ultraviolet rays appear to catalyse the reaction. The process is simple and gives almost pure compounds in laboratory preparations. Recently, a patent appeared on this reaction[54].

The mechanism of the reaction was dealt with in detail by Kharasch *et al.*[55]. The following mechanism of the photochemical sulphonation was proposed by them:

$$SO_2Cl_2 \xrightarrow{\text{Pyridine}} SO_2 + Cl_2 \quad \ldots\ldots(1)$$

$$Cl_2 \xrightarrow{h\nu} 2Cl\cdot \quad \ldots\ldots(2)$$

$$Cl\cdot + RH \rightarrow R\cdot + HCl \quad \ldots\ldots(3)$$

$$R\cdot + SO_2 \rightarrow RSO_2\cdot \quad \ldots\ldots(4)$$

$$RSO_2\cdot + Cl_2 \rightarrow RSO_2Cl + Cl\cdot \quad \ldots\ldots(5)$$

They found that by using different catalysts in this reaction the end products may be converted to either chlorinated or sulphochlorinated derivative or both.

The formation of the sulphochlorinated product in this reaction was also followed. The following changes take place in the organic radicals formed:

$$R\cdot + SO_2Cl_2 \rightarrow RCl + \cdot SO_2Cl \quad \ldots\ldots(a)$$

$$R\cdot + Cl_2 \rightarrow RCl + \cdot Cl \quad \ldots\ldots(b)$$

$$R\cdot + SO_2 \rightarrow RSO_2\cdot \quad \ldots\ldots(c)$$

Reaction (a) requires the maximum energy, reaction (b) requires less energy, and reaction (c) requires no energy. Hence the formation of sulphochlorination product is the most favoured reaction.

In the above process, different investigators used various catalysts to induce the reaction. The photochemical formation of sulphonyl chloride with sulphuryl chloride appears to be catalysed by the carboxyl group in the aliphatic chain[56], pyridine, quinoline and thiophenols[57,58] and phosphate compounds[49].

It was reported by Brown[59] that even aromatic sulphonyl chlorides could be prepared using sulphuryl chloride catalysed by aluminium chloride.

Aqueous chlorination of sulphur containing compounds — Sulphochlorination of aliphatic hydrocarbons may lead to side reactions forming chloro compounds, which are undesirable for certain purposes. Reasonably pure compounds, however, could be prepared by the chlorination[60-70] of mercaptans, sulphides and thiourea compounds. But chlorination of thioureas or isothioureas like alkyl isothioureas may lead to explosion hazards with the formation of nitrogen trichloride[71]. Apart from the methods described, different sulphonyl chlorides have been prepared from organic thiosulphates[72], thiocyanates[73-75], thiols[76,77] and sulphinates[78].

Of all the methods described so far, Reed's process is the simplest and the most widely used method for the manufacture of aliphatic sulphonyl chlorides.

Preparation of Aromatic Sulphonyl Chlorides

The general method of preparation of aromatic sulphonyl chlorides involves the action of chlorosulphonic acid on the aromatic hydrocarbons. Recently, the use of sulphuric acid or thionyl chlorides has also been recommended. Suter[79] and Suter and Weston[80] made an exhaustive survey on the preparation of aromatic sulphonyl halides. Several reviews on the preparation of aromatic sulphonyl chlorides cover the period[13-15] up to 1952. Some of the methods developed after 1950 are outlined below.

Aromatic sulphonyl chlorides have been prepared in high yields by reacting chlorosulphonic acid with sodium sulphonate[81]. By this method many aromatic compounds having substituent groups and also various benzene rings have been prepared. In yet another method, the products formed are purified directly using strong acids[82].

Some novel methods of preparing aromatic sulphonyl chlorides have been reported recently. The use of sulphuric acid as the sulphonating agent has been reported[83]. The reaction is:

$$RH+H_2SO_4+2CCl_4 \rightarrow RSO_2Cl+2COCl_2+3HCl$$

Both *ortho* and *para* toluene sulphonyl chlorides[84,85] have been obtained as byproducts in the manufacture of saccharin and chloramine-T. Here again, higher yields are obtained on using sodium salts.

Another method of preparing reasonably pure aromatic sulphonyl chloride is by the use of sulphonic acids in the presence of thionyl chloride and dimethyl formamide as a solvent base. *p*-Toluene sulphonyl chloride, *p*-acetyl benzene sulphonyl chloride[86,87] and 2-naphthalene sulphonyl chloride[88] were prepared by this method using sulphonic acids as the starting materials[89].

The preparation of other sulphonyl halides like sulphonyl bromide, iodide and fluoride has not been described; these compounds are of minor importance.

Purification of Sulphonyl Chlorides

The preparation of sulphonyl chlorides is often accompanied by side reactions leading mostly to the formation of chlorinated compounds, and occasionally of di- and polysulphonyl chlorides, depending upon the experimental conditions. In addition, free unreacted hydrocarbons may also be present in the reaction product. Hence, it is necessary to purify the products obtained by the different methods. The simplest methods to achieve this are distillation and fractional crystallization, which are followed widely. But at elevated temperatures, sulphochlorides tend to split off and consequently result in loss of the material. Even under reduced pressures, the separation is found to be difficult. Hence, other methods are to be resorted to for the separation of sulphonyl chlorides from the other reaction products. Certain solvents like liquid sulphur dioxide, dimethyl formamide, etc., are used for the purpose.

One of the most commonly employed methods involves the use of liquid sulphur dioxide at freezing temperature[90-94]. The organic sulphonyl halides are relatively soluble in liquid sulphur dioxide. In most of the systems, the sulphonyl halides are made to dissolve in the solvents and the insoluble products are removed. In addition to these, acetonitrile[94], dimethyl formamide[95], light hydrocarbon oils[96,97], β,β′-dichlorodiethyl ether[98], with a catalyst and high pressure[99] and with anhydrous fluoride[100] at freezing temperature have been used successfully to separate the sulphonyl halides formed.

Stability of Sulphonyl Chlorides

Sulphonyl chlorides tend to be unstable on storage, forming darker coloured compounds. Splitting up of sulphochlorides may also take place on storage over long periods. Data on this aspect are scanty. The addition of 1% of a bicyclic terpene, such as α-pinene, β-pinene, camphor, camphor oil and dipentene has been found to be effective in the stabilization of sulphochlorides against discolouration on storage[101]. A German patent[102] refers to the use of ammonia and formalin to improve the storage stability of sulphonyl chlorides, while the use of water and caustic alkali is recommended in a Hungarian patent[103].

Since pyridine, amines, azo compounds, etc., are used as catalysts in the preparation of sulphonyl chloride, these may also act as stabilizing agents. It is worth while studying the effect of the above agents on the stability of sulphochlorides.

Estimation of Sulphonyl Chlorides

The universal method of estimating sulphonyl chlorides is by estimating sulphur by barium sulphate method after oxidizing the sulphochloride using peroxide fusion mixture.

The sulphonyl chlorides are hydrolysed in the presence of alkali and the excess alkali is subsequently back-titrated for determining the sulphonyl chlorides[104,105]. Yet another method is to hydrolyse the sulphonyl chlorides in the presence of pyridine to sulphonic acid and subsequently titrating with stronger alkali[106,107]. In the direct titration method, hexamethylenimine is used in the presence of mixed methylene yellow and methyl red indicators[108]. In another method, the sulphonyl chloride is burnt in a Grote-Krekeler apparatus and the combustion products absorbed in alkaline peroxide solution[109]. A non-aqueous titration method for the estimation of sulphonyl chloride involves the use of pyridine

as solvent and tetrabutyl ammonium hydroxide as titrant[110,111].

Sulphonyl chlorides could also be estimated by various instrumental techniques like polarography, amperometry, colorimetry and spectrophotometry.

The polarographic reduction method was employed by several workers[109,112,113]. Ashworth *et al.*[114] determined both aliphatic and aromatic sulphonyl chlorides through visual and polarovoltric titrations using sodium sulphide as titrant. The end point could also be determined biamperometrically[115]. Very recently, Ashworth and Bohnstedt[116] reported that the sulphonyl chlorides could be estimated by spectrophotometric techniques. Chromatographic separation of sulphonyl chlorides has also been reported recently[117]. Colorimetric estimation of the sulphonyl chlorides using the Aldridge's reagent has been reported[118].

Spot tests specific for functional groups were developed for the characterization of sulphonyl chlorides. Aryl sulphonyl halides with ammonium thiocyanate gave a yellow precipitate[119] and 2-aminofluorene in pyridine produced a violet colour[120] when sulphonyl chlorides reacted with pyridine-alkali mixture; this produced a purple red colouration[117,121]. These colour reactions can be utilized for the estimation of sulphonyl chlorides.

Use of Sulphonyl Halides in Leather Manufacture

Sulphonyl chlorides in tanning — The use of sulphonyl chlorides in tanning has been recognized for a long time[4-8]. Immendorfer[3] described the use of synthetic oil for chamois tanning in 1944. Brown *et al.*[122] attempted to make sulphochlorinated oil from Pennsylvania (American) crude oil and used it in chamois tanning. Skelt, a compound similar to Immergan, the sulphochlorinated mineral oil, was produced by du Pont; details of the tanning procedure using skelt were described by Jurney[123]. The use of formaldehyde pretanning and alkali washing in chamois tanning using sulphonyl chloride was stressed by different workers[122-127]. A French patent[128] describes the preparation of chamois leather by pretanning with sulphonyl chloride and formaldehyde followed by iron tanning.

Sulphonyl chloride was also used along with aluminium salts to produce white, wash-fast glove leathers[129,130]. Another method reported concerns paraffin sulphonyl chloride tanning of glove leather after formaldehyde pretanning[131]. It is worth while attempting chrome sulphonyl chloride tanning in the case of glove and garment leathers. It may produce a soft supple leather, fast to washing and dry cleaning. By pretanning or retanning sulphonyl chloride tanned leather with zirconium or syntan, a white leather having desirable characteristics like fastness to light and washing can be produced.

Many investigators attempted to prepare sulphochlorinated oils from various petroleum fractions like diesel and kerosene oils and waxes[132-134]. The products were found to give satisfactory leather. Srinivasan and Nayudamma[135] made a systematic study of aliphatic and aromatic sulphochlorides produced from different hydrocarbons, aliphatic fatty acids and alcohols. They found that both aliphatic and aromatic sulphonyl chlorides possess tanning property, the degree of tanning with respect to aromatic sulphonyl chlorides depending upon the number of aromatic rings present. Further they reported that the sulphochlorination of aliphatic hydrocarbons depends on the nature of the hydrocarbon — chain length, degree of saturation and branching of the chains. The tanning potency of sulphochlorinated fatty acids and alcohols was influenced by the OH and COOH groups.

Many improvements in sulphochlorinated oils in order to impart special characteristics to the tanned leathers have also been suggested. When an aromatic sulphonyl chloride is reacted with amino arylacylarylamides which still contain an acylable amino group, a soluble product containing at least 4 sulphonimide groups and 2 sulphonic groups is obtained[136]. The product obtained is a good tanning agent and a useful dye auxiliary. An improved product[137] was obtained by treating sulphochloride radicals with alkylene oxides or polybasic alcohols. The product gave a white, soft, compact and tough leather. Sulphochlorinated phthalic acids[138] or other similar products resulted in improved tanning. Before and after tanning, the leather was treated with salts of metals which were able to form complexes with the said groups. In another patented process reported[139], the insoluble sulphonyl chlorides of long chain hydrocarbons are mixed with the corresponding sulphamides and alkali salts; a water-soluble product suitable for use as a tanning agent is obtained.

Sulphonyl chlorides as fatliquoring agents — Several chlorinated and sulphochlorinated oils are used as fatliquoring agents for chrome and other leathers after amidation and condensation. Das[1] and Spiers[2] worked on Derminol oils (chlorinated mineral oils) and the products of their amidation and condensation with chloroacetic acid. The condensed product was subsequently sulphonated for use as a fatliquoring agent with or without natural oils. Similar fatliquoring agents were reported in two other patents[140,141]. Otto[142] found that the use of sulphochlorinated oils along with sulphated oils resulted in the avoidance of excess neutralization, a tighter grain and a more uniform distribution of the oil. Yet another patent[143] concerns the use of chlorinated or sulphochlorinated polyolefins like polypropylene for fatliquoring leathers.

Sulphonyl chlorides as leather impregnants — Sulphochlorinated oils after amidation and emulsification were used as impregnating agents for chrome, vegetable and other leathers to get improved properties. Several patents[144-148] have been taken on the impregnation of vegetable and other leathers.

Other Uses of Sulphonyl Chlorides

The use of sulphonyl chlorides in tanning industry has been very limited compared to their application in pharmaceuticals and detergent industries. Sulphonyl chlorides are used as rubber additives[149-154], emulsifying agents, polymeric sulphonamides[155-159], lubricant additives[160-164], photographic colour developing agents[165-170], wetting and surface active agents[171-184], textile auxiliaries[185-189], dye auxiliaries[190-199], in electroplating industries as metal surface

improvement agents[200-203], antioxidants in petroleum products[204-207], softeners and solvents for plastics[208-211], fire fighting agents[212,213], rust preventing agents[214,215], in accelerating the rapid drying of printing inks[216-218], pesticides and disinfectants[219] and as hair waving compounds[220].

Mechanism of Sulphonyl Chloride Tanning

Many workers have suggested that sulphonyl chloride reacts with pelt and forms a definite chemical compound, which cannot be removed easily or extracted by alkali or solvent. In 1932, Clarke and Gillespie[221] reported the reaction of benzene sulphonyl chloride with the amino acid arginine. Later, Gurin and Clarke[222] reacted gelatin with benzene sulphonyl chloride in the presence of caustic alkali. In sulphonyl chloride tanning, the main reactive group involved was found to be the amino group. Immendorfer[223], using aliphatic sulphonyl chloride for tanning, suggested that sulphonyl chloride tannage consists mainly of reaction between the uncharged amino groups and RSO_2Cl to form sulphonamides. Patterson[224], from his comprehensive study, showed the existence of $-SO_2-$ groups in hide powder tanned with RSO_2Cl; through the infrared spectra of the hydrolysed proteins the formation of sulphonamide group was established.

Nayudamma and Ranganathan[225] suggested, from shrinkage temperature measurements on sulphonyl chloride tanned leather individually and in combination with other tannages, the formation of sulphonamide linkages without involving any crosslinks, since there was no difference between the shrinkage temperature of the original collagen and that of RSO_2Cl tanned leather. Kenchington and Lauder[226], using solution viscosity as a measure of crosslinking, studied the reaction of bifunctional sulphonyl chloride benzene *m*-disulphonyl chloride with gelatin. They found that the crosslinking efficiency of benzene *m*-disulphonyl chloride increases with increase in *p*H.

On reacting $PhSO_2Cl$, a monofunctional sulphonyl chloride and diphenyl methane — 4,4′-bis(sulphonyl chloride), a bifunctional sulphonyl chloride, with various substrates like amino acids, gelatine, hide powder and acetone dehydrated pelt, Stather *et al.*[227] found that crosslinking with collagen molecules is unlikely. By studying the effect of hydrothermal shrinkage and recovery on the optical birefringence of the tanned collagen fibres, Ramanathan and Nayudamma[228] found that sulphonyl chloride reacts with collagen without forming additional crosslinkages. Additional work is essential to throw light on the crosslinking mechanism of sulphonyl chlorides, especially to show whether a bifunctional sulphonyl chloride forms crosslinks with collagen or not.

By pretreating protein with sulphonyl chloride Nayudamma *et al.*[229] demonstrated that sulphonyl chloride activates and fixes approximately double the quantity of formaldehyde as compared to the ready reactivity of the sulphonamides formed with the acid amide groups at low *p*H values and with amino and guanidino groups at higher *p*H values. They further showed that the likely site for the fixation of sulphonyl chloride may be amino, carboxyl, peptide, acid amide, guanidino and hydroxyl groups. An elaborate study by Srinivasan and Nayudamma[230] using modified hide powder, polyamide and combination tanned systems showed that the amino groups are involved. This finding was also substantiated by the results obtained on the isoelectric point of the RSO_2Cl treated collagen.

Summary

The literature up to 1966 on the various methods of manufacture of sulphonyl chlorides, their purification and estimation is reviewed. The different uses of sulphonyl chlorides in the leather industry are discussed, indicating the possibilities for future application. The other uses of sulphonyl chlorides are also enumerated.

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Chemical Modification & X-ray Crystal Structure of Proteins

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IN the last several years, many proteins have been isolated, purified and characterized. Each one of them has a specific biological function to perform. The mechanism with which a protein or an enzyme performs its function is, therefore, of considerable interest. The biological function of a protein depends not only on the sequence of its amino acids or the primary structure but also on its secondary and tertiary structures. Well-known types of secondary structure of a polypeptide chain are the α-helix and the random coil. Tertiary structure is the three-dimensional arrangement of secondary structure, so that the particular amino acid residues that comprise the ' active site ' are brought into a unique position suitable for biological function.

The primary structures of insulin, ribonuclease, myoglobin, haemoglobin, TMV protein, trypsin, chymotrypsin, lysozyme, cytochrome *c*, and a few others have been determined by laborious chemical methods. But the determination of their secondary and tertiary structures has been a difficult problem until recently.

Physical methods, such as electrophoresis, sedimentation, diffusion, light scattering, osmotic pressure, viscosity and optical rotary dispersion have been used to obtain information about the homogeneity, molecular size and shape and conformation of proteins in solution. In the last several years, X-ray crystallography has been applied to the study of three-dimensional structure of crystalline proteins. But the important problem in X-ray analysis is the determination of the relative phases of the diffracted waves.

The multiple isomorphous replacement (MIR) technique has become the basis for the determination of phases in the X-ray crystallography of globular proteins. This technique requires excellent crystals of the native protein and at least two isomorphous heavy atom derivatives. Heavy atom derivatives isomorphous with the native protein have been used not only for the solving of phases in the determination of three-dimensional structure of proteins, but also to locate the amino acid residues at the ' active site '.

Only myoglobin and haemoglobin have yielded nicely to X-ray investigation because of their high helix content and the availability of an excellent series of isomorphous heavy atom derivatives. An extraordinary feature of the helical structure of proteins is that it is a highly organized structure and responds well to X-ray analysis. However, many proteins such as lysozyme, chymotrypsin, ribonuclease and insulin are low helix proteins; furthermore, good isomorphous heavy atom derivatives are not always available with these proteins. But still their structures also have been solved only recently.

Most of the heavy atom derivatives used for X-ray investigation are complexes in which the heavy atoms are not covalently linked to the protein. Also, the site on a protein to which such a complex will be attached cannot be predicted easily in advance. At present, the search for heavy atom derivatives is largely by trial and error and their success is judged by the appearance of changes in the X-ray pictures. Actually, hundreds of derivatives have to be tried before getting a few satisfactory ones. Therefore, there is a great need for systematic methods based on a better understanding of the fundamental chemistry of the proteins in which specific sites on protein molecules are labelled with heavy atoms. Specific single site labelling techniques would be of great help to the protein crystallographer rather than the non-specific methods of labelling. Thus, chemical modification of proteins for X-ray investigation has become a very important step in the determination of their tertiary structures. In fact, in the early stages of three-dimensional structure determination the protein chemist plays a more vital role than the crystallographer. Once good derivatives have been found, the solution of the structure is in principle only a matter of time and hard work.

The purpose of this article is: (1) to give a brief account of the important stages in the determination of three-dimensional structure of a protein by X-ray diffraction and to discuss the importance of isomorphous heavy atom derivatives in the determination of phases; (2) to give an account of the heavy atom reagents used to label specifically certain amino acid residues in proteins with heavy atoms and to discuss their utility in the determination of phases of reflexions; and (3) to describe and discuss the results obtained from X-ray diffraction studies of various proteins. In the past few years, the amount of information accumulated on the tertiary structures of proteins is so enormous that only material on a few typical proteins such as myoglobin, haemoglobin, lysozyme, ribonuclease, α-chymotrypsin, papain and insulin, irrespective of whether or not chemically modified derivatives have been used for the determination of phases, has been included in this article. Here, only globular proteins are considered, since the multiple isomorphous replacement technique is at present applicable only to such proteins.

X-ray Crystallography of Proteins

The X-ray diffraction technique is a powerful tool in the determination of molecular structures by single crystal analysis. The technique of single crystal X-ray analysis is used to solve the three-dimensional structures of not only small molecules but also macromolecules like proteins. By judicious use of this method it is possible to obtain information on the arrangement of individual atoms in the molecule and hence its three-dimensional structure. This method along with the mathematical treatment has been reviewed by Dickerson[1].

The Unit Cell

The unit cell is the smallest unit of a crystal; it will generate the entire crystal when repeated in three dimensions. It has all the essential features of crystal structure; therefore, a statement of the unit cell structure is a complete account of the pattern in the crystal. The unit cell is always a parallelopiped having the sides a, b and c, and the angles between them α, β and Υ. Unit cell axes need not be mutually perpendicular, although the calculations will be simplified if they are.

Crystals are composed of groups of atoms repeated at regular intervals, with the same orientation in three dimensions. For some considerations, it is sufficient to regard each group of atoms as a representative point. The collection of points so formed is called the space lattice or real lattice of the crystal. The space lattice consists of repeated unit cells. In X-ray diffraction, X-rays are scattered by the electrons in atoms. The intensity of spots or reflexions in the diffraction pattern depends, therefore, upon the number of electrons at the lattice points in the crystal.

Bragg's Equation

The fundamental equation in X-ray crystallography is the Bragg equation

$$2d \sin \theta = n\lambda$$

which is the condition for diffracted beams to be in phase or for constructive interference; d is the distance between the real lattice planes; θ, the angle which the incident X-ray beam makes with these planes (this angle is known as the Bragg angle); λ, the wavelength of monochromatic X-rays; and n, an integer which specifies the order of reflexion. Only when this equation is satisfied, X-rays will be reflected by lattice points in the crystal. There will be a spot in the diffraction pattern for each of the reflexions.

Reciprocal Lattice

There is a reciprocal relation between the diffracting object and its diffraction pattern; the diffraction pattern is called the reciprocal lattice. This represents a lattice with points whose distance from the origin is equal to the sine of the Bragg angle, given by the relation involving the reciprocal of d: $\sin \theta = \lambda/2d_{hkl}$. All reflexions for any particular wavelength are represented by points or spots in the reciprocal lattice that fall within a sphere of unit radius (since the sine of the Bragg angle cannot be greater than unity) known as the sphere of reflexion. The diffraction pattern can be considered as a three-dimensional arrangement of spots, with three indices (h, k, l) being required to locate and identify each spot.

The photographs taken in an X-ray camera are those of reciprocal lattices of single crystals. Therefore, reciprocal lattices are very valuable in the determination of unit cell parameters and the symmetry of crystals. The unit cell dimensions are determined from a knowledge of the spacings of spots in the reciprocal lattice, wavelength of the X-radiation used and crystal to film distance.

Symmetry Elements

The packing of molecules in a crystal is usually not very simple. Factors such as the shape of the molecule, charge effects and intermolecular hydrogen bonding play a very important role. In order to understand this, it is essential to know something about the symmetry elements. The symmetry of the crystal is described in terms of certain symmetry operations which are the result of certain symmetry elements. The possible symmetry elements are twofold, threefold, fourfold and sixfold rotations, as well as combination of rotation with displacement along the rotation axis (screw rotations), reflexions across a mirror plane, combinations of reflexion with displacement (glide reflexions) and inversion of the entire molecule through the centre of symmetry. Reflexions, glides and inversions are not encountered in proteins, because they require equal numbers of right- and left-handed molecules. The screw rotation which is a helix is the most commonly occurring element of symmetry in protein crystals.

Space Groups

Crystals have been classified into seven systems on the basis of the symmetry of the lattice, and there exists a particular relationship between unit cell axes in these systems. Although it would appear that an infinite number of arrangements for packing irregular objects in a regular manner is possible, crystallographers have actually shown mathematically that there exist only 230 symmetrically distinct arrangements called 'space groups'. Each one of these space groups falls into one or the other of the seven crystal systems.

The space group expresses the sum total of the symmetry properties of a crystal structure, and mere external form or bulk properties do not suffice for its determination. They can be determined from the symmetry of the diffraction pattern and from the distribution of extinctions or systematic absences of reflexions or spots in the pattern or the X-ray photograph.

Molecular Weight

X-ray diffraction technique is an accurate method of determining the molecular weight of protein. If ρ is the density of the crystal in grams per cm^3, V, the volume of the unit cell in cm^3 as found from X-ray measurements, and N, the Avogadro number, then the molecular weight M is given by the relation: $M = N\rho V/n$, where n is the number of molecules in the unit cell. In any real crystal, however, liquid of crystallization will be present between molecules and the value of M must be corrected by multiplying with the percentage of protein by weight in the crystal to obtain the true molecular weight.

Asymmetric Unit

The portion of the unit cell which is completely independent of symmetry and which by the action of symmetry operations fills the cell completely is called the asymmetric unit. The number of molecules per asymmetric unit depends upon the nature of the molecule, the space group and the

symmetry elements of the crystal system. For example, in monoclinic myoglobin, the unit cell contains two molecules; but the asymmetric unit consists of only one molecule. So the molecular weight is equal to that calculated for the asymmetric unit. On the other hand, haemoglobin consists of half the molecule per asymmetric unit, the other half being related by twofold axis. Then, it has a molecular weight twice that of the asymmetric unit. Therefore, the number of molecules per asymmetric unit and the number of asymmetric units per unit cell are very important in characterizing the crystal.

Resolution

Bragg's equation indicates that for any given wavelength of X-rays there is a lower limit to the spacings that can give an observable diffraction pattern. Since the maximum value of $\sin\theta = 1$, this limit is given by

$$d_{\min} = \frac{\lambda}{2\sin\theta_{\max}} = \frac{\lambda}{2}$$

This is what is called 'resolution'. The resolution of an electron density map in Å is a rough measure of the smallest details one can expect to see in the map. Higher the resolution, the finer the details that one can observe. Since the diffraction pattern, like the crystal, is three-dimensional, the number of reflexions or spots in a sphere of given radius in the pattern increases as the inverse cube of the resolution. Therefore, the amount of data required for a given resolution will be greater the higher the resolution. For example, in the X-ray analysis of myoglobin, only 400 reflexions were needed for 6 Å resolution, 9600 at 2 Å, and over 25,000 at 1·4 Å resolution. This means that it may be wise to study a protein structure first at low resolution to be sure of the methods and derivatives employed before investing an enormous amount of time and hard work required for high resolution analysis.

Fourier Series Representation

A crystal is essentially a three-dimensional periodically repeating structure. It is considered as a continuous distribution of electron density, reaching maxima at the atomic centres and falling off asymptotically to zero in the space between atoms. Since this distribution is periodic in three dimensions, it can be represented by a three-dimensional Fourier series

$$\rho(x,y,z) = \frac{1}{V}\sum_{-h}^{h}\sum_{-k}^{k}\sum_{-l}^{l}|F_{hkl\ obs.}|\cos[2\pi(hx + ky + lz) - \alpha_{hkl}]$$

where x, y, z are fractional coordinates; V, the unit cell volume; and h, k, l, the indices of reflexions. The summation is carried out over all the values of h, k, l, so that there is one term for each spot or reflexion on the X-ray diffraction picture. α_{hkl} is the phase of the reflexion h, k, l, and $|F_{hkl\ obs.}|$ is the positive square root of $|F_{hkl\ obs.}|^2$, which is related to the intensity of the reflexions. The Fourier coefficient, $\boldsymbol{F_{hkl}}$, known as the structure factor, is a complex number, representable either in terms of its real and imaginary components or in terms of a magnitude (structure amplitude $|F_{hkl}|$) and a phase ϕ_{hkl}:

$$\boldsymbol{F}_{hkl} = A + iB = |F_{hkl}|e^{i\phi_{hkl}}$$

The only experimentally measurable quantity in X-ray diffraction studies is the intensity of the reflexions collected under a specific resolution. It is related to the structure amplitude by the relations:

$$I_{hkl}\,(\exp) \propto |F_{hkl\ obs.}|^2 = \frac{|F_{hkl\ obs.}|^2 KLp}{A}$$

$$\therefore\ |F_{hkl\ obs.}|^2 = \frac{I_{hkl}\,(\exp)}{K}\cdot\frac{A}{Lp} = I_{hkl}(rel.obs)\frac{A}{Lp}$$

$$|F_{hkl\ obs.}|^2 = I_{hkl}(abs.\ obs.)$$

where K is the scaling factor, obtained by scaling all the reflexions with reference to a particular set appearing on all the levels; I_{hkl}, intensity of each of the reflexions; L, Lorentz factor — a correction for the geometry of the X-ray camera; p (polarization correction) $= (1+\cos^2 2\theta)/2$; A (absorption correction) $= e^{-\mu t}$; μ, absorption coefficient; and t, thickness of the reflecting path in the crystal.

To determine the crystal structure by Fourier synthesis, relative phases of the reflexions must be known. However, the measured intensities are free from phases. Therefore, they should have to be determined indirectly. This is a major problem in X-ray crystallography. But for this problem, the determination of three-dimensional structure would have been a very simple process.

Calculation of Theoretical Structure Factor

Here, one has to take a close look at the Fourier coefficient. This coefficient, known as the structure factor $\boldsymbol{F_{hkl}}$, is the corner-stone in X-ray diffraction studies, and its determination is the ultimate aim in the solution of a structure. This quantity is a measure of the intensity of the beam scattered by the whole unit cell expressed in terms of the amount scattered by a single electron as a unit. The structure factor is defined by the relation:

$$\boldsymbol{F}_{hkl} = \sum_r f_r e^{2\pi i[hx_r + ky_r + lz_r]} = \sum_r f_r e^{i\alpha_r}$$

where the summation is carried out over r number of atoms for a particular reflexion with the indices $(h;\ k,\ l)$, and $f_r = f_0 e^{-B\sin^2\theta/\lambda^2}$ where f_r and f_0 are respectively the scattering factors of the vibrating atom and stationary atom, which depends upon $\sin\theta/\lambda$, and B is the temperature factor, which is different for different atoms in a structure, and may also vary with direction.

The quantity f_r is equal to the atomic number for zero deflexion of the X-ray beam and falls off smoothly with increasing angle of scattering. The phase factor $\alpha_r = 2\pi(hx_r + ky_r + lz_r)$ acknowledges the fact that all atoms are not at the same place and specifically that the rth atom is located at $(x_r,\ y_r,\ z_r)$. Scattering from any one atom may, therefore, be in or out of phase with that of a neighbour, and the summation of contributions to the total scattered beam must be complex rather than simply algebraic.

$$\therefore F_{hkl} = \sum_r f_r \cos 2\pi(hx_r+ky_r+lz_r) + i\sum_r f_r \sin 2\pi(hx_r+ky_r+lz_r)$$

$$F = A+iB \quad \therefore FF^* = |F|^2 = A^2+B^2$$

Then,

$$\tan \alpha = B/A$$

Therefore, in order to calculate the phase α_{hkl} for each reflexion needed for Fourier synthesis, the coordinates of the atoms in the unit cell must be known.

Patterson method — An attempt to locate the positions of atoms and to solve for the relative phases of the reflexions was first made by Patterson in 1934. Instead of the structure factor, $F_{calc.}$, he used the squares of structure amplitudes as Fourier coefficients. These quantities are directly related to the observed intensities which are measured without any ambiguity. He showed that the resulting synthesis was related in some manner to the crystal structure. This function, known as the Patterson function, $P(u, v, w)$, is defined by the relation:

$$P(u,v,w) = \frac{1}{V}\sum_{-h}^{h}\sum_{-k}^{k}\sum_{-l}^{l} |F_{hkl\ obs.}|^2 \cos 2\pi(hu+kv+lw)$$

The summation in the Patterson function is carried out for every grid point over all the reflexions (h, k, l). $P(u, v, w)$ is real for all values of u, v, w. This does not give the positions of atoms, but only all the possible vectors (u, v, w) between atoms in the unit cell. For an array of N atoms, there will be $N(N-1)$ possible vector peaks which are essentially distinct. These peaks are the ends of the interatomic vectors with one end situated at the origin.

Since proteins contain thousands of atoms other than hydrogens, it is not possible to resolve the very large number of peaks obtained in their Patterson maps. It is here that the protein chemist comes to the crystallographer's aid by preparing excellent heavy atom isomorphous derivatives. The interpretation of the diffraction pattern will be simpler if only a few heavy atoms are introduced into the protein. Then, it would be easier to resolve a smaller number of vector peaks.

Straight Patterson maps of proteins are, therefore, virtually unintelligible. But difference Patterson maps of one type or another are quite useful in locating the heavy atom sites in the unit cell. They use some function of the change in intensities, produced by the heavy atom groups, as coefficients.

The vector peaks in the difference Patterson map are located and their coordinates (u, v, w) determined. Afterwards, the positions (x, y, z) of the heavy atoms are derived from the knowledge of interatomic vectors. Heavy atom parameters obtained are refined by difference Fourier and least squares methods[2,3]. Proper allowance for anomalous scattering of X-rays by various heavy atoms in the derivatives must also be made[4,5]. Once the heavy atoms are located in the unit cell, the structure factor f_H of the heavy atom is calculable both in magnitude and phase for each of the reflexions in the diffraction pattern. The phases of each of the reflexions from the protein crystal are calculated using the multiple isomorphous replacement technique.

In the early stages of an analysis it is often convenient to work with two-dimensional projections of a structure which may possess a centre of symmetry. Then the structure factor is just a scalar quantity with a plus or minus sign or with the phase angles limited to 0° or 180°C. The reflexions of this type will have then only signs, and not phases, to be determined. Such a situation can greatly help the search for heavy atom sites.

Multiple isomorphous replacement technique — This method which has been used successfully in nearly every protein structure analysis for phase determination has been reviewed elsewhere[6-8]. Here it will be described only briefly. The technique involves the introduction of a heavy atom or group of atoms at specific sites on the protein molecules without disturbing the rest of the structure. Here, the heavy atoms scatter X-rays more strongly than the atoms contained in the protein, and thus produce only detectable intensity changes, and do not alter the mode of crystallization. In other words, the heavy atom derivative must be isomorphous with the parent protein.

The ideal heavy atom derivative is one in which the heavy atom is bound with high occupancy to a single site on the protein without altering its mode of crystallization. Under these conditions, it should be possible to locate the position of the heavy atom without difficulty. Complete isomorphism can be achieved only rarely, since the substitution of the heavy atom will in general effect some change in the position of the protein molecule relative to its neighbours in the crystal. Small departures from isomorphism may, however, be overlooked at low resolution, but for high resolution structure analysis large departures from isomorphism, characterized by changes in the unit cell dimensions of more than 0·5%, may not result in accurate phase determination.

The lower limit of atomic weight for an acceptable heavy atom was discussed by Crick and Kendrew[7]. This will depend upon the degree of isomorphism and on the percentage occupancy of the heavy atom binding site. In general, it appears that for small protein molecules with molecular weights less than 20000 such as myoglobin or lysozyme, atoms such as iodine may be used satisfactorily. Indeed, iodine has given promising results in the case of α-chymotrypsin (mol. wt 25000) under conditions where a high degree of isomorphism was achieved. With proteins of higher molecular weight, heavier atoms such as mercury are needed.

The structure factor of a heavy atom derivative of a protein, F_{PH}, is the sum of the contributions from the protein alone, F_P, and that from the introduced heavy atom group, f_H: $F_{PH} = F_P + f_H$. Once the heavy atoms are located in the unit cell, the heavy atom contribution f_H is calculable both in magnitude and phase for each of the reflexions in the diffraction pattern. When the intensity of each reflexion has been measured from crystals with and without the heavy atom groups, the magnitudes $|F_P|$ and $|F_{PH}|$ are also known. The phase problem then becomes simply a geometric one of finding the orientation of a triangle when the lengths of all three sides and the direction of

one side are known. There will be two acceptable solutions, but the ambiguity can be resolved by finding a second heavy atom derivative. This will produce two possible phase angles as before, one which is the true one will be the same as one of the choices from the first derivative. Actually, due to errors in the measurements of intensities of reflexions, and in the location of heavy atoms, the minimum of two derivatives is rarely satisfactory, and the accuracy of phase determination is greatly improved by the use of three, four, five or even six derivatives.

At low resolution when the number of reflexions is not large, phases are determined by graphical means[9]. But when the number of reflexions at high resolution is very large, phase determination is carried out on the computer rather than graphically. The 'best' phases and amplitudes were computed and used in the final Fourier synthesis. Questions of proper treatment of errors, obtaining the best electron density map and adapting phase analysis for machine computation have been discussed by Blow and Crick[10], Dickerson *et al.*[11] and Rossmann and Blow[12].

Electron density map — With the knowledge of both the phase and the amplitude of each of the reflexions, an image of the diffracting material of the crystal, namely the electron density of the protein, is calculated. In the electron density map thus obtained, more atoms in addition to those included in the phase determination can be seen. The positions of these new as well as old atoms are found out and used to calculate the improved $F_{calc.}$ and the phase. The cycle is repeated until the observed and the calculated structures converge.

The calculation is computed at a series of equally spaced grid points over the entire unit cell. The resulting values are tabulated in sections along one unit cell axis in a form suitable for contouring. The contours are then traced on to transparent 'perspex' sheets and the sheets stacked in parallel, so that the electron density could be viewed in three dimensions. In general, it is expected that the electron density will be highest at the atomic positions, less along the bond lengths, and zero elsewhere. The experienced eye can recognize the distinctive side chains of the amino acid residues and identify peptide groups and the course of the main chain.

Equipments and facilities —X-ray diffraction studies are carried out using single crystals of the native protein and at least two isomorphous heavy atom derivatives and copper K_α ($\lambda = 1{\cdot}54$ Å) X-rays. Largely, photographic techniques are used for data collection: Buerger's precision camera (Weissenberg's camera is not used in the case of proteins since the spots in the diffraction photographs are too closely spaced for accurate interpretation) for taking X-ray photographs and microdensitometer for measuring the intensities of reflexions on them. But recently, automatic diffractometers which do both these tasks simultaneously have come into use. With these automatic equipments the intensities of successive reflexions, measured with a proportional counter, are recorded on punched tape which can be fed directly into a high speed computer.

Calculations involving Fourier summation to plot the electron density map, phase determination, and other problems in X-ray diffraction studies of proteins cannot be carried out by hand. An enormously large amount of time would be required. Therefore, very fast and large computers are highly necessary, and used largely for all such studies. The delay experienced till 15 years ago in the determination of three-dimensional structures of a number of proteins was due to the non-availability of computers, especially large and very fast computers. In addition, the availability of suitable heavy atom derivatives isomorphous with the native protein is one of the greatest problems in the determination of the tertiary structure of a protein.

Heavy Atom Reagents

The synthesis of heavy atom reagents, their physical characteristics, their specificities for the particular amino acid residues in proteins, the conditions for their reaction and their utility in the evaluation of phases needed for the determination of three-dimensional structure of a protein are discussed below.

p-Chloromercuribenzoic acid (*PCMB*) — This is synthesized by the method of Whitmore and Woodward as presented by Blatt[13]. It can be prepared by the oxidation of *p*-tolylmercuric chloride with alkaline potassium permanganate. It is also commercially available, m.p. 273°C.

This reagent is specific for cysteine residues. Many reactions of —SH groups in proteins take place preferentially as the mercaptide ions. The *p*H range for such reactions varies from 7 to 10. This is also the actual range of *p*H for the ionization of these —SH groups in proteins. However, the actual *p*H for the reaction will depend upon the nature of the protein and of the medium, and also on the environment in which the group is situated in the protein.

p-Chloromercuribenzoic acid can be used only in the case of proteins which possess sulphydryl groups. Unfortunately, however, many proteins do not possess such groups. Haemoglobin consists of four polypeptide chains, two α- and two β-chains. Each chain contains an —SH group, a definite site for the attachment of a heavy atom like mercury, readily reactive on the β-chains, but much less so on the α-chains.

The reaction of PCMB with horse haemoglobin was carried out as follows: Haemoglobin crystallized at *p*H 6·9 was allowed to react with PCMB in a salt solution consisting of 0·17 and 0·83 mole % of $(NH_4)_2HPO_4$ and $(NH_4)_2SO_4$ respectively. Excellent monoclinic crystals suitable for X-ray diffraction studies were formed below *p*H 7·1 in one or two weeks.

Papain is a proteolytic enzyme which contains only one —SH group. Its reaction with PCMB reagent was carried out as follows: orthorhombic crystals of papain C obtained from methanol-water mixtures were soaked in 0·0015*M* PCMB. The solvent was a mixture of 20 ml methanol and 10 ml of 0·1*N* aminoethanol buffer at *p*H 9·3.

These PCMB derivatives of haemoglobin and papain are isomorphous with the native proteins

and, therefore, they have been very useful in the evaluation of phases needed for the determination of three-dimensional structures.

p-Iodobenzenesulphonyl chloride (pipsyl Cl) and p-iodobenzenesulphonyl fluoride (pipsyl F) — The synthesis of pipsyl chloride is based on an adaptation of the method of Roger Adams and Marvel as presented by Blatt[14]. It can be prepared by the action of PCl_5 or $POCl_3$ upon the salts of *p*-iodobenzene sulphonic acid or by the action of chlorosulphonic acid on iodobenzene. It can be recrystallized from *n*-hexane. This reagent is also commercially available.

Pipsyl fluoride is synthesized from pipsyl chloride by the method outlined for benzenesulphonyl fluoride by Sigler *et al.*[15], m.p. 86°C.

Both pipsyl chloride and pipsyl fluoride are specific for active serine residues in proteinases, e.g. their use in labelling the active serine residue in α-chymotrypsin (α-CHT) with a heavy atom.

Kallos and Rizok[16] used pipsyl chloride to label the active serine residue in Υ-CHT with a heavy atom. The reaction was carried out as follows: Υ-CHT (50 μmoles) dissolved in phosphate buffer (*p*H 7·8) at 0°C was treated with two portions of pipsyl chloride (35 μmoles) in ice-cold acetonitrile added successively at 20 min intervals. (Acetonitrile concentration should not exceed 10%.) After 1 hr, the reaction mixture was dialysed extensively at 4°C against $10^{-4}M$ HCl. The pipsyl-CHT isolated by freeze drying was crystallized from ammonium sulphate at $0{\cdot}5M$ saturation.

Inhibition with pipsyl fluoride of the enzyme in solution as well as in crystalline form was carried out by Sigler *et al.*[15]. Inhibition of α-CHT in solution was carried out as follows: a 1% α-CHT solution ($4\times10^{-4}M$) in $0{\cdot}05M$ phosphate buffer (*p*H 7·2) containing 5% acetonitrile was mixed with an equal volume of a $4{\cdot}2\times10^{-4}M$ sulphonyl fluoride solution in the same solvent and allowed to stand for not over 4 hr. The addition of the inhibitor solution in acetonitrile to the enzyme must be regulated so as to maintain a single phase with minimum agitation of the enzyme solution. The enzyme solutions were dialysed exhaustively against $10^{-3}M$ HCl in the cold and lyophilized. The inhibited enzyme was crystallized from a 1·25% protein solution $0{\cdot}5M$ saturated with ammonium sulphate.

The activity of the crystals of α-CHT ($4\times10^{-8}M$) was inhibited by exposing them for 6 days to 5 ml of standard $(NH_4)_2SO_4$ supernatant solution containing 2% acetonitrile and 3×10^{-5} mole of the inhibitor. The reacted crystals were rinsed free of unreacted inhibitor with standard $(NH_4)_2SO_4$ supernatant solution and crystals removed for X-ray diffraction studies.

Derivatives prepared from pipsyl fluoride were found to be more suitable for crystallographic studies than those prepared from pipsyl chloride using the method of Kallos and Rizok[16].

Pipsyl-α-CHT is a very good heavy atom derivative isomorphous with tosyl-α-CHT, and has been used for the determination of three-dimensional structure of α-CHT by Blow and his associates.

Pipsyl halides also react with other amino acids like lysine, cysteine and tyrosine in peptides and proteins, e.g. pipsyl chloride has been used by Fletcher[17] to label lysine and tyrosine selectively with a heavy atom in insulin with a view to locating the concerned residues by chemical means.

p-Chloromercuribenzenesulphonyl fluoride (PCMBSF) — This has been synthesized by two alternate methods as described by Sigler *et al.*[15]. In one of these a mercury atom can be introduced into an aromatic nucleus at the position of an amino group, by proceeding through the diazonium chloride. In the other method, potassium salt of *p*-chloromercuribenzenesulphonic acid is converted to the sulphonyl chloride by refluxing with $POCl_3$. The crude sulphonyl chloride is converted directly to the sulphonyl fluoride with potassium fluoride, m.p. 254-256°C.

This reagent, like pipsyl halides, is specific for serine residues in proteinases. It has been used to covalently link the active serine residue in α-CHT with a heavy atom. The method of preparation of PCMBS-α-CHT is similar to the one used for pipsyl-α-CHT.

PCMBSF was expected to be an ideal reagent for preparing heavy atom derivative isomorphous with tosyl-α-CHT, as it inhibits the enzyme readily and stoichiometrically. However, difference Fourier projections of several preparations showed that the mercury atom was distributed with a variable relative occupancy. Therefore, this derivative was considered to be unsuitable for phase determination.

Phenyl mercuric acetate (PMA) — Phenyl mercuric acetate can be synthesized by the direct mercuration of benzene with mercuric acetate. Mercurated benzene may be obtained by this method in 80% yield provided the acetic acid formed in the reaction is neutralized with mercuric oxide or alcohol. This reagent can also be synthesized by a method described by Sigler and Blow[18].

This reagent is also specific for serine residues. It has been used to label the active serine residue in α-CHT with a heavy atom. α-CHT is first converted into tosyl-α-CHT, which is treated with phenyl mercuric acetate to give phenyl mercuric tosyl-α-chymotrypsin (PMA-tosyl-α-CHT).

The method of preparation of PMA-tosyl-α-CHT has been described by Sigler and Blow[18]. This derivative, which is isomorphous with tosyl-α-CHT, was of immense help in the determination of the three-dimensional structure of α-CHT. The electron density map at 2 Å resolution phased only with the $PtCl_4$ and pipsyl derivatives did not lead to a total interpretation of the molecular structure. The additional phase information derived from the PMA-tosyl derivative limited to 2·5 Å resolution improved the map to the point where a confident interpretation could be made.

Since pipsyl halides, PCMBSF and phenyl mercuric acetate used to label the active serine residue in α-CHT with a heavy atom have not been tried with other proteins, it is not necessarily true that they would work well with other proteins.

Chloromercurinaphthalene-1 sulphonyl fluoride — This reagent has come into use only very recently. It offers a systematic method for introducing heavy atoms into a protein molecule.

Chloromercurinaphthalene 1-sulphonyl fluoride has been synthesized by the method described by Fenselau *et al.*[19]. Aminonaphthalene sulphonyl fluoride is first prepared from aminonaphthalene sulphonic acid and fluorosulphonic acid followed by the introduction of a heavy atom into the naphthalene ring at the position of an amino group by proceeding through the diazonium chloride.

This reagent reacts specifically with active serine residue in α-CHT. Its specific reaction with α-CHT was carried out as follows: The heavy atom reagent (25-30 μmoles) was dissolved in 0·5 ml dioxane and the solution brought to 2 ml by adding methanol. α-CHT (20 μmoles) dissolved in 30 ml phosphate buffer (0·05*M*, *p*H 7·0) was allowed to react with sulphonyl fluoride, which was added in small proportions over a period of 60 min. When an enzymic activity of less than 0·5% was observed (generally within 3-5 hr at room temperature), the solution was transferred to the top of Sephadex G-25 column, which had been previously packed in deionized water. Thus, the protein derivative was separated from smaller peptides produced by autolysis and the unaltered organic compound, and after lyophilization it was stored as dry powder.

If this derivative could be crystallized and if it is isomorphous with the native α-CHT or tosyl-α-CHT, it will be a very good heavy atom derivative for X-ray crystallographic studies of α-CHT. This reagent again has not been tried with other proteins.

4-Iodo-2-nitrofluorobenzene (*INFB*) — Venkatappa and Steinrauf[20], in their search for heavy atom derivatives isomorphous with native lysozyme for its three-dimensional structure determination, synthesized a heavy atom reagent which is less reactive, but more selective than fluorodinitrobenzene (FDNB). This heavy atom reagent, 4-iodo-2-nitrofluorobenzene (INFB), is highly specific for lysine residues at neutral or slightly alkaline *p*H. FDNB itself is very reactive and less selective, reacting not only with lysine residues but also with histidine, tyrosine and cysteine residues in proteins. INFB is sensitive to changes in environment and absorbs in a region of the spectrum where the protein is transparent, but is not very sensitive to changes in *p*H. Therefore, it will be very useful in the modification of protein for X-ray diffraction studies.

INFB reagent has been synthesized by the method of Venkatappa and Steinrauf[20]. The synthesis involves diazotization of 4-fluoro-3-nitroaniline and subsequent treatment of the diazotized aniline with aqueous potassium iodide in the presence of a crystal of copper sulphate.

Venkatappa and Steinrauf used this reagent to block lysine residues in lysozyme selectively. In the *p*H range 7-8, they have been able to label one lysine only, residue No. 13, in the polypeptide chain of lysozyme with a heavy atom. The reaction with lysozyme was carried out as follows: crystals of lysozyme (7×10^{-6} mole) in 0·05*M* phosphate buffer (*p*H 7·0) were reacted with an excess of the heavy atom reagent ($1{\cdot}12\times10^{-4}$ mole) in acetonitrile. The concentration of acetonitrile in the reaction mixture should not exceed 10%. The reagent was added in small amounts at a time and the mixture shaken gently. The crystals were left for one week in the reagent solution to attain equilibrium. The reacted crystals were dissolved in dilute acetic acid and dialysed exhaustively with frequent changes of distilled water. From the pale yellow solution, INP lysozyme was obtained by crystallization in the triclinic form with 2% sodium nitrate and acetate buffer (*p*H 4·5), and also in the orthorhombic form with 2% sodium chloride and borate buffer (*p*H 9·0). The concentration of INP lysozyme used for crystallization must be 1%. Excellent crystals of both the forms obtained were of appropriate size suitable for X-ray diffraction studies.

INP lysozyme is isomorphous with the native protein. Consequently, it should be usable for phase information. Furthermore, since it is biologically active it can be used in X-ray studies with confidence.

p-Iodophenyl isothiocyanate — Phenyl isocyanate and its thioanalogue have been used by Edman for the determination of N-terminal residues in proteins. These reagents react not only with NH_2 groups of N-terminal residues but also with ϵ-NH_2 groups of lysine residues at alkaline *p*H.

Low and her colleagues[21,22] used *p*-iodophenyl isocyanate to prepare isomorphous heavy atom derivatives of insulin required for the determination of its three-dimensional structure (personal communication). Similarly, Drenth *et al.*[23-25] used the thioanalogue of the above reagent to prepare heavy atom isomorphous derivatives of papain C required for phase determination.

The synthesis of *p*-iodophenyl isothiocyanate is based on the method of Dains, Brewster and Orlander as presented by Blatt[26]. It can be prepared by the action of CS_2 and liquor ammonia on *p*-iodaniline, and of lead nitrate on ammonium *p*-iodophenyl thiocarbamate (*p*-$IC_6H_4NHCSSNH_4$), which is the intermediate product of the first reaction.

The reaction of *p*-iodophenyl isothiocyanate with papain C was carried out by soaking orthorhombic crystals of the protein in a dilute solution of *p*-iodophenyl isothiocyanate (*p*H 9·3), the soaking medium being 65% methanol in water.

This heavy atom derivative was found to be isomorphous not with the native protein but with its phenyl isothiocyanate derivative. Drenth and Jansonius (personal communication) were able to locate the positions of the heavy atoms: the iodine atoms, in difference-Fourier projections. They lie at the expected distance of 7 Å from the S-positions.

Iodine — A solution of iodine in potassium iodide is a very good reagent to iodinate histidine or tyrosine residues selectively in proteins under mild conditions for preparing isomorphous heavy atom derivatives needed for X-ray diffraction studies of the protein. The attacking agent in iodination reaction is the electrophile I^+; so the reaction is usually carried out at acid *p*H. However, the actual *p*H of the reaction will depend upon the nature of the protein and of the medium, and also upon the environment in which the amino acid residues are situated in the native protein. Generally, aqueous medium and acetate or phosphate buffers are used for the reaction.

Venkatappa and Steinrauf[20] iodinated the single histidine in lysozyme in acetate buffer (*p*H 4·7)

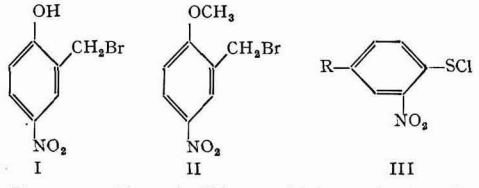
(tyrosines in lysozyme are very unreactive, since they are deeply buried in the interior of the molecule and hence they are not iodinated) in their attempt to prepare isomorphous heavy atom derivatives needed for X-ray investigation of the protein, and to find out the role of histidine in the biological function and dimerization of lysozyme. This 'iodolysozyme' crystallized in the triclinic and orthorhombic form was found to be isomorphous with the native protein and it can be used in the evaluation of phases of reflexions. They have also found that the histidine is not at the active site, but it could be at the dimerization site[27].

Recently, Kretzinger[28] monoiodinated two of the tyrosine residues in metmyoglobin (tyr H22 is deeply buried in the interior of the molecule and hence not iodinated), the crystals of which were taken in 75% saturated $(NH_4)_2SO_4$ and phosphate buffered to *p*H 5·5. This iodination was intended for a crystallographic investigation of the positions of some of the amino acid residues, particularly tyrosines, in the nonhelical regions of the molecule, which are very poorly defined even in the most fully refined Fourier synthesis.

Thiolation of certain amino acid residues in proteins — Since many proteins do not contain cysteines, it is very difficult to get excellent heavy atom derivatives for X-ray diffraction studies, using specific reagents such as PCMB. Recently, Polgar and Bender[29] replaced the reactive serine residue in subtilisin with a cysteine. This method is of great interest not only to workers in enzyme catalysis but also to crystallographers, particularly if it could be extended to other enzymes containing reactive serines.

Shall and Bernard[30] and Avey *et al.*[31] used Benesch reagent, N-acetyl homocysteine thiolactone, to introduce a thiol into ribonuclease. Acylation by Benesch reagent occurs at the freely accessible amino groups in proteins. This technique would be of great interest to protein crystallographers if it could be used in other proteins also. When once a protein is thiolated, the specific heavy atom reagent can be easily tagged on. In ribonuclease, residue No. 41, which is a lysine, has been thiolated to introduce a heavy atom for the purpose of X-ray investigation.

'Potential heavy atom' reagents — A number of reagents which are highly selective for tryptophan residues have come into use for the chemical modification of proteins. They are: 2-hydroxy-5-nitrobenzyl bromide (I), 2-methoxy-5-nitrobenzyl bromide (II), and a sulphenyl halide (III).



I II III

Reagents (I) and (II) are highly selective for tryptophans at neutral or acid[32,33] *p*H. They have some tendency to react with cysteine and methionine residues also. However, by varying the reaction conditions, they can be made to react specifically with tryptophans in proteins, which do not contain cysteines. In fact, they have been used successfully in α-CHT. Both the reagents are sensitive to changes in environment; but the former is sensitive to changes in *p*H of the medium, whereas the latter is not affected by changes in *p*H.

Since the $-NO_2$ group in 2-methoxy-5-nitrobenzyl bromide and 2-hydroxy-5-nitrobenzyl bromide has no influence on the reactivity of the reagent for tryptophans in proteins, the presence of this group is not crucial. Therefore, reagents which contain a heavy atom like —I instead of $-NO_2$ at this position, if synthesized, might serve to label tryptophans in proteins with heavy atoms.

Sulphenyl halides[34] react with tryptophan residues selectively in proteins in acid medium (acetic acid or formic acid) at the 2-position of the indole nucleus allowing the introduction of a chromophoric group. Sulphenyl halides containing heavy atoms such as —I in place of R might serve well to introduce a heavy atom systematically into a protein.

Discussion

The results obtained from the X-ray diffraction studies of a few typical globular proteins are given and discussed below. The relation between the structure and biological function of the protein is also described briefly.

Myoglobin

Myoglobin, the protein present in muscles, consists of a single polypeptide chain of 153 amino acid residues and one haem group, and has a molecular weight of 17000. Like haemoglobin, it combines reversibly with molecular oxygen. However, whereas the role of haemoglobin is to transport CO_2 from the tissues to the lungs and of oxygen from the lungs to the tissues, that of myoglobin is to store oxygen in the tissues. On account of its smaller size, the high percentage of α-helix, the availability of a large number of heavy atom derivatives, and of its important biological function, myoglobin was the first protein to have its three-dimensional structure established.

The determination of three-dimensional structure of myoglobin by X-ray diffraction method was started in 1948 and was completed in 1963 at Cambridge. This investigation was carried out by a team of scientists led by John C. Kendrew. Max F. Perutz, also at Cambridge, determined the three-dimensional structure of haemoglobin at low resolution.

X-ray analysis was carried out on the monoclinic crystals of sperm whale metmyoglobin with unit cell dimensions: $a = 64{\cdot}6$ Å; $b = 31{\cdot}1$ Å; $c = 34{\cdot}8$ Å; $\beta = 105{\cdot}5°$; and space group $P2_1$. Each unit cell contained two molecules. Even though the protein did not possess any sulphydryls, it gave five very good isomorphous heavy atom derivatives, all of them being complexes of the protein with the heavy atom reagents. They were used to determine the phases of reflexions.

Kendrew[35] has given an excellent discussion of the results of high resolution X-ray analysis of myoglobin. His Nobel lecture[36] gives not only a brief account of the determination of its three-dimensional structure, but also describes the general

nature of the molecule. Dickerson[1] also gave a good review of the X-ray diffraction studies of myoglobin.

At 2 Å resolution, the course of the polypeptide chain was clearly visible. All the straight segments of the polypeptide chain, which appeared as rods of high electron density in 6 Å Fourier synthesis, have now been found to be hollow cylindrical tubes of high density. Study of the density distribution on the surface of these cylinders showed that it did fit the arrangement of atoms in the α-helix as postulated by Pauling and Corey in 1951. Furthermore, that the α-helix is right-handed was decided from the known absolute configuration of L-amino acids. Thus, the α-helix, which was predicted by Pauling and Corey for polypeptide chains, was seen directly for the first time in three dimensions in the case of myoglobin. The X-ray studies have also revealed that myoglobin contains 65% helix, which is in good agreement with the value obtained from optical rotary dispersion measurements.

Initially, Kendrew and his colleagues were not very optimistic about the identification of side chains at this resolution. However, by carefully studying the shapes and sizes of electron density projecting out at regular intervals from the polypeptide chain, they were able to identify these shapes reasonably well with one or another of the 17 different types of side chains known to be present in the myoglobin molecule. Their X-ray analysis of the amino acid sequence was confirmed by chemical studies carried out by Edmundson at the Rockefeller Institute in New York.

Another view of the electron density map shows the haem group edge on, which appears as a flat disc with the iron atom at its centre. Surprisingly, it was also found that the iron atom was slightly out of the plane of the group. The haem itself was found to have a polar and a nonpolar end, one formed by the propionic acid groups and the other by the vinyl and methyl groups. The arrangement of amino acid side chains in globin is such that it offers the haem group an oily pocket into which it can slide with the vinyl end first, and extend the propionic acid side chain out into the exterior. The fifth coordination position of the iron atom is occupied by a ring nitrogen atom of a histidine residue, the so-called haem-linked histidine. On the other side of the iron atom, occupying its sixth coordination position, is a water molecule in ferri- or metmyoglobin at acid *p*H. Beyond this water molecule, in a position suitable for hydrogen bond formation, is a second histidine residue. It is important to note that the same type of arrangement of two histidines also exists in haemoglobin. The group is held in place by a large number of van der Waals' interactions. The orientation of the haem groups in the unit cell was determined independently from electron spin resonance measurements.

Fourier synthesis at 1·4 Å resolution was calculated by successive Fourier refinement technique on 17000 observable parent myoglobin intensities alone, as the MIR method was not feasible at this resolution. Three cycles of refinement were sufficient to give the desired structure. Many of the disturbances found in the region of the heavy atom sites in the 2 Å synthesis have disappeared. The nature of the molecule became progressively clearer at this resolution. Out of the 153 amino acid residues present in the polypeptide chain of myoglobin, some 118 residues make up 8 segments of right-handed α-helix, 7-24 residues long. These segments are joined by two sharp corners and five nonhelical segments. There is also a nonhelical tail of five residues at the carboxyl end of the chain. The whole is folded in a complex and unsymmetrical manner to form a flattened, roughly triangular prism with dimensions of about $45 \times 35 \times 25$ Å. The whole structure is extremely compact. There is no water inside the molecule except for a very small number of single water molecules, presumably trapped at the time of folding.

Nearly all the polar and charged groups lie on the surface. The interior of the molecule is almost entirely made up of nonpolar residues, except for a few which are exposed to the surface, generally close-packed and in van der Waals' contact with their neighbours.

Most of the stabilizing energy of the tertiary structure appears to come from the relatively non-specific van der Waals' attractions between nonpolar residues which make up the bulk of the interior of the molecule. Further, the haem group also plays a very important role in stabilizing the myoglobin structure.

The oxygenation reaction of myoglobin is biologically very important and interesting. X-ray crystallographic studies have indicated that there is no change in the structure of myoglobin due to oxygenation or deoxygenation of the molecule. The iron atom in both oxy and deoxy myoglobin is in the ferrous state. The crystal structures of met- or ferrimyoglobin and oxy myoglobin are indistinguishable, so that it does not matter much which of them is used for the X-ray analysis.

Kendrew and his colleagues have demonstrated that the difference Fourier technique is capable of detecting very small modifications in proteins, once the native crystal structure of the protein is known with considerable accuracy. This method has been used to investigate the binding of azide, *p*-chloromercuribenzene sulphonate (PCMS), Cu^{2+} and Zn^{2+} and xenon to myoglobin[37-41]. Using the same technique, the crystal structure of deoxy myoglobin also has been examined at 2 Å resolution[42].

Haemoglobin

Haemoglobin, the protein present in blood, consists of four myoglobin-like subunits. The subunits occur in two identical pairs: the α- and β-chains. Each chain has one haem group and one sulphydryl, readily reactive on the β-chains, but much less so on the α-chains. It has a molecular weight of 67000. In spite of its large size, its X-ray investigation was taken up because of its close relation with myoglobin, and of its important biological function.

X-ray analysis at low resolution was carried out on the monoclinic crystals of horse oxyhaemoglobin with space group *C*2, in which two halves of each molecule are related by a twofold crystallographic

axis. The asymmetric unit is, therefore, composed of one α- and one β-chain. The unit cell dimensions are : $a = 108{\cdot}95$ Å; $b = 63{\cdot}51$ Å; $c = 54{\cdot}92$ Å; and $\beta = 110°53' \pm 10'$. Six excellent heavy atom isomorphous derivatives have been used along with the native protein for the determination of phases of reflexions. They were readily available with haemoglobin on account of the presence of sulphydryl groups in the molecule[1]. At 5·5 Å resolution, 1200 reflexions were measured from the parent protein and from each of the six heavy atom derivatives. The positions of heavy atoms were determined first by difference Patterson and Fourier projections on the centrosymmetric plane of monoclinic crystals, and later by three-dimensional correlation functions, $(|F_{H_1}|-|F_{H_2}|)^2$ being used as coefficients, where F_{H_1} and F_{H_2} are the observed structure factors for the two different heavy atom derivatives. The parameters and anisotropic shape factors of the heavy atoms were refined by three-dimensional least squares methods.

Even though the X-ray analysis was carried out at low resolution, it gave sufficient information on the general nature of the structure of the molecule, so that its biological function could be understood readily. Perutz[43], in his Nobel lecture, discussed the structure of haemoglobin exhaustively. Kraut[44] and Dickerson[1] have also reviewed the work on haemoglobin.

The haem groups are seen to lie in 4 separate pockets on the surface of the molecule. Each pocket is formed by the folds in one of the polypeptide chains, which appears to make contact with the haem group at 4 different points at least. The iron atoms in the neighbouring pockets between the β- and the α-chains are 25 Å apart. This is the closest distance between any two iron atoms in the structure. The distances between symmetrically related iron atoms in the α- and β-chains are 36 and 34·4 Å respectively. The iron atoms are, thus, much too far apart for the combination with oxygen of any one of them to affect the oxygen affinity of its neighbours directly.

The packing of the molecules in the crystals is pseudo face-centred cubic, and in its overall shape the molecule resembles a spheroid with the dimensions $64 \times 55 \times 50$ Å.

Reversible oxygenation is the important biological function of haemoglobin and of myoglobin. However, haemoglobin displays some additional features not found in myoglobin. One is the sigmoid shape of its oxygen binding versus partial pressure curve. In contrast, the binding curve for myoglobin is hyperbolic, as would be expected for any well-behaved system of non-interacting binding centres. The sigmoid oxygen-binding curve of haemoglobin is physiologically advantageous, since the difference between the partial pressure of oxygen in the lungs and in the tissues is a comparatively small one, and this feature allows haemoglobin to be charged with the oxygen and to be discharged completely within this narrow range. Theoretically, the sigmoid-shaped curve requires some kind of interaction to take place between the four haem groups, such that the binding of an oxygen molecule by one affects the oxygen-binding affinity of the others. Since the closest approach between haem iron atoms of α- and β-chains in horse oxyhaemoglobin is 25 Å, the haem-haem interaction must be of a subtle and indirect kind.

Haemoglobin shows another important property known as the Bohr effect. This phenomenon is related to the oxygen affinity and pH of the medium. Myoglobin shows only a very weak Bohr effect. The pH of the medium affects the affinity of haemoglobin for oxygen; it is greater at high than at low pH, so that the presence of lactic acid and bicarbonate in working muscle speeds up the liberation of oxygen. As with the haem-haem interaction, this property is of importance for the role of haemoglobin in oxygen transport. Crystallographic and kinetic studies indicate that the interaction between the haem groups and the Bohr effect are due to a major structural change which accompanies the reaction of haemoglobin with oxygen. Recent X-ray crystallographic studies show that upon oxygenation the two β-chains move closer together by 7 Å, without any apparent gross structural changes having occurred in either the α- or the β-chains themselves.

Since the original structure determination was carried out on horse oxyhaemoglobin, it would have been better if the three-dimensional crystal structure of the oxygen-free form of the same protein was also solved. Unfortunately, the oxygen-free horse haemoglobin crystallized in a form not suitable for detailed X-ray analysis. Therefore, the structure of oxygen-free human haemoglobin was examined, since its primary structure has been established[45] and it differed from the horse haemoglobin[46] only in a smaller number of residues. Furthermore, oxygen-free human haemoglobin crystallized in a more favourable space group. A three-dimensional Fourier map at 5·5 Å resolution[47] was obtained based on three isomorphous derivatives. The relative movement of the β-chains was immediately obvious, the distance between the two iron atoms attached to the β-chains having changed from 33·4 Å in the oxygenated to 40·3 Å in the oxygen-free haemoglobin. At this resolution, no alteration could be detected in the structures of the individual subunits, nor could any change be found in the distance between the two α-chains. Also, it was not possible to determine whether the orientation of the haem groups had changed. Thus, the results suggest that the oxygenated and oxygen-free forms do have some important differences in structure, and that a molecular rearrangement accompanies the reaction of haemoglobin with oxygen.

The haem-haem interaction and the Bohr effect, which are due to the major structural changes that accompany the oxygenation reaction, will no doubt be better understood if the three-dimensional structure of one of the two forms of haemoglobin is solved at high resolution. Recently, Perutz and his associates[48,49] have obtained a three-dimensional Fourier synthesis of horse oxyhaemoglobin at 2·8 Å resolution. The phase angles of 8000 reflexions were determined by the method of multiple isomorphous replacement, using crystals of native haemoglobin and of three heavy atom derivatives. The electron density map shows the positions of nearly all the

amino acid residues and some details of the haem groups. It also reveals that the secondary structure of the haemoglobin chains is similar to that of myoglobin, but some of the helical segments are more irregular and some parts of the nonhelical segments have different conformation. The structure of the contact between unlike subunits suggests that the tetramer, rather than the $\alpha\beta$ dimer, is the functional unit of haemoglobin.

Perutz and Lehmann[50] have also found out that the human haemoglobin molecule is insensitive to replacement of most amino acids on its surface, but extremely sensitive to even small alterations of internal nonpolar contacts, especially those near the haems. Any replacement at the contacts between α and β subunits affects the respiratory function.

Lysozyme

Lysozyme is an enzyme present in hen egg-white. It has the power of rapidly dissolving certain bacteria such as *Micrococcus lysodeikticus*. It splits the chemical skeleton of bacterial cell walls by hydrolysing the $\beta(1\rightarrow4)$ linkages between N-acetyl muramic acid and N-acetyl glucosamine.

Lysozyme consists of a single polypeptide chain of 129 amino acid residues crosslinked by four disulphide bridges. It is a highly basic protein of molecular weight 14400, which is stable at acid *p*H without significant loss of activity, and which undergoes denaturation above *p*H 12. Its isoelectric point lies between *p*H 10·5 and 11·0. It contains six lysines, eleven arginines, six tryptophans, one histidine and eleven carboxyl groups. Of these carboxyls, some possess abnormally high pK_a values, and some others abnormally low pK_a values. It does not contain any sulphydryls which would have facilitated the attachment of specific heavy atoms for X-ray studies. Its primary structure has been established by Pierre Jollès and his colleagues at the University of Paris and by Robert E. Canfield of the Columbia University College of Physicians and Surgeons.

The determination of three-dimensional structure of lysozyme by X-ray diffraction studies at low resolution on several different crystal forms was carried[51-53] out in 1962. This was followed in 1965 by a complete structure determination of the molecule at 2 Å resolution[54]. The crystal form studied is tetragonal with unit cell dimensions: $a = b = 79{\cdot}1$ Å; $c = 37{\cdot}9$ Å; and space group $P4_32_12$. The unit cell contains eight lysozyme molecules. Three heavy atom isomorphous derivatives have been used to calculate the phases of reflexions. They are only complexes of the protein with the heavy atom reagents: ortho-mercury hydroxytoluene parasulphonic acid, $UO_2F_5^{3-}$ and an ion derived from $UO_2(NO_3)_2$. Anomalous scattering data were used in conjunction with the isomorphous replacement intensity differences[4] to form a joint probability distribution for the phase of each reflexion. The position of the centroid of each probability distribution gave a phase angle and weighting factor for each reflexion from which the electron density map with minimum rms error was calculated[10]. The necessary diffraction measurements were made with the Hilger & Watts Ltd linear diffractometer[55] adapted to measure three reflexions at a time[56,57] and the data were processed on the Elliott 803 B computer at the Royal Institution, London. The Fourier synthesis was done on the University of London Atlas computer.

Davies[58] has discussed in detail the work on hen egg-white lysozyme. Here it will be described briefly. The electron density map of lysozyme at 2 Å resolution was interpreted using the amino acid sequence of Canfield[59] and of Jollès *et al.*[60]. The distinctive side chains of amino acid residues can be recognized and the peptide groups and the course of the main chain can be identified. Phillips and his colleagues were able to determine the spacial arrangement of each of the amino acid residues and to locate the positions of some 95% of the atoms in the molecule. Four elongated peaks of high density could correspond to the four disulphide bridges, and the ring-shaped and forked side chains could be identified readily. Most prominent in the map are the indole rings of tryptophan residues 28, 108 and 111 and the phenol ring of tyrosine 23, which are seen almost edge on. These four residues come together to form part of a curiously regular hydrophobic box which surrounds and shields the sulphur atom at the methionine residue 105. The lysozyme molecule appears to have a hydrophobic spine consisting mainly of the six tryptophan side chains and including this hydrophobic box. Three of these tryptophan side chains 62, 63 and 123 protrude beyond the molecular boundary and there are, in addition, a few strongly hydrophobic side chains exposed to the surface (val 2, phe 3, leu 17).

Most of the charged and polar side chains are distributed on the surface of the molecule. On the other hand, most of the markedly nonpolar and hydrophobic side chains lie inside the molecule. Examination of the primary structure shows that nonpolar side chains are not uniformly distributed along the chain. The deep cleft divides the molecule into two parts: the right hand one has many nonpolar groups and would appear to be held together principally by hydrophobic bonds, whereas the left hand one has relatively few which seem to be stabilized mostly by hydrogen bonds.

The polypeptide chain of 129 amino acid residues is folded up in three dimensions to form an ellipsoid with dimensions $45\times30\times30$ Å. The molecule may be considered to consist of three parts. The first 40 residues form a right hand wing in which the chain is coiled around a core of nonpolar residues in a stable looking conformation which includes two stretches of helix (residues 5-15 and 24-34). Residue 40, which is a threonine, is hydrogen-bonded back to the terminal amino end of the molecule, so that the chain is now close to its starting position. The second part of the molecule consists of residues 41-95 which branch out to form a left hand wing to the molecule that looks to be in a less rigid conformation and contains a high proportion of polar residues. In this region, three lengths of chain are roughly anti-parallel in the extended β-conformation and two of them, involving the residues

41-54, form a closed loop and are in the antiparallel pleated sheet conformation. The third part of the molecule consists of the remainder of the chain, residues 96-129, which partially closes the gap between the two wings and wraps around the outside of the right hand wing, leaving a pronounced cleft between the two parts of the molecule.

Lysozyme has a number of segments of helix; of these only three are important. They include the residues 5-15, 24-34 and 88-96. Although the height per residue and the number of residues per turn correspond fairly closely to those of the α-helix, there are other types of helix also. X-ray analysis has revealed a helix content of nearly 42% which is in good agreement with the predictions of optical rotary dispersion measurements.

The difference Fourier technique which was used by Kendrew and his colleagues to study the binding of azide, Cu^{2+} and Zn^{2+} to myoglobin, has been used by Phillips and his associates[61,62] to locate the region of the lysozyme molecule where small inhibitor molecules are bound. This aided them in locating the amino acid residues supposed to be at the active site in lysozyme. The inhibitors used for binding studies are N-acetylglucosamine, di-N-acetylchitobiose, 6-iodo, α-methyl N-acetylglucosaminide, tri-N-acetylchitotriose, N-acetylmuramic acid and NAG-NAM dimer, and they are all competitive inhibitors. As a result of the crystallographic study of these enzyme-inhibitor complexes they have been able to locate the amino acid residues at the active centre. In particular, the glutamic acid (residue No. 35), aspartic acid (residue No. 52) and three tryptophans (residue Nos. 62, 63 and 108) are involved; but the histidine is not considered to be implicated. Phillips[63] has proposed a mechanism for the enzymic action of lysozyme, which is of considerable interest, since it is the first mechanism of enzymic action put forward on the basis of a detailed three-dimensional structure. Even though lysozyme has not been seen in action, Phillips has succeeded in building up a detailed picture of how it may perform its function.

Ribonuclease

Ribonuclease, an enzyme isolated from bovine pancreas, has been the subject of biological, chemical and crystallographic studies for many years. It acts on pentose ribonucleic acid, but the mechanism of its enzymic action is not known. It is a particularly interesting protein for X-ray study because of its size, availability and the fact that it was one of the first proteins to have its primary structure established[64]. It consists of a single polypeptide chain of 124 amino acid residues cross-linked by four disulphide bridges, and has a molecular weight of 13683. The molecule does not possess any free SH or similar groups which facilitate the attachment of specific heavy atoms for X-ray studies.

Two groups, Harker's at Buffalo and Carlisle's at Birbeck College, London, have obtained low resolution three-dimensional maps from crystals grown from aqueous alcohol solution. Richards at Yale University[65] has taken up the crystallographic study of an enzymatically modified form called ribonuclease S obtained by limited proteolysis of the native enzyme with subtilisin.

Harker *et al.*[66] obtained a three-dimensional electron density map of RNase at a resolution of 4 Å using monoclinic crystals crystallized from 75% 2-methyl-2,4-pentanediol and a series of five heavy atom isomorphous derivatives. All the derivatives are the complexes of the protein with the heavy atom reagents. The interpretation of the Fourier map was not completely satisfactory; it was not possible to correlate the length of the high density regions with the known length of the polypeptide chain in ribonuclease. There appeared to be more 'cross-points' in the continuous regions than can be put into correspondence with the four disulphide bridges in the ribonuclease molecule. X-ray studies suggest a helix content of about 17% in agreement with the optical rotary dispersion measurements.

Avey *et al.*[31] have recently prepared a low resolution electron density map of ribonuclease in which only reflexions to a spacing of 5·5 Å were used. They have used monoclinic crystals of the native protein and five isomorphous heavy atom derivatives: *p*-hydroxy mercuribenzene sulphonate, $IrCl_6$, cytidylic acid-uranyl pentafluoride, uranyl pentafluoride and Benesch reagent, N-acetyl homocysteine thiolactone. The Benesch reagent was used to introduce a thiol at the position of a lysine in ribonuclease for the purpose of attaching a heavy atom. This was the only chemically modified heavy atom derivative of RNase, whereas all the others are complexes of the protein with the heavy atom reagents. Using the known primary structure of the enzyme, the interpretation of the map reveals a cleft in the molecule. Inhibitors have been located in this cleft which identify it as the probable active site of the molecule. High regions in the electron density map were found to correspond with the disulphide bridges. The lysine which has been thiolated was found to be residue No. 41 in the polypeptide chain of ribonuclease. Now it is known that this residue along with two histidine residue Nos. 12 and 119 comprises the active site of the molecule.

Very recently, Kartha and his colleagues[67] at Buffalo have studied an electron density map at 2 Å resolution involving 7294 reflexions from the native protein and from seven heavy atom isomorphous derivatives. Crystals of RNase used in X-ray studies were grown from 55% 2-methyl-2,4-pentanediol at *p*H 5·0 usually in the presence of phosphate buffer, and were monoclinic with the unit cell dimensions: $a = 30{\cdot}13$ Å; $b = 38{\cdot}11$ Å; $c = 53{\cdot}29$ Å; $\beta = 105{\cdot}75°$; and space group $P2_1$. There are two molecules per unit cell and the unit cell contains 40% by weight of the solvent. All the heavy atom derivatives used in this study are complexes of the protein with the particular heavy atom reagents. The heavy atom positions were determined and refined by difference Patterson and Fourier syntheses and least squares methods. The protein phase angles were evaluated by a combination of isomorphous series and anomalous dispersion data from the different derivatives, taking care to give proper weights to the phases

obtained using any given derivative, and to the reliability of the data from that derivative.

The electron density map shows clearly the structure of the molecule. With the help of the primary structure, Harker and his associates have been able to locate all the amino acid residues of which the molecule is made. Four disulphide bridges have been located unambiguously. In contrast to myoglobin, the absence of any appreciable amount of α-helix makes a complete description of this molecule more difficult. The molecule is roughly kidney-shaped with the dimensions of $38 \times 28 \times 22$ Å and it has a deep depression in the middle of one side.

Harker and his colleagues have sufficient reason to believe that they have located the active site of this enzyme molecule. From an examination of the position of the PO_4^{3-} ion, they have indirect information about the location of the enzymatic centre. It is seen that the residues closest to the PO_4^{3-} are residue No. 119 near the C-terminal end and residue No. 12 near the amino end. Both of them are histidines, and chemical evidence indicates their close relation with the activity of the molecule. Other residues further out, but which might be of importance, are lysine 7, lysine 41 and histidine 48, all of which are reasonably close to the phosphate site.

This model proposed by Harker and his associates disagrees with the one put forward[68] by Carlisle and his associates.

α-*Chymotrypsin* (α-*CHT*)

Chymotrypsin is a term used to designate the members of a closely related group of enzymes (α, β, γ, δ and π), all having the same proteolytic activity, and all derived from the inactive precursor, chymotrypsinogen, present in pancreas. It is produced from chymotrypsinogen by the enzymatic cleavage of four peptide bonds forming three polypeptide chains called A, B and C, all of which are inter- and intrachain linked with five disulphide bridges. The chymotrypsinogen-chymotrypsin system of zymogen and proteolytic enzymes has been investigated more thoroughly over a longer period of time than any other enzyme system. A general review by Niemann[69], and an article by Bender and Kezdy[70] which discusses the mechanism of chymotrypsin's enzyme action, are of great interest. The enzyme catalyses the hydrolysis of peptide bonds not only in proteins but also in synthetic substrates like esters and amides. It hydrolyses CO-NH linkages in which the carboxyl group is provided by an aromatic amino acid.

Of all the chymotrypsins, only α- and γ-chymotrypsins have been investigated largely both by chemical and crystallographic means. Chymotrypsinogen consists of a single polypeptide chain of 246 amino acid residues crosslinked by five disulphide bridges, whereas α-chymotrypsin consists of 241 amino acid residues in three linear polypeptide chains which are held together by two of the five disulphide linkages. Its primary structure has been established by Hartley[71] and Keil and Sorm[72]. It has a molecular weight of 25000.

Single crystal X-ray analyses on the chymotrypsins have yielded interesting information. Sigler and Skinner[73] have found that γ-CHT in the crystalline state reacts with diisopropyl fluorophosphate (DFP) to give inhibited crystals of the enzyme which are indistinguishable crystallographically from those obtained after reaction in solution. The diffraction patterns of both the inhibited and the native γ-CHT crystals are very similar. Only small differences in the unit cell dimensions and diffraction intensities were found between the native and DFP-inhibited enzymes. This probably indicated that any changes in enzyme conformation upon phosphorylation must be minor.

Sigler *et al.*[74] and Kallos and Rizok[16] have prepared crystalline pipsyl-γ-CHT. This compound was found to be isomorphous with the tosyl derivative, and not with the native enzyme. The iodine atom attached at the active site was sufficiently heavy to be located crystallographically by difference Patterson projections at 4 Å resolution. Small differences in the unit cell dimensions of native and inhibited enzyme crystals may be due to some change in the enzyme configuration around the active site.

X-ray diffraction studies on crystalline α-CHT have been in progress since 1960. In crystals of α-CHT, two molecules form a single asymmetric unit of structure. Blow *et al.*[75] used isomorphous platinum halide derivatives to solve the phase problem; but these were not enough to reveal the molecular structure or even the individual molecules. However, together with rotational and translational analysis of the Patterson function[76,77], the isomorphous replacement data were used to define the arrangement of molecules within the asymmetric unit. They expressed the hope that the structure will be deduced sventually by making use of the non-crystallographic symmetry between two molecules in the same asymmetric unit[75].

Sigler *et al.*[15] have prepared and examined, by X-ray diffraction methods, a series of crystalline derivatives of α-CHT using DFP and several sulphonyl fluoride inhibitors, including the two heavy atom reagents: pipsyl fluoride and *p*-chloromercuribenzene sulphonyl fluoride. Using phase angles determined from the single isomorphous $PtCl_4^{2-}$ derivative[75], difference Fourier projections have been used to locate the sites at which these inhibitors bind to the enzyme molecule. All the inhibitors bind to the same sites. These sites, which are the catalytic sites, lie close to a non-crystallographic dimer axis which relates adjacent molecules in the crystal.

Recently, Mathews *et al.*[78] determined the three-dimensional structure of tosyl-α-CHT by X-ray diffraction studies at 2 Å resolution using the monoclinic crystals grown at *p*H 4·2 with the space group $P2_1$ and the unit cell dimensions: $a = 49{\cdot}1$ Å; $b = 67{\cdot}4$ Å; $c = 65{\cdot}9$ Å; and $\beta = 101{\cdot}8°$. The unit cell contains 4 molecules each with a molecular weight of 25000. Although the enzyme normally functions as a monomer, the crystallographic asymmetric unit contains two molecules, related by local non-crystallographic twofold axes. Three heavy atom derivatives: $PtCl_4$ derivative,

pipsyl derivative and PMA-tosylated derivative, isomorphous with the inhibited enzyme tosyl-α-CHT, were used to determine phases by the MIR technique. Separate phase probability distributions were calculated for the native enzyme using the $PtCl_4$ derivative and for the tosylated enzyme using pipsyl and PMA-tosylated derivatives. A joint probability distribution was evaluated, simply as the product of the two distributions. This is equivalent to assuming that the small differences between the tosylated and the native enzyme have a negligible effect on the phase angle. The assumptions implicit in this procedure for calculating the phase angles may account for certain deficiencies in the electron density map discussed below.

Starting with the amino acid sequence of Hartley[71], Blow *et al.*[75] have constructed an atomic model of the protein which conforms directly to the observed electron density distribution. Two positions in the molecule could be assigned with confidence to definite points in the amino acid sequence. The side chain of ser 195 was easily indentified by its connection to the sulphonyl group. In addition, tyr 171 was selectively diiodinated in the crystal, and the positions of the iodine atoms were located by difference Fourier projections. Four features were readily recognized as cystine bridges by their strong density and their characteristic staggered appearance. Continuous chains of density could be readily followed from these, corresponding in length to the A, B and C chains, assuming the polypeptide chains to be fully extended. This interpretation was confirmed by the positions already assigned to ser 195 and tyr 171, and by the clear appearance of many aromatic residues at appropriate positions along the chains.

A region of the map adjacent to the active centre and attributed to residues 190-193 does not appear as an obvious chain of continuous density. A difference Fourier synthesis between the native and the tosylated enzymes shows that the largest shifts in density occur in this region. Lack of clarity in this critical region is probably caused by the way in which the phase information for the native and tosylated enzyme was combined. The fifth cystine bridge (191-220) appears as a strong feature of electron density in this region. Some regions of the molecule, especially the ' methionine loop ' of the C-chain (168-182), and a sequence in the middle of the B-chain (70-80) which are at the extremes of the molecule in the *a*-direction showed relatively weak density in each of the individual molecules.

With the exception of eight residues at the C-terminus which form a short segment of α-helix, the chains tend to be fully extended. As these chains trace through the molecule, they often run parallel to one another at a distance of 4·5-5 Å for stretches of several residues. In a fully extended polypeptide chain, the peptide groups are available for hydrogen bonding, and, therefore, the tertiary structure is presumably stabilized to a large extent by hydrogen bonds between the chains. There is a strong tendency for a chain to interact with a neighbouring region in the sequence. This occurs frequently when a chain turns back and runs parallel to its previous course. The overall appearance is of a chain ' piled on itself ' rather than rolled, coiled or knotted. This suggests that the molecule might be folded around nuclei of highly stabilized local conformation.

The terminal residues leu 13, ile 16, tyr 146 and ala 149 are in positions consistent with the cleavage of peptides from the surface of the zymogen. The electron density map shows regular humps along the main chains, indicating the positions of side chains. Although many of the bulky side chains can be clearly identified, short side chains, and especially flexible ones extending into the solution regions, would certainly not be identified without the aid of the sequence. With only two exceptions (ala, val), all charged groups point towards the solvent. Much of the interior of the molecule is composed of regions where hydrophobic groups from several chains interact, although it is not uncommon for the smaller hydrophobic groups to be exposed to the surface.

There is no pronounced cleft in the molecule. However, in the vicinity of the active serine, there are open regions on the surface of the molecule where the structure is not closely packed. The tosyl group bound to ser 195 extends across one of these open regions. His 57 extends from one side pointing directly towards the sulphur atom of the tosyl group, and approaching within 5 Å of it. The positions of ser 195 and his 57 support the accepted view that they exert a concerted action in the catalytic mechanism. The mechanism by which the inactive precursor is converted into an active enzyme is also revealed clearly in the electron density map.

Papain

Papain, a proteolytic enzyme of molecular weight 22000, has been isolated from papaya latex. It is an SH enzyme which contains a single polypeptide chain of 211 amino acid residues. Its sequence also has been established almost conclusively[79].

The three-dimensional structure of papain has been studied by Drenth and Jansonius at the University of Groningen, Netherlands. It has been crystallized in various forms from water-methanol systems: two monoclinic forms, A and B, and two orthorhombic forms, C and D. All crystallizations took place between 0° and 5°C. The A form is formed at alcohol concentration below 60%, whereas C is dominant at higher alcohol concentrations. The C form grows as well-developed needles suitable for X-ray diffraction studies. Therefore, papain C was used for the determination of its three-dimensional structure.

X-ray investigation was carried out on the orthorhombic crystals of papain C with unit cell dimensions: $a = 45$ Å; $b = 104{\cdot}3$ Å; $c = 50{\cdot}8$ Å; and space group $P2_12_12_1$. Each unit cell contains four molecules. Four heavy atom isomorphous derivatives have been used along with the native protein for the determination of phases of reflexions by the MIR technique. The heavy atom reagents used for their preparation are PCMB, Na_2PtCl_6, K_2HgI_4 and Na_3IrCl_6. Of these derivatives, only the

first one is a chemically modified one, whereas all the others are complexes.

The heavy atoms were located by means of two-dimensional difference Patterson and Fourier syntheses. It appeared that the papain molecule has one site available for chemical reaction with PCMB, while the inorganic ions were complexed with the molecule in two positions, which were approximately the same for all the three ions. One of the latter positions is close to the PCMB binding site. Site occupancy factors vary from 25 to 100%. Two 2-dimensional Fourier projections of the papain structure have been prepared with these derivatives to a resolution of 5 Å (ref. 23). The positions of the molecules have been predicted from these projections, assuming their shape to be spherical.

Since the derivatives containing Na_2PtCl_6, K_2HgI_4 and Na_3IrCl_6 gave rise to the same heavy atom sites, they provided very little independent sign information. Therefore, Drenth and Jansonius decided to use only PCMB and Na_2PtCl_6 derivatives for phase determination. However, another heavy atom derivative used for improvement in the accuracy of phase determination was that of $HgCl_2$. This derivative, which was rejected in the two-dimensional work, because it complexed with papain at four sites, proved useful when sodium benzoate was added to the soaking solution at a 0·03*M* concentration.

A three-dimensional Fourier synthesis of papain at 4·5 Å resolution was performed by Drenth *et al.*[24] by using these three isomorphous heavy atom derivatives. The heavy atom parameters, already roughly known from two-dimensional Fourier projections, were refined by the least squares methods. In the subsequent phase calculation, a mean figure of merit of 0·85 was obtained. The absolute heavy atom configuration could be found from anomalous dispersion measurements, which made it possible to choose the correct phases for finding the protein structure from the subsequent ' best ' Fourier synthesis. The molecules could be separated fairly easily. They are more or less spheroidal with dimensions $36 \times 48 \times 36$ Å. Their centres lie close to the positions previously predicted and all heavy atom binding sites are at the surface. The structure is very complex and probably has only a small number of short stretches of α-helices. The course of the polypeptide chain cannot be followed, owing to the large number of intersections. The —SH group, known to be part of the active centre, has been located. It lies on the surface in a slight depression, which enlarges the area of contact with the substrate.

In order to get a better picture of the molecule, a three-dimensional Fourier synthesis at higher resolution is necessary. For this, a search for more isomorphous heavy atom derivatives is underway. Recently, Drenth *et al.*[25] have obtained a three-dimensional Fourier synthesis at 2·8 Å resolution, involving 8000 reflexions from the native protein and from five isomorphous heavy atom derivatives which are chemically modified ones. The electron density map has revealed that the single polypeptide chain is folded into two distinct portions which are divided by a cleft. The active site, consisting of a cysteine and a histidine, lies at the surface of the cleft. Apart from four short α-helical segments and one short segment of β-structure, the conformation of the chain is irregular.

Insulin

Insulin is a hormone which plays a very important role in the metabolism of carbohydrates. It is a small protein consisting of two polypeptide chains of 30 and 21 amino acid residues crosslinked by two disulphide bridges, with a third disulphide within the shorter chain. It was the first protein to have its primary structure established[80], and has a molecular weight of 5733. In spite of its important biological function, its small size, and the fact that it was the first protein to have its amino acid sequence worked out, its three-dimensional structure has not yet been solved. This is chiefly due to the difficulties encountered with the heavy atom derivatives.

The three-dimensional structure of insulin has been studied for many years, mainly by two groups: one at Columbia University and the other at Oxford University. The orthorhombic form of insulin has been studied extensively by Low *et al.* as the acid sulphate[21] and the citrate[22]. A three-dimensional Patterson map has been calculated for the sulphate and shrinkage stages reminiscent of those of haemoglobin have been examined in both the forms. They also studied the gross molecular shape on the basis of intensity distributions and Patterson functions from various orthorhombic forms. They are continuing their search for more heavy atom derivatives. Recently, they have used *p*-iodophenyl isocyanate to prepare a heavy atom derivative of insulin.

The Oxford group led by Dorothy Hodgkin has studied the rhombohedral form of insulin containing six monomers and either two or four zinc atoms per unit cell. Three-dimensional Patterson maps of both the 2Zn and 4Zn forms have been prepared, and attempts have been made to interpret them. Rossmann, in cooperation with the members of the Oxford group, recently used the rotation and translational functions[76,77] to attack the structure of insulin without derivatives, but not with much success.

Recently, Adams *et al.*[81] prepared rhombohedral insulin crystals containing lead for X-ray diffraction studies. They obtained X-ray photographs showing marked anomalous dispersion effects, whereby two lead atoms per unit cell can be placed by difference Patterson calculations along the threefold axis. Measurements have been made of the intensities of all reflexions to spacings of 4·5 Å and phase angles assigned to about two-thirds of these. Very approximate electron density maps derived from these show features suggesting some parts of the molecule to have α-helix character.

Since suitable heavy atom isomorphous derivatives are not available with this protein, the determination of three-dimensional structure of insulin is still an unsolved problem. Chemically modified derivatives of insulin can be prepared by using specific heavy atom reagents for histidine, tyrosine

or lysine. If they are isomorphous with the native protein, they will be very useful in the evaluation of phases of reflexions which are needed for the structure determination.

Summary

The important stages involved in the determination of the tertiary structure of a protein by X-ray diffraction, using the principle of multiple isomorphous replacement technique, are described briefly. The importance of isomorphous heavy atom derivatives of a protein in the evaluation of phases, a problem highly vital in the X-ray crystallography of proteins, is also discussed.

The heavy atom reagents used to label specifically certain amino acid residues in proteins with heavy atoms are enumerated and their utility in the determination of the phases of reflexions is discussed. The results obtained from the X-ray diffraction studies of a number of proteins such as myoglobin, haemoglobin, lysozyme, ribonuclease, α-chymotrypsin, papain and insulin are described. It is brought out that only in the case of insulin, its three-dimensional structure has not been established, even though its primary structure was determined first.

The relation between the structure and biological function of a protein is also discussed briefly.

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REVIEWS

COLLECTED PAPERS OF THE INSTITUTE OF PHYSICAL AND CHEMICAL RESEARCH, 1967 (The Institute of Physical and Chemical Research, Yamato-Machi, Saitama, Japan), 1968

This volume of over 1000 pages contains more than 175 research papers by several scientists of the institute and published during 1967 in different scientific periodicals of the world. The subjects of the papers range over the diverse ramifications of physics and chemistry and are broadly characterized under the following heads: Nuclear physics, Solid state physics, Applied physics, Physical chemistry, Inorganic chemistry, and Biochemistry. There is also a general section on Engineering.

The papers collected here are exact reproductions in the original language and format from the journals where they were originally published, except for a suitable dimensional reduction to conform to a uniform size for this volume. Many of the papers are in Japanese and the rest mainly in English, with a few in French. As a result of this heterogeneity, there is naturally some jerkiness and a little odd appearance in type form and size. However, their bringing together, under one cover, gives a good idea of the nature and output of the research work from this institute. The volume gives evidence of the rapid strides and significant contributions that the Japanese are making to the fast developing fields like solid state physics and nuclear physics on both the theoretical and experimental fronts. The volume contains an author index which indicates the authors' names both in English and Japanese.

FUNDAMENTALS OF INTERNAL COMBUSTION ENGINES by Paul W. Gill, James H. Smith & Eugene J. Ziurys (Oxford & IBH Publishing Co., Calcutta), 1967. Pp. xviii+559. Price Rs 10.00

This is a good text-book for beginners in understanding the fundamentals of IC engines. The earlier chapters deal with the basic theory of engineering thermodynamics, power cycles and fuels. This is very essential in understanding the performance of IC engines. The later chapters deal broadly with 3 different types of power plants, viz. Spark ignition engines, Compression ignition engines, and Gas turbine. A brief note is added at the end on nuclear power and sophisticated types of power plants. In dealing with various chapters the authors show their skill in making the subject matter very interesting and clear for the beginners.

This book will be very useful for undergraduate classes studying IC engines. The worked out examples and the inclusion of air tables increase the utility of the book both as a text-book and reference book for the teacher.

A. V. SHREENATH

FREEZING PRESERVATION OF FOOD: Vol. 2, edited by Donald K. Tressler, Wallace B. van Arsdel & M. J. Copley (AVI Publishing Co. Inc., Westport), 1968. Pp. ix+397

This is the second volume of the four-volume treatise on the freezing preservation of foods and is an augmented and completely rewritten edition of the 1957 two-volume edition by Tressler and Evers. Several specialists closely associated with the work have contributed the subject matter of each of the different chapters. The volume is an up-to-date one, comprehensive and authoritative in its exposition. The book has been divided into 14 chapters with an appendix (containing selected methods of analysis) and conversion tables. The get-up is excellent with a number of tables and figures.

The physical phenomena of freezing of both plant and animal tissues are considered in the first chapter. Freezing and thawing induce some physical changes which influence the quality of the frozen foods and are dealt with in detail. Chapter 2 presents data on thermal properties of various foods. Hitherto calculations on heat removal of foods during freezing were based on specific heat and latent heat of frozen foods, which could lead to significant errors because foods do not freeze at constant temperature. This discrepancy has been overcome by using enthalpies and the heat removed during freezing has been calculated on this basis. These results are tabulated for various commodities and will be invaluable data for frozen food manufacturers (viz. enthalpy of frozen meats, fruits, vegetables, juices and egg products). Data on thermal conductivity of frozen meats, fruits, vegetables, eggs and butter fat and also various other thermal properties of the above materials in fresh form are tabulated. Most of these data are based on recent publications.

Measurement of frozen food quality and quality changes is a brief summary of the authors' (Kramer and Twigg, 1966) book on 'Fundamentals of quality control in food industry' with a slight orientation towards frozen foods and is discussed in Chapter 3.

Chapters 4-11 are concerned mainly with the characteristics of all kinds of fresh and frozen foods with a bias on the factors affecting the quality changes during freezing and storage of various foods (viz. vegetables, fruits, poultry, meats, frozen fish and shell fish, dairy products, eggs, etc.). Among the quality factors discussed, flavour has been adequately covered. Various aspects on the role of monosodium glutamate on the quality of frozen meats have been emphasized. Pre-freezing treatments to prevent gelation of egg yolk have been dealt with in detail. Chapter 12 deals with the evaluation of packaging materials generally used for frozen foods and Chapter 13 with the microbiology of frozen foods besides considering the effect of freezing and low temperature storage on the bacteria, yeasts and moulds found in foods. The last chapter rightly discusses the

nutritive value of frozen foods, with particular reference to loss of vitamins and other nutrients during preparation for freezing and freezing storage of foods. The appendix includes several selected methods of analysis.

The presentation is highly commendable and the book is an invaluable one for students, research workers and others engaged in frozen food industry.

In short this volume will serve as a very useful reference book.

L. V. L. SASTRY

PHYCOLOGIA INDICA (ICONES OF INDIAN MARINE ALGAE): Vol. I, by K. S. Srinivasan (Botanical Survey of India, Calcutta), 1969. Pp. xix+52+51 plates. Price Rs 31 or Sh. 60 or $ 7.00

This publication is the first volume in a series of illustrated catalogues planned to bring together many of the marine algae recorded in our country. So far no effort has been made to study this fascinating group of plants, in spite of the fact, there are a number of places along our long coast line abounding in them. Unlike land plants which have drawn attention from various workers both outside and inside India, these marine plants have so far attracted very little attention, except for their enumeration in some of the publications made by European botanists, while on travel to some of the neighbouring countries. The only work of importance on Indian marine algae has been that of Boergesen who visited India on a short tour on his way to Ceylon and who enumerated also many algae collected by others. It is, therefore, welcome to see a volume, depicting in colour, some of the species belonging to the red, brown and green seaweeds collected along our coast, and studied first hand by the author himself. This first volume includes 51 species, 13 belonging to Chlorophyceae, 14 to Phaeophyceae and 24 to Rhodophyceae. The illustrations are excellently executed and each one provided with a fairly detailed description. Further a few line drawings are reproduced from some of the published literature, to explain certain important characteristics.

The book opens with a short introduction, comprising a general account of the ecological aspects of marine algae, particularly the factors influencing their growth, the nature of sub-stratum on which they are found and the seasonal changes that affect their occurrence. This is followed by a short account of the nature of the Indian coast line and its physiography and how these marine plants can be collected, preserved and studied.

The text of the book is confined to a detailed description of each of the 51 species illustrated. Besides description, the habitat of the algae is mentioned with a few notes regarding the ecological conditions under which it has been found.

The book provides a valuable and detailed guide to all those interested in the Indian seaweeds and will greatly help in identifying them and understanding them. The author, the artists and the publishers are to be congratulated for the excellent manner in which they have brought out this volume. It is gratifying to note that the Botanical Survey of India has sponsored this work and we earnestly look forward to further volumes of this useful series at an early date. It is befitting that this entire work is dedicated to late Prof. Iyengar, the eminent algologist, who laid the foundation of algological studies in India.

While appreciating all efforts that have been made to bring out such an excellent work, it may not be out of place to make a few suggestions, which are likely to improve its usefulness. The descriptions of the various species are evidently based on some important reference works and for taxonomic assistance, it would have been helpful if some of the works on which the type descriptions have been based are cited, so that they will be of value to those research workers who are interested in pursuing their studies. Secondly, the author could have mentioned where the collections on which these illustrations are based are located, by indicating the specimen number and herbarium, so that for purposes of later taxonomists they would have been useful.

The book is well printed and produced and the price is fairly cheap for the fine colour plates included.

K. R. RAMANATHAN

PUBLICATIONS RECEIVED

INTRODUCTION TO MEDICAL ELECTRONICS by S. K. Guha (Bharati Bhawan, Patna), 1969. Pp. x+131. Price Rs 20.00

METHODS OF MULTIVARIATE ANALYSIS by Keith Hope (University of London Press Ltd, London), 1968. Pp. 165. Price 30s.

EVOLUTION OF LIFE by M. S. Randhawa, Jagjit Singh, A. K. Dey & Vishnu Mittre (Publications & Information Directorate, CSIR, New Delhi), 1969. Pp. 360. Price Rs 45.00

NOTES & NEWS

A new technique for optical pumping at high temperatures

A significant extension of the optical pumping technique, that will greatly increase its utility in rf spectroscopy, has been reported from the Department of Physics, University of New Hampshire, New Hampshire. Optical pumping utilizing the alkalis has in the past provided very precise values for free atom hfs and g_J values. The high precision is especially useful in looking for effects caused by higher order multiple interactions. The present technique will make possible the pumping of a considerable number of new elements which have orbital S-states with $J \neq 0$ and, therefore, can potentially be polarized by spin-exchange collisions.

The technique has enabled optical pumping experiments over temperatures up to 750°C, with the result that even Zeeman transitions in silver could be detected. The technique has also been successfully applied to study spin-exchange polarization of electrons, atomic hydrogen, and atomic nitrogen up to 850°C. Besides considerably increasing the number of elements that can be polarized, this relatively simple technique enables one to investigate the energy dependence of spin-exchange cross-sections, spin relaxation times and hyperfine pressure shifts.

The basic optical arrangement is shown in Fig. 1.

Circularly polarized Rb-$D1$ radiation is used to polarize the Rb atoms in a 300 cm³ flask containing a buffer gas and atomic silver. The absorption flask is an oven whose temperature is varied from 700° to 750°C during the course of the measurements. At this temperature, silver has a vapour pressure of approximately 10^{-5} torr. Spin-exchange collisions between the silver atoms and the polarized Rb atoms cause the orientation of the silver atoms. The equilibrium polarization of Rb and thus the amount of light absorption is altered when a resonant rf magnetic field is applied to the silver atoms. The rf field is amplitude-modulated and the signal is detected in a phase-sensitive detector. In order to optically pump Rb in a flask at high temperature, the vapour densities of Rb and Ag need to be controlled independently of the temperature of the flask [*Phys. Rev. Lett.*, **22** (1969), 161].

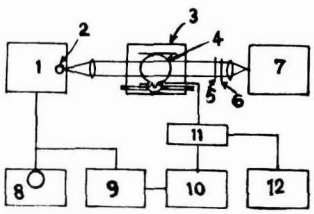


Fig. 1 — Basic optical pumping arrangement [1, pre-amplifier; 2, photocell; 3, oven; 4, pyrex optical pumping flask; 5, circular polarizer; 6, D-1 filter; 7, light source; 8, oscilloscope; 9, phase-sensitive detector; 10, audio oscillator; 11, relay switch; and 12, rf signal generator]

Photoelectron spectroscopy

The technique of 'photoelectron spectroscopy' measuring the binding energies of the electrons in molecules directly, in contrast to absorption spectroscopy where only the difference between the two energy levels is recorded, has gained considerable importance during the past few years. The importance of the technique was recently focused in the first international conference on photoelectron spectroscopy held during March 1969 in London under the auspices of the Royal Society.

It is perhaps surprising that although the photoelectric effect has been common knowledge for more than 60 years, until recently nobody had thought of applying it to analyse molecular levels. There are two chief sources of monochromatic photons in regular use. The first resonance line of helium produces a strong output at 584 Å in the far ultraviolet, and soft X-rays from magnesium and aluminium are also used. Higher energy photons tend to produce better resolution in the photoelectron spectra, but usually a lower intensity as well. Two review papers set the scene for the conference. Dr D. W. Turner of Oxford University and Prof. K. Seighbahn of University of Uppsala, the originators of two principal branches of the technique, discussed how photoelectron measurements have been used to determine molecular electronic structure and for chemical analysis. The halogens are elements which give sharp lines in the helium-584 spectra, and Prof. W. C. Price of King's College, London, gave a paper on the spectra of the halides of group III elements.

An incisive theoretical paper on general theory of diatomic molecules was given by Dr J. M. Sichel of Bristol University, in which he analysed the angular distribution of the photoelectrons from such molecules. An interesting paper on photo-ionization cross-sections by Dr J. A. R. Samson of GCA Technology Division, Bedford, Massachusetts, contained an account of how the angular distribution of photoelectrons can be estimated from measurements at two particular angles.

Several papers referred to the phenomenon of auto-ionization, which is a major contributor to the difficulties in absorption spectroscopy. This process occurs when an electron is not emitted directly from an atom, but jumps to a resonant state and is then emitted later. The line shape for such processes in photoelectron spectra is under investigation at present. As Dr Turner pointed out in his paper, it is now also possible to analyse the fine structure of the spectra to give the vibration frequencies of the molecular ion, and from these the electron orbits of complicated molecules can be inferred [*Nature, Lond.*, **221** (1969), 903].

Structure of glass by NMR and ESR

Nuclear magnetic resonance and electron spin resonance techniques have been used to acquire more insight into the structure of glass and its defects. Known structural groupings and bonding

configurations in oxide compounds yield specific chemical shifts, quadruple interactions, and dipolar broadening in the NMR spectra for the constituent nuclei. The same parameters are then measured for glasses made from the oxides and correlations arrived at. Very often it is observed that the groupings and configurations present in the glasses resemble those found in the various crystalline compounds under study. In such systems, the NMR spectra can often be used to determine the amount of each configuration that is present in glass. Glasses contain a wide variety of point defects involving impurity ions and vacancies. Point defects affect the optical, thermal and mechanical properties of glasses. Relaxation studies on several paramagnetic defects in silica and germania show that spin relaxation between 10° and 200°K is dominated by low frequency vibrations, characteristic of defects but not of the host. ESR and relaxation studies of silica and germania glasses and crystals have led to models suggesting the structure of defects such as atomic hydrogen centres wherein hydrogen atom is probably trapped in a large void. However, in silica crystals the hydrogen atom is at an interstitial site, probably between two silicon atoms [*Chem. Engng News*, **47** (No. 13) (1969), 50].

Formation of ammonia by a new route

In Haber's method for the manufacture of ammonia, the reaction necessitates the use of high temperature and pressure. Findings at Stanford University on nitrogen fixation research reveal that the reaction steps take place even at room temperature. In a typical experiment, commercially available titanium tetraisopropoxide is mixed with a reducing agent, such as sodium naphthalide in tetrahydrofuran or diglyme. The reactants are stirred at room temperature in an atmosphere of nitrogen at atmospheric pressure. The sodium naphthalide reduces the tetravalent titanium compound to titanium disopropoxide, each molecule of which then binds nitrogen molecule. Additional sodium naphthalide reduces this nitrogen complex to the ammonia level. Subsequent hydrolysis by isopropyl alcohol yields ammonia. Coproduct titanium isopropoxide can be reused.

One of the most intriguing aspects of the results is that the reaction still takes place even when air in place of pure nitrogen is used. It is obvious that atmospheric nitrogen competes significantly with oxygen for reaction with titanium.

Though this route has not been accepted as a commercial route to ammonia, the new reaction looks promising. One of the points intended to be studied is to explore the use of electric current as a source of electrons in place of a reducing agent. Another aspect to be studied involves a series of experiments employing organic substances to see if these can react with molecular nitrogen to form organic amines or similar nitrogen compounds [*Chem. Engng News*, **47** (No. 13) (1969), 48].

A new route to aldehydes

A series of new syntheses for numerous aliphatic, unsaturated and cycloalkane aldehydes, potentially useful for synthetic organic chemists has been developed at Louisiana State University, New Orleans. Commercially available dihydro-1,3-oxazines, typically 2,4,4,6-tetramethyl-5,6-dihydro-1,3(4H)-oxazine, are the starting materials. In place of a hydrogen on the 2-carbon, other substituent groups like phenyl or carboethoxy can be used.

An oxazine used in the synthesis is converted quantitatively to a lithio salt at −70°C by reaction with phenyl, *n*-butyl or *t*-butyl lithium in tetrahydrofuran. The anion remains stable below −50°C for several days. Alkylation of anion results from adding an alkyl halide along with a base such as *n*-butyl lithium. The alkylated oxazine which can be isolated and purified is then reduced as such with $NaBH_4$ (or deuteride) to tetrahydro-1,3-oxazines in THF-ethanol-water solution. The aldehyde is obtained by hydrolysis of crude tetrahydro-1,3-oxazines. The products are separated by steam distillation or extraction from acidic solution. The reduction step which allows simple addition of deuterium or tritium to the molecule offers one of the most versatile methods to prepare C_1-labelled aldehydes. The deuteride reduction of the starting materials followed by cleavage provides a convenient source of C-1 deuterated acetaldehyde and phenyl acetaldehyde. This technique for the preparation of unsaturated aldehydes allows a stereospecific two-carbon chain extension of carbonyl compounds. For example, *trans*-β-ionylidene acetaldehyde has been prepared in 54% overall yield from β-ionone.

Besides deuterated aldehydes other aldehydes can be made with labelled carbons using commercially available acetonitrile containing ^{14}C. Various α,β-unsaturated aldehydes can be made starting from suitable carbonyl compounds and following the same procedure [*Chem. Engng News*, **47** (7) (1969), 32].

Apparatus for undisturbed addition of solids to refluxing solutions

Currently, the addition of solids to hot reaction mixtures is made either down the condenser or via the side arms. Both these approaches give rise to complications, condensers becoming choked and refluxing solvent being lost from opened side arms. These problems have been overcome in a simple apparatus designed in which solid materials can be placed and transferred to the reaction without causing the escape of the refluxing solvent from the reaction flask.

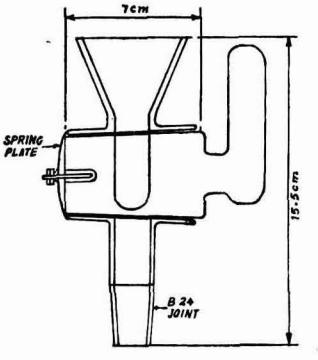


Fig. 1 — Apparatus for addition of solids to refluxing solutions

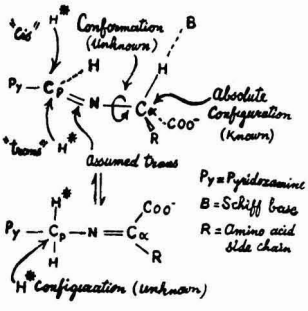
The principle on which the apparatus (Fig. 1) is based is the provision of a large hollow stopcock into which part of a test tube has been fitted. As long as the container is not filled beyond about 50% of its capacity, the tap does not get bound. Even when clogging takes place, it usually gets cleared in a short time due to the washing effect of the refluxing solvent. A typical application of the apparatus is in reactions where sodium has to be added in small pieces [*Chemy Ind.*, (1968), 684].

Stereochemistry of enzymatic transamination

An approach to the determination of complete stereochemistry of enzymatic transamination has been described by Dunathan, Davis, Kury and Kaplan [*Biochemistry*, **7** (1968), 4532]. The large family of enzymes utilizing pyridoxal phosphate as cofactor catalyse a great variety of transformations of amino acids. It is involved in amino acid synthesis, transformation and degradation. Two forms of B_6 cofactor-pyridoxal phosphate and pyridoxamine phosphate are involved during enzymatic transamination. These bound factors are interconverted by tautomerism of the cofactor substrate (PLP amino acid Schiff bases).

The fundamental step of transamination is the transformation of 1,3-prototropic shift. It is suggested that the symmetry of the hydrogen labilized at the pyridoxamine-4-methylene group may be related to the symmetry of the amino acid substrate.

It is postulated that the mechanism of transfer must be *cis*, in which both proton removal and addition take place on the same side of the cofactor amino acid Schiff base. Having established both the stereospecificity of the cofactor 4-methylene carbon and a probable *cis* mechanism for transfer, the only unknown point left is the absolute configuration of the asymmetric monodeutero pyridoxamine samples from the enzyme reaction. According to Dunathan the 'S' monodeutero pyridoxamine must contain deuterium in the position where it is enzyme labile [*Chem. Engng News*, **46** (53) (1968), 52].



Preparative fractionation of genetically specific nucleic acids

A general procedure for separating genetically specific DNA and RNA on preparative scale has been reported from the Biology Division, Oak Ridge National Laboratory, Tennessee. The method, which is essentially a modification of the RNA isolation technique of Beutz and Reilly [*Science, N.Y.*, **151** (1966), 328], involves the preparation of a DNA-nitrocellulose complex, drying of the complex at high temperature, preincubation in a polymer solution and batch incubation of the complex with the preparation to be purified by elution of the required nucleic acids. The technique is applicable to a variety of experimental solutions in which a relatively large amount of genetically specific nucleic acid is desired; mixtures of labelled DNA from bacteria and bacteriophage have been obtained in 50-fold purification with 70% recovery and mRNA specific for T_2 bacteriophage in milligram quantities.

The technique appears to meet the requirements of a specific separatory system: (i) separation of both DNA and RNA on the basis of genetic specificity is feasible, (ii) relatively large amounts of the nucleic acids of interest can be separated, (iii) it is applicable to microbial systems in general, (iv) it allows estimation of the (radioactive) specific activity of the DNA or RNA. Separation of nucleic acids on the basis of sequence homology assumes considerable importance because the availability of specific nucleic acids in large amounts will render *in vitro* syntheses of specific proteins possible. Although the technique has so far been applied to microorganisms only, the DNA-nitrocellulose column may prove to be a useful tool for separating RNA transcribed from the unique and the imperfectly complementary fractions of DNA in higher organisms.

Nitrocellulose (Hercules type RS, 5-6 sec) ground with an approximately equal volume of 2×SSC [0·15*M* NaCl-0·015*M* sodium citrate (*p*H 7·2)] in an electrically driven mortar and pestle for 1 hr is passed through a 40 mesh sieve. The resulting paste is washed ten times with 4 vol. of 2×SSC; each time, the nitrocellulose is allowed to settle for 10 min, and the fine material is decanted. The nitrocellulose is washed with 4 vol. of 2×SSC in a Buchner funnel, suspended in 4 vol. of 6×SSC, allowed to stand for 10 min, decanted and finally suspended in 3 vol. of 6×SSC and stored at 4°C until use.

DNA is dissociated by exposure to 0·15*N* NaOH for 10 min, followed by neutralization with HCl. The ionic strength of the solution is then raised by the addition of 4/10 vol. of 20×SSC. This DNA is referred to as DNA-I. The solution is added to the appropriate amount of a nitrocellulose slurry in 6×SSC and stirred for 30-60 min at room temperature, filtered through Whatman No. 3MM. The powder is dispersed in a petri dish and dried *in vacuo* at room temperature overnight. This DNA-nitrocellulose powder is preincubated in a medium (PMG) containing 0·02% each of Ficoll (mol. wt, 400000), polyvinylpyrrolidone (mol. wt, 360000), and bovine serum albumin in 6×SSC. The preincubated complex is incubated in the DNA to be analysed (DNA-II) and the suspension in GSSC is shaken at 66°C for 16-24 hr and transferred to a jacketed chromatography column.

The required nucleic acid fraction in the form of duplexes is then obtained by elution of the column first with successive portions of G×SSC and then with successive portions of water [*Biochemistry*, **8** (1969), 222].

Regional Research Laboratory, Hyderabad

The annual report of the laboratory for 1967-68 highlights its main activities and achievements in basic and applied research. During the year, a pilot plant of 1 tonne/day capacity for the fluidized carbonization of non-caking Singareni coals and an externally heated high temperature carbonizer for the production of metallurgical coke from low grade coals were set up. An important finding reported from the laboratory concerns the mechanism of the toxic action of insecticides, viz. that neurosecretions are liberated by the action of insecticides on the nervous system of the insects. A method, using zinc ions, for the quantitative separation of ribosomal and soluble ribonucleic acid has been worked out.

A new system, benzimidazole (1, 2C) quinazoline, has been synthesized by the reaction of 4-chloroquinazoline with *o*-aminophenol followed by ring closure with PPA. Many new compounds were prepared by nucleophilic displacements on 3 methylthio phenmorpholone and their pharmacological actions are being studied. Three new compounds, iso-diospyrin, compound A and compound B, were isolated from the chloroform extract of wood and bark of *D. chloroxylon*. From the heartwood of *Diospyros melanoxylon* Roxb., two new naphthol derivatives N_3 and N_4 and an *ortho*-quinone have been obtained. Using different solvents and varying other process conditions, 85-90% yield of 2,4-dichlorophenol has been obtained by the chlorination of phenol with gaseous chlorine. The dichlorophenol was then condensed with monochloroacetic acid to give pure 2,4-dichlorophenoxyacetic acid in 74-98% yield by a single step procedure.

Diepoxyoctadecanoic acid (m.p. 76-77°) required for the preparation of diepithio derivative was prepared by epoxidizing pure linoleic acid. Linelaidic acid (m.p. 29°) was prepared from linoleic acid by isomerization with sodium nitrite and nitric acid, passage through a silica gel column to remove the nitrogenous matter and three recrystallizations from acetone at −20°. A method for reducing the high acid value of commercial sardine oil has been worked out.

That the neurosecretions are liberated by the action of insecticides on the nervous system of insects has been found for the first time. When insecticides belonging to two different groups are used together, they interfere with each other's action, resulting in less mortality. When insecticides belonging to the same group were used, an increase in mortality was observed. This observation is useful in preparing effective formulations of insecticides. The degree of sterilization produced with various concentrations of Apholate on *Achoea janata* has been studied. In males, 100% sterility was observed by exposing the animals for 4 hr on an Apholate-treated surface (200 mg/sq ft). In females, 2 hr exposure on Apholate-treated surface (100 mg/sq ft) produced complete sterility.

A column chromatographic method using ion-exchange resins has been worked out for the quantitative separation of mono-, di- and triphosphates. A method worked out for the isolation of newly synthesized RNA from spermatozoa involves delipiding of the incubated spermatozoa with alcohol and alcohol-ether mixture, followed by digestion with protonase in the presence of thioglycollic acid, carrier RNA and inhibitors of RNase, and extraction with phenol.

A two-stage process for making an ammonia synthesis catalyst has been developed. The catalyst prepared has been found to possess more than 90% activity compared to commercial catalysts. Adjustments are being made in the catalyst preparation to improve its efficiency. To obviate the need for expensive imported replacement in the existing electronic switchgear for autoclaves, an alternate design using indigenous components for switching of inductive loads has been developed.

CSIRO, Australia

The annual report of the Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia, for the year 1967-68 records details of the various projects undertaken towards developing and utilizing Australia's natural resources as well as accelerating the pace of its industrial development. During the year a new laboratory for the Radiophysics Division was completed.

During the year, the 3000-acre solar observatory for the Divisions of Radiophysics and Physics was opened and its automatically steerable radio heliograph was commissioned. This unique instrument is used for obtaining continuous radio pictures of the sun. The observatory is the only one in the world with facilities at the same site for making high resolution radio and optical observations of the sun. These facilities will enable a better understanding of the phenomena like sunspots and solar flares. In collaboration with radioastronomers in North America, the Division of Radiophysics is planning to measure, for the first time, the diameters of some of the more interesting quasars. A possible theoretical explanation of west wind (of more than 200 miles an hour), required to be postulated to explain the observations on the gradual tilt of satellite orbit planes towards the end of the satellite's life, has been put forward by the Upper Atmosphere Section.

A technique developed at the Division of Applied Physics makes it possible to determine the density of a sample of low viscosity liquid only 1 cc in volume with an accuracy of two parts in 100,000.

A project in hand at the Division of Entomology concerns the isolation and identification of the chemical compound(s) concerned in the spread of fungus infecting pinewood (as a result of laying eggs in it by Sirex).

As a part of its research on possible new insecticides, the Division of Applied Chemistry has developed several unchlorinated test compounds, which compare with DDT in their toxicity to flies, but which are relatively harmless. The chance of insects developing resistance to these compounds seems to be much less than with DDT.

Studies conducted at the Division of Animal Health have

revealed that a virus which causes mucosal disease in cattle can also infect pigs under natural conditions. Pigs which are infected with mucosal disease virus show no obvious symptoms, but they produce an antibody which makes them resistant to subsequent infection with swine fever virus. These animals give misleading positive reaction for swine fever when subjected to the blood tests. As a result of these findings, a new blood test which unambiguously distinguishes between pigs infected with mucosal disease virus and those infected with swine fever virus has been developed.

A knowledge of the amount of heat produced by sheep can help in devising ways of improving the efficiency with which sheep utilizes its food for growing wool. Scientists of the Division of Animal Physiology and the University of New England are working together on a new technique that should make it possible to determine heat production in both grazing animals and laboratory animals as a matter of routine. The technique is based on the fact that an animal's heat production is related to its carbon dioxide production.

The research undertaken by the Division of Wild Life Research on the social behaviour of rabbit has led to an efficient technique for poisoning rabbits. Studies on the function of scent glands found in the anal region of the rabbit, on the part played by the strong smelling secretion by the glands in the social behaviour of rabbits and on new methods of rabbit control, based perhaps on the use of chemical attractants, are in progress. Studies being conducted on the history and the various factors causing plague through the house mouse indicate the possibility of predicting plagues 3 or 4 months in advance.

In an attempt to find quicker and cheaper methods of wool testing, a system that will measure the yield and fineness of wool samples semi-automatically is being explored in the Textile Physics Division. A modified dump, which constrains vacuum pressed wool bales during dumping and prevents bursting, has been developed jointly by the Textile Physics and Protein Chemistry Divisions. Work is also being done on sampling methods to find out the smallest number of samples which need to be taken if the characteristics of all the wool in a particular lot are to be measured with the required degree of precision.

The Division of Applied Mineralogy has developed a sensitive method for determining tellurium in rock samples. The method employs atomic absorption spectroscopy and can detect two parts of tellurium in one hundred million parts of rock. The method will be useful as a preliminary indicator of gold prospecting sites since Te has often been associated with gold in Australia and traces of Te in a rock sample could be an indication that gold is nearby. At the Divisions of Applied Mineralogy and Mineral Chemistry a new sintering method has been devised in which oxide shapes are heated in a plasma of ionized gas. The method is much quicker than the normal heating techniques and greater densities and strengths can be achieved in the final product.

As a result of a 10-year survey project conducted in the Division of Soils an 'Atlas of Australian Soils', a series of 10 maps which together provide the most detailed picture yet available of various Australian soils and their distribution, was completed in 1968. The atlas makes it possible to compare and contrast soils from different parts of Australia. A novel way of turning low grade rock phosphate into a more useful fertilizer named 'biological superphosphate' or 'biosuper' has been devised.

The Division of Land Research is trying to find out the contribution that carbon dioxide, water and sunlight each make to the plant. This information is being analysed in a computer so that mathematical equations which describe in detail how plant growth is related to environment could be established. These equations could provide plant breeders with an additional guide to the sort of characters they should select when breeding varieties to suit a particular environment.

An instrument designed by the Division of Soil Mechanics to measure the humidity of the air just above the soil sample quickly and accurately has provided foundation engineers with an improved technique for determining moisture potentials. Several versions of the instrument have been made depending upon the need.

Investigations by the Division of Building Research into the curing of concrete under closely controlled conditions have led to the development of a system of curing based on the use of hot flue gases from a burner instead of steam. The test results showed that curing with the gas was just as effective as steam curing and costs much less. The Division has developed a range of concrete glasses that fire at temperatures as low as 500-600°C. At these temperatures the strength of concrete is not seriously affected. There is no need to treat the concrete surface specially before glazing and a variety of colours and patterns is possible. Glazed blocks can be made durable, easy to maintain and with non-fading colours. They are much less permeable to water than unglazed blocks and white efflorescent crusts cannot form on their surface.

Research is continuing in the Division of Animal Physiology to ascertain the reasons for the remarkable increase in wool growth which can be obtained when proteins or certain amino acids are put into the fourth stomach of sheep.

The Food Preservation Division is studying the oxidation products of α-farnesene which seems to be the most likely cause of superficial scald in apples. At the request of the Victorian Grain Elevators Board, the Division of Mechanical Engineering undertook the design of the installation of equipment for passing air through grain to minimize insect infestation in giant sheds. The Division has also devised a method of rapidly cooling grain in large sheds with refrigerated air and is undertaking tests in Queensland where the temperatures are too high for adequate cooling.

Optics Communications

This new journal, started by the North-Holland Publishing Co., Amsterdam, will serve as a medium for the rapid publication of short contributions in the fields of optics and interaction of light with matter. The journal, to be issued

monthly, will accept short communications (not exceeding 4 printed pages) on instrumental and physical optics (including holography, optical information processing, sources, detectors, coherences, etc.), spectroscopy (atomic, molecular, condensed matter) and quantum electronics (optical pumping, masers, lasers, non-linear optics, etc.). Manuscripts for publication should be communicated to the Editor, Prof. F. Abeles, Laboratorie d'Optique, Tour 33, Faculté des Sciences, 9, Quai St. Bernard, 75 Paris, 5. The rate of subscription for Vol. 1 is $ 20.

OMR (Organic Magnetic Resonance)

This new bimonthly international journal, launched from February 1969 by Messrs Heyden & Son Ltd, London, is devoted specifically to all branches of magnetic resonance as applied in the field of organic chemistry. The journal will include papers on NMR, NQR and ESR and the more recently developed technique of ion cyclotron resonance. A spectral supplement to each issue will contain spectral reproductions in full. The annual subscription for the journal is £ 16 10s for institutions and £ 5 for individuals.

Dr A. K. De

Dr Arun Kumar De, Professor and Head of the Department of Mechanical Engineering, Indian Institute of Technology, Bombay, has been appointed Director, Central Mechanical Engineering Research Institute, Durgapur.

Born on 2 November 1925, Dr De received his early education in Calcutta and after graduation in mechanical engineering in 1947 from the College of Engineering and Technology, Jadavpur, had industrial training at the Jay Engineering Works Ltd, Calcutta, during 1947-49, followed by advanced training in UK from 1949 to 1951. On completion of the training, Dr De served the Jay Engineering Works as a development engineer till 1958. The same year he joined the Indian Institute of Technology (IIT), Bombay, as an Assistant Professor. It is mostly due to his initiative and drive that the master's degree course in machine tools engineering was started at IIT, Bombay, in 1960. In 1962, he proceeded to USSR and worked with Prof. N. S. Acherkan in the Machine Tools and Small Tools Institute, Moscow, for his doctorate degree. On his return, he was appointed Professor and Head of Mechanical Engineering Department at IIT, Bombay.

Dr De has specialized in production engineering and machine tools and has to his credit a number of research papers dealing with machine tool rigidity and its measurement, dynamic errors in metal cutting operation, static rigidity of bed plate, static rigidity of lathe beds by model analysis, rigidity characteristics of clamped columns and shaping machines, etc., and effect of cutting fluids on tools wear.

Announcements

▪ *The Second International Air Pollution Conference* will be held in Washington DC during 6-11 December 1970 under the auspices of the International Union of Air Pollution Prevention Associations. The programme of the conference includes separate technical sessions (cocurrent) for the following subject areas: (1) Air pollution chemistry and physics (sampling, analysis, instrumentation, aerosols and effects of non-biological systems); (2) Air pollution meteorology (transport, diffusion, modelling, forecasting and vegetation); (3) Air pollution medicine and biology (effects on people, animals and vegetation; air quality criteria); (4) Air pollution engineering (sources, their engineering control and control equipment); (5) Air pollution control administration (legislation, regulations, inspection and control programme operation, air pollution standards); and (6) Air pollution surveys (community and area studies). Proposals to present papers (including a provisional title for the paper, abstract limited to 200 words and names, titles, affiliations of the proposed authors and address of the principal author) should be sent by 31 January 1970 to the national Chairman of the Programme Committee for India, Shri Jaydev M. Dave, Deputy Director, Central Public Health Engineering Research Institute, Nagpur, or directly to Prof. Arthur C. Stern, Dept of Environmental Sciences & Engineering, School of Public Health, University of North Carolina, PO Box 630, Chapel Hill, NC 27514, USA.

▪ *The Sixth Congress of the International Federation of Prestressing* will be held in Prague during 6-13 June 1970. The congress will have four technical sessions devoted to (i) special lectures covering various aspects of research, design and development in prestressed concrete; (ii) discussions on reports of FIP commissions, (iii) reports from member groups on outstanding prestressed bridges, buildings and other structures; and (iv) technical contributions on various aspects of prestressed concrete.

The official languages of the congress will be English, French, German and Russian. Further details can be had from the Organizing Committee of the Sixth Congress of FIP, PO Box 107, Praha-6-Dejvice, Czechoslovakia.

▪ *A Symposium on Macromolecules in Storage and Transfer of Biological Information*, organized by the Biology and Medical Committee of the Department of Atomic Energy, will be held at the Bhabha Atomic Research Centre, Trombay, Bombay 85, about mid-December 1969. The scientific programme will include sessions on Ultrastructure of cells and electron microscopy of DNA, Nucleic acid structure and function, Transcription and translation of nucleic acids, Regulatory mechanisms, Function of cellular membranes, Nature and repair of radiation damage. The last date for sending abstracts for contributed papers is 30 September 1969. The abstracts may be sent to Dr N. K. Notani, Biology Division, Bhabha Atomic Research Centre, Trombay, Bombay 85.

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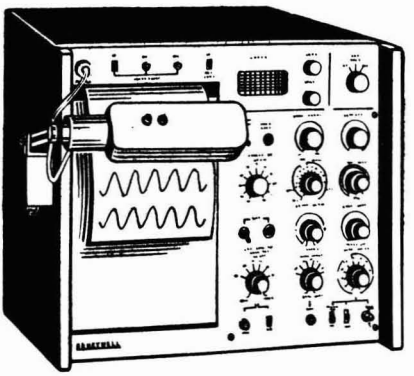

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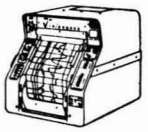

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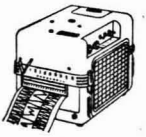

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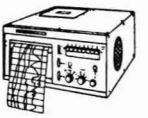

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