BIOCHEMISTRY AT LIVERPOOL 1902–1971

by

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THIS paper begins by surveying the state of biochemical knowledge at the turn of the century. It then records how in 1902 the first British Chair of Biochemistry was founded. It goes on to describe some contributions to the subject made at Liverpool prior to the 1939–1945 war and notes that many of these advances were made by people trained in other disciplines. Finally some highlights from a steadily increasing post-war output of papers will be mentioned.

This is not a record of uninterrupted success; there have been notable achievements and some failures. Major problems were tackled before an adequate scientific background existed or the necessary techniques had emerged. Erroneous received ideas imposed heavy constraints. The path of the pioneers was hard and if they did no more than clear the ground they deserved respect and sympathy.

Biochemistry grows exponentially; with a doubling period of about fifteen years 95 per cent of the subject as we know it did not exist in 1900. Physiological chemistry in Germany had provided a firm base for a study of the roles of carbohydrate, fat and protein. Animals and men had been placed in respiration chambers so that oxygen consumption and carbon dioxide output could be measured. Respiratory quotients and calorific values had been obtained. Basal metabolism had been linked to body surface area and the specific dynamic action of food had been discovered. The application to patients of respiratory quotients and the measurement of basal metabolic rates in thyroid disfunction had opened a new chapter. In broad terms human requirements of nutrients were being established and the picture of the animal organism maintaining its body temperature and matching to its physical activity the utilization of energy from food was a challenge to the rising generation of biochemists. This challenge they accepted—as best they could; in fact not enough was known about the relevant organic chemistry.

Emil Fischer's work reached fruition in the present century. In 1868 six aminoacids were known and by 1900 sixteen. Valine and proline were identified by Fischer in 1901 and Hopkins and Cole discovered tryptophan; methionine, threonine and thyroxine came much later. Detection of sugar in urine had been feasible for many years but insulin was not prepared until 1921 and purified much later. The significance of metabolic and biosynthetic pathways could be imagined but factual evidence was scarce. Chevreul had given to lipid chemistry a sound descriptive basis; lecithin was known but the distinction between it and kephalin was not made until 1913. Cholesterol had been obtained from gallstones and carotene also was known but the organic chemistry of plant and animal sterols and carotenoids began to be systematic only in the period 1923–33. The word *hormone* was introduced in 1902 by Bayliss

https://doi.org/10.1017/S002572730001797X Published online by Cambridge University Press

and Starling and systematic endocrinology also advanced step by step with the organic chemistry of natural products.

The first enunciation of the vitamin concept was probably by Lunin[1] in 1881 when he postulated 'besides the principal ingredients [of foods] small quantities of material essential to life'. Although Pekelharing[2] repeated the work in 1905 it did not become generally known until 1926. The notion of nutritional deficiency as a cause of specific disease gained ground with the work of Eijkman[3] (1897) and of Grijns[4] (1901) on beri beri. Even scurvy was not fully recognized as a deficiency disease until the work of Holst and Fröhlich[5] (1907) who induced it in guinea pigs kept on an artificial diet.

In 1900 Gowland Hopkins had twenty papers to his name. He had described a yellow pigment in the wings of butterflies, a pigment which half a century later was to acquire renewed interest. But he was known mainly for his rather pedestrian studies on the analytical chemistry of urine. After his work with S. W. Cole.[6] on tryptophan he realized (1906) that the protein zein was 'incomplete' nutritionally.[7] It lacked lysine and tryptophan. Hopkins showed that a tryptophan supplement to zein stimulated growth and the concept of 'essential' aminoacid was established.

The *vitamin* idea was not adopted with undue alacrity. When the British Medical Association held its annual meeting in 1920 Hopkins[8] refused to speak about the vitamin *hypothesis* and claimed that 'vitamines, though still of unknown nature in the chemical sense are not merely hypothetical'. Sir James Barr was sceptical, 'vitamines, so far as their composition is concerned seem to be a figment of the imagination'. In 1913 Hopkins[9] had himself been a little taunting when he said 'It is yet a rare thing in this country to meet a professed biologist . . . who has taken the trouble so to equip himself in organic chemistry as to understand fully an important fact of metabolism stated in terms of structural formulae'.

This was perhaps harsh when it is remembered how little there was to 'understand fully'. Animal experiments allowed problems to be studied and were necessary to monitor chemical separations. The problems of isolation and characterization, seen in relation to the chemical and physical techniques available, were formidable. Indeed the advance of biochemistry depended on simultaneous growth in fields such as organic and physical chemistry, nutrition, physiology and clinical medicine. Improved statistical methods were important too, and the appeal to the experimental animal was final.

From about 1925 onwards great advances occurred in the study of vitamins and hormones. The discovery of co-enzymes as catalysts of metabolic processes had a liberating effect on biochemistry and the central processes of electron transport, oxidative phosphorylation, photosynthesis and metabolic cycles generally began to be vulnerable.

It is pertinent to note that Miescher's[10] great work on the nucleic acids (1869–71) following Liebig's discovery of inosinic acid (1847) had been followed up only slowly. Structural formulae began to appear in 1911 (Levene and Jacobs)[11] and the main bases and D-ribose were known by 1914. The first nucleotide co-enzyme was reported in 1906 by Harden and Young[12] but the modern era did not begin until about 1944. MacMunn's work[13] on cytochromes (1884) was rejected and neglected until Keilin[14] 'rediscovered' these important catalysts in 1925. Kühne's work[15] on visual purple

(1877) had to wait half a century before further progress took place.

In surveying biochemistry at a provincial university over a period of seventy years in the twentieth century two comments are necessary although not very original: (1) at all times the pedagogic problems have been serious and complicated and the explosive growth of the subject has made it necessary frequently to revise the content of courses provided for different student groups; (2) biochemistry reflects the strengths and weaknesses of research in many fields; it invades and is invaded by other disciplines on an increasing scale, so that the subject must be seen broadly.

Very early in this century the far-sighted people, academic and lay, who were responsible for Liverpool's University College (at that time a constituent part of the Victoria University) decided to foster medical education and research. Prominent in the scheme was 'Physiological Chemistry' or as they were beginning to say 'Bio-Chemistry'. After discussions,* records of which have not been traced, the following letter was sent to Principal (later Sir Alfred) Dale:

18 Water Street, Liverpool.
February 1st 1902

Dear Principal Dale,
As you are aware I promised £5,000 towards the University scheme. I have now reconsidered the matter, and in lieu of this promise I propose to devote £25,000 to help forward the movement. I wish this sum to be devoted to the advancement of Medical Science, and I propose, with the approval of the Council, that it shall be divided as follows:—
£10,000 to found a Chair of Biological Chemistry,
£9,000 to be used for building purposes,
£6,000 to be devoted to permanently endowing my two Fellowships of Colonial and International Medical Research, and for founding a third Fellowship in Gynaecological Research. I am desirous that the sum of £9,000 shall be made to cover all expenses of erecting and fitting the proposed building, which I suggest shall be simply constructed and designed to give a maximum amount of space to research and teaching, and to adjoin the Thompson-Yates Laboratories. I wish the building to have accommodation for research in Physiology and Pathology, for the Tropical School of Medicine, for the new Professor of Bio-Chemistry, and for Clinical Pathology. Trusting this will have the approval of the Council, I remain,
Dear Principal Dale, Yours sincerely,
Signed: William Johnston.

The Council of University College accepted Mr. Johnston's† splendid offer on 4 February 1902 'upon the conditions stated in his letter'.

In March 1902 Senate approved a proposal to establish a Chair of Physiological Chemistry and regulations for the professorship were drawn up in April. Dr. A. S. F. Grünbaum was already lecturing to medical students on the subject and in May

* I am indebted to Mr. Michael Cook, M.A. the University Archivist and to Mr. Adrian Allan of his Department for making available information and press cuttings. The material collected in the course of the present study will be handed over to the Archivist.

† William Johnston (1841–1917) of Woodslee, Bromborough, Cheshire, Born Northern Ireland, began business in Liverpool 1863; established the ship-owning business of Wm. Johnston & Son; retired 1916 when his firm was taken over by Furness Withy & Co. On 21 February 1903 he appointed three trustees, Henry Garvey, Robert Sutherland and William Stewart Johnston to administer the Chair's endowment of £10,000. One of his daughters married Sir Rupert Boyce and another married Percy Newberry. Both were professors at Liverpool. Newberry was an Egyptologist. Mrs. Boyce died at the birth of her first child and to commemorate her life her father founded the Ethel Boyce Fellowship in Gynaecology. An obituary notice of Sir Rupert Boyce FRS signed Ernest Glynn appeared in 1911 (*J. Path. Bact.*, **18**, 276–82).

1902 Council decided that the occupant of the new Chair 'be not expected to undertake teaching for the existing medical degree course but that he devote his time to teaching advanced students and original research'.

The title of the Johnston Chair was changed from Physiological Chemistry to Bio-Chemistry, the hyphenation persisting for some years. Dr. Benjamin Moore* of the Royal University of Ireland was invited to be the first holder of the Chair from October 1902.

The Johnston Laboratories were officially opened in May 1903 by the Right Hon. Walter Long, President of the Local Government Board. The *Liverpool Courier* (11 May 1903) contained a full account of speeches made at the ceremony and listed the names of a large number of guests. A celebratory dinner was given by Mr. Johnston at the old Adelphi Hotel.

The architects were Messrs. Willink and Thicknesse advised by Professor Simpson. Neither at the opening ceremony nor at the dinner was there any (recorded) reference to the short time interval between February 1902 and May 1903. Even if design studies preceded the announcement of Mr. Johnston's gift, to erect and equip the building in less than fifteen months seems a staggering achievement. We may experience a wry astonishment that seventy years ago, before building construction became mechanized, such a rate of progress called for no public comment. I am however indebted to the University Archivist for a letter dated 30 December 1902 to Principal Dale from Professor Rupert Boyce the pathologist, which included the following passage:

> Since April 1st of this year I have literally been in almost daily contact with the Architects (Messrs. Simpson, Willink & Thicknesse), and I have on all occasions found them to be most accessible, and always ready to receive and work out suggestions, to alter pre-conceived plans for more simple ones. The result has been that so far from incurring extra expense in the course of the construction of the present Johnston Laboratories, we have been able to effect reductions in almost every direction . . . . Johnston Laboratory has been erected in probably shorter time than any other laboratory on the grounds . . . (Professor Simpson) has had the charge of the external and internal decorations . . . on many occasions (he has) taken his students over the building, and has given them a practical demonstration. If when we erect our College Buildings, we can also make them an object lesson to our Students, I think we are doing something good.

At the official opening there was an impressive gathering of foreign and British savants, industrialists, politicians and administrators. Mr. E. K. Muspratt presided and his speech showed that already there was at Liverpool a keen interest in Public Health, in medical research and especially in Tropical Medicine. All the speeches suggested that although the pursuit of natural knowledge was admirable in its own

* An obituary notice initialled F. G. H. (Frederick Gowland Hopkins) appeared in 1927 (*Proc. Roy. Soc.*, 1927, CIB, XVII). Benjamin Moore's first degree was in Engineering. He then worked with Ostwald at Leipzig on physical chemistry and at University College, London under Sharpey-Schafer on Physiology. From there he went to Yale as Associate Professor of Physiology and back to England to lecture on Physiology at Charing Cross Hospital Medical School, there to become medically qualified (Conjoint Board). From the Royal University of Ireland Moore was elected to the Johnston Chair at Liverpool (1902–1914). He later joined the Staff of what is now the National Institute of Medical Research at Hampstead. In 1920 he was elected to the new Whitley Chair of Biochemistry at Oxford. A notice in *Nature* (16 March 1922) signed L. H. (probably Leonard Hill) referred very appreciatively to Moore's wartime work on trinitrotoluene poisoning among munition workers. Moore showed that TNT entered the body mainly through the skin. Despite opposition his view was accepted and effective measures were taken to diminish the poisoning.

right, it could also help to alleviate hardship and help to promote the general welfare.

Beneath the urbanity of Mr. E. K. Muspratt's speech was more than a hint to Mr. Walter Long that the State had a duty to participate in research, especially medical research. Mr. Long in a polished reply took the point that 'the Chairman meant something more than sympathetic appreciation'. A seed was no doubt sown, perhaps state-aid was in the air, but it was not until 1913 that the forerunner of the Medical Research Council was invited to make use of one penny per annum per head of the population insured under the 1911 Insurance Act.

It seems probable that the celebrations did much more than give publicity to the Johnston endowments, they may well have helped to establish a favourable 'image' of the university in the eyes of other potential donors.

When the present very large 'Life Sciences' laboratories were built to house the Biochemistry Department at a cost of £1.4m. for construction and £0.5m. for equipment there was no official opening, or celebration. I feel that a great opportunity was missed.

Under Professor Moore a splendid group was soon formed and the Johnston Laboratories provided a focal point for widely ranging activities. Moore and Edward Whitley* founded the *Bio-Chemical Journal* as a private venture from the University of Liverpool. There were already three German journals including Hoppe-Seyler's *Zeitschrift für Physiologishe Chemie* started in 1877. The American *Journal of Biological Chemistry* first appeared in 1905 and the *Bio-Chemical Journal* and *Biochemische Zeitschrift* both in 1906.

A Biochemical Club was founded in London and developed into the Biochemical Society and after prolonged and tough negotiations (see 'History of the Biochemical Society', Morton, 1969) acquired the *Bio-Chemical Journal*. The first volume under the new auspices (Volume 7) appeared in 1913 with new Editors. The first six volumes contained a good deal on researches done in Liverpool. The range of topics and the quality of the papers testifies to the intellectual vigour of the young University College. Moore must have been at the height of his powers. There were studies on diabetes by Moore, Edie and Hill Abram and by O. T. Williams with an analytical paper by Edie and Spence. Roaf and Whitley carried out biochemical studies on tadpoles and frogs while Moore and his collaborators worked on marine organisms, on photosynthesis, on renal calculi and on the toxicity of heavy metals. With Stenhouse Williams he studied conditions for growing micro-organisms. He also found time to investigate the relationship between the size of an experimental animal and the dosage of drugs. Among the other contributors to the early volumes were R. E. Kelly, Warrington Yorke and W. C. M. Lewis.

* I am indebted to Dr. T. Moore and to Mr. E. Whitley of Clive, Shropshire, for the following information:

Edward Whitley, 1879–1945, was the eldest son of Edward Whitley, M.P., some time Mayor of Liverpool. He was educated at Liverpool College, at Trinity College, Oxford (where he read Physiology and Psychology) and at Gottingen and Nancy. His association (through Professor W. A. Herdman) with Liverpool University began about 1903. He undertook research in biochemistry in collaboration with Professor Benjamin Moore and also worked at the University's Marine Biological Station, Port Erin, Isle of Man. He moved to Oxford in 1911 and from there to Torquay in 1936.

With Moore he launched the *Biochemical Journal* in 1906. In 1920 he provided £10,000 for the endowment of the Whitley Chair of Biochemistry at Oxford.

The 1914–18 war came soon after the departure of Moore to London, and biochemistry at Liverpool suffered—as indeed did scientific research generally. The first decade of the century saw the establishment at Liverpool of a centre of excellence in preclinical subjects. Sherrington in the adjacent building was doing research work of the greatest importance to physiology and his intensive approach differed from the more dispersed effort of Moore's group. Physiology was ready for Sherrington's rifle but Biochemistry at that stage perhaps needed the shot-gun. In 1911 Moore published a book entitled *The Dawn of the Health Age* (London, J. & A. Churchill). Its aim was to establish the need to remodel 'the present system of medical service in the interests of the whole community'.

Although he disclaimed party-political interest Moore was a passionate reformer; he set great store on hygiene and on the prevention of infectious disease; he was scornful about 'the clap-trap of faith-healing or homeopathy—and fraudulent quack medicines'. He was no less severe on what he described as 'tinkering with disease instead of stopping it' and he exposed unsparingly some of the follies of the Public Health Service in the first decade of the century. In all this he was well informed in addition to being pungent. He regarded the Friendly Societies, clubs and tontines as no more than poor alternatives to a National Medical Service based on universal state medical insurance. Such a service would be as beneficial to the doctors as it would be to the public. (Moore put the average income in 1911 of the 32,000 doctors practising in the British Isles at £200–£250 per annum.) In spite of his academic preoccupation Moore understood the national state of affairs and his book has a ring of truth. A chapter on the hospital system is forthright and at times brutally logical. Moore illustrated his argument in terms of the 'Royal Charity Infirmary, Cottonport' and the 'Guardian Angel Workhouse Infirmary, Cottonport'. I have tabulated some of his facts.

	Royal Charity Infirmary, Cottonport	Guardian Angel Workhouse, Infirmary, Cottonport
Beds	300	Nearly 1,000
Visiting Medical Officers	21*	2
Resident „ „	10	4
Operating theatres	4 (large)	2 (both small)
Passenger lifts	2	0
Baths	Every ward	2/floor—150 beds

* including:

3 senior physicians	2 assistant physicians
3 senior surgeons	3 assistant surgeons
1 gynaecologist	1 assistant gynaecologist
1 ophthalmic surgeon	1 laryngologist
1 dermatologist	1 X-ray surgeon
4 anaesthetists	

(Information from B. Moore, *Dawn of the Health Age*, 1911, p. 72 ff.)

Moore insisted that 'for the serious surgical diseases and injuries amongst the working classes, hospitals are absolutely indispensable'. He spared nobody; doctors, politicians and administrators, the Voluntary Hospitals and the Poor Law all came under the lash. All the same, the impression on the reader some sixty years later is that Moore's clarity of mind prevented his zeal from ever becoming really unfair.

His discussion of the measures to be taken to control infectious disease demonstrated how ready he was to bend his mind to urgent practical problems. The final chapter of the book deals again with a national medical service. Moore was in tune with the more enlightened and constructive thought of the time. Perhaps he overestimated the predictability of medical advance; perhaps he idealized human nature. Here and there his prose became poetical but throughout the book one senses the voice of a man happy in his profession and determined that additions to knowledge should be applied with driving force, intelligence and compassion.

Moore published a little volume on the *Origin and Nature of Life* (London, Home University Library, 1913). It reveals a lot about the period and the author. The first chapter ends as follows: ' . . . science just as much as religion possesses its dead mythology. It by no means follows that these myths, of science and religion, did not serve a useful purpose in those days when they were living and powerful beliefs, but when new knowledge, power and methods arise, they must be cast on one side, and replaced by better machinery to lead to new revelations. They are the scaffolding of the structure in the course of erection, and not an intrinsic part or any permanent adornment of it'. The book goes on to discuss electrons and atoms and chemical evolution in the suns, followed by chemical compounds on earth and the building materials for living matter. Moore had a profound belief in 'colloid' chemistry. His account of theories of 'the origin of life and how it appeared on earth' deals faithfully with spontaneous generation and its refutation but refrains from slamming doors.

The final sections on the living organism at work and on cyclic activities of life show Moore's prescience in assessing timeliness and promise at the frontiers of biological and medical science. Although he had fully grasped the vitamin concept he spoke of 'external hormones from outside the body required in the daily food'.

In common with many other scientists of his day Moore liked a peroration: 'There is a scheme in it all and an eternal purpose which is ever progressing. It means something that this much has been revealed to us, and having once seen it there comes a touch of illumination and faith, that kindles something sacred within the mind akin to reverence and love. One must needs work for the highest and for more knowledge of this revelation, whatever the future may hold in store, for we do not now know how more and more glorious things may yet be'.

In 1921 after he had taken up his duties at Oxford as Whitley Professor, Moore published another substantial book. The title *Biochemistry* (London, Arnold, 1912) was accompanied by a sub-title 'A study of the origin, reactions, and equilibria of living matter'. The omissions are almost as interesting as the contents. The index for instance does not contain calorie, carotene, cholesterol, diabetes, tyrosine or vitamin! Moore had a sharp appreciation of the scientific importance of photosynthesis and he knew that a role for chlorophyll had to be defined. Photosynthesis must however have begun before anything so complicated as a chlorophyll molecule had arrived on the terrestrial scene. This strengthened his interest in a possible photosynthesis of organic matter from carbonic acid in which inorganic ions or colloids acted catalytically. Such an approach to the origin of life had a big place in his mind. Almost half the book is devoted to one aspect or another of photosynthesis. Von Baeyer's idea that formaldehyde arose by union of carbonic acid and

water under the influence of light was widely accepted and although Moore very fairly set out the objections he remained under the spell like Willstätter and later Baly. The fact that the photosynthetic process involves the decomposition of water rather than carbonic acid was too strange an idea to be accepted at the time. Moore's account of the whole subject must have been influential and Baly and Heilbron were considerably indebted to his survey—and indeed they made some of the same mistakes.

The rest of the book shows Moore expounding the fundamentals of biophysical chemistry applied to enzymology. He also wrote appreciatively about secretin and gastrin. The total absence from the book of structural formulae is striking. No doubt had he lived Moore would have welcomed the enormous contribution of natural product organic chemistry to biochemistry but in 1921 the tidal wave had hardly begun.

In his obituary notice on Benjamin Moore, Gowland Hopkins referred to a paper with Roaf[16] dealing with osmotic pressures in colloidal solutions and said of Moore that 'he possessed at the time a fairly definite conception of the membrane equilibrium which, four years later, was quantitatively studied and clearly defined by Donnan'.

The enterprising authorities at Liverpool had induced Sir John Brunner to endow the first British Chair of Physical Chemistry and the first occupant was Frederick George Donnan, who was at Liverpool from 1904 to 1913. One of his first tasks was to supervise the erection of the Muspratt Laboratory. He left Liverpool to succeed Sir William Ramsay at University College, London. Donnan is best known for a paper entitled 'The theory of membrane equilibria in the presence of a non-dialysable electrolyte'.[17] The theory was tested experimentally[18,19] and applied to the swelling of gelatine.[20] The work did not attract the attention it deserved until after the 1914–1918 war when Jacques Loeb persuaded the scientific public that the Donnan principle was a powerful tool.

Loeb[21] had shown that proteins were amphoteric and that the ionic dissociation of the carboxyl and amino groups conformed with mass action. He added electrolytes to solutions of proteins and measured viscosities, osmotic pressures and changes in membrane potentials, all of which could be accounted for in terms of Donnan's theory. Textbooks of biophysical chemistry still contain full expositions of the theory but the application of Donnan's ideas to biological systems has become very complex. The simple artificial membranes like copper ferrocyanide are very different from let us say, the mitochondrial membranes or myelin in health and disease. Membranes vary greatly in composition and properties with the site, the cell and the species; the biophysics and biochemistry of membranes now requires multi-author treatises at frequent intervals. It is perhaps not surprising that so shrewd a person as Donnan, having achieved a theory which in its original scientific setting approached perfection, decided to leave well alone.

Walter Ramsden (1868–1947) succeeded Benjamin Moore in October 1914 and held the Johnston Chair until he retired in 1931. He qualified in medicine (1897) and in 1899 he was elected at Pembroke College, Oxford, to the Sheppard Medical Fellowship which he held to the end of his life. (Under the old statutes the Fellowship is tenable for life unless forfeited by marriage; Ramsden never married.) Ramsden's

main interest was in surface phenomena particularly in relation to the behaviour of proteins.[22] He observed that some proteins were dissolved by urea and some coagulated proteins went into solution in concentrated aqueous solutions of urea. With N. G. Chavasse he noted the effect of urea solutions on the viscosity of solutions of denatured egg albumin.[23] It was shown that urea unmasked the thiol and disulphide groups in egg albumin—a modern-sounding effect. During the 1914–18 war he worked on the estimation of quinine[24] but soon afterwards returned to the study of surface phenomena. Ramsden continued to work in the laboratory when he retired to Pembroke College in 1931. Those who knew him best felt that he published little largely because he was almost exasperatingly modest and meticulous. He wrote and rewrote but rarely reached the point of submitting papers for publication—something very uncharacteristic of twentieth-century scientists. Tributes to Ramsden are to be found by Sir Rudolph Peters[25] and by Dr. R. Coope.[26]

During Ramsden's tenure of the Chair, Beit Memorial Fellowships were held in the Department by Henry Cohen (now Lord Cohen of Birkenhead) and by E. Noble Chamberlain.

Cohen studied the inorganic phosphorus content of cerebrospinal fluid[27] and extended his work to the magnesium content of cerebrospinal fluid and other body fluids[28]. He made the important observation that in meningitis the magnesium content of the cerebrospinal fluid was consistently less than the value for normal subjects (2.5 as compared with 3.28 mg./100 ml.). Inorganic phosphate on the other hand was above the normal value. Cohen formulated a law of meningitis (since known as Cohen's Law) to the effect that in that disease those substances which in the c.s.f. were normally present in higher concentrations than in plasma (namely Mg. and Cl.) would show falls, whereas substances normally present in lower concentrations than in plasma (inorganic phosphorus, K and protein) would exhibit rises. Since they were first made the observations have been amply confirmed.[29] Over-simplified 'explanations' of Cohen's Law have been discarded and a sophisticated treatment in terms of membrane theory will be required. The work is discussed by H. Davson in his recent standard work on the physiology of the cerebrospinal fluid (London, Churchill, 1967).

The interest in cholesterol which never seems to have been absent from biochemistry at Liverpool was evident in papers by Chamberlain.[30] Various tissues of male rabbits were analysed for cholesterol by the Liebermann-Burchard colour test. Brain and suprarenals were found to be the richest with the figures for the latter very variable while the former gave much more reproducible results. Sodium oleate emulsions of cholesterol were injected into the rabbit ear-vein and the effects observed included a decrease in adrenal cholesterol and a marked increase in spleen cholesterol; hypercholesterolaemia was quite transient. The suprarenals of non-pregnant doe rabbits contained more cholesterol (7.7 to 20 per cent average 12.3) than those of bucks (3.9 to 11.1 per cent average 7.4) but the difference was narrowed in pregnant does. The ovaries were very rich in cholesterol. It was suggested that the adrenals were important in cholesterol physiology. A later paper with Corlett[31] recorded cholesterol levels in buck rabbits made anaemic by loss of blood or the subcutaneous injection of phenylhydrazine hydrochloride. Bleeding resulted in a temporary fall in

blood cholesterol followed by very marked hypercholesterolaemia and a concomitant depletion of suprarenal cholesterol. The phenomenon was also seen after injection of phenylhydrazine. This was valuable exploratory work, in a sense ahead of its time.

A Liverpool Cancer Research Organization* was founded soon after World War I. It was later designated 'The Liverpool Medical Research Organization' directed by W. Blair Bell, Professor of Obstetrics and Gynaecology. The word organization was used because the emphasis was on a planned co-operative study in which the heads of various departments could make the fullest use of the facilities of their own laboratories and could engage graduate research assistants and technicians to pursue particular aspects of the larger investigation. The holder of the Brunner Chair of Physical Chemistry (Professor W. C. McC. Lewis) gave unstinted support to the scheme and supervised experimental work carried out by several post-doctoral workers.

Blair Bell considered (this was in the nineteen-twenties) that malignant cells resembled chorionic epithelium. He recognized that lead ions were acutely toxic but entertained the hypothesis that normal cells were less susceptible to damage than malignant cells. By administering colloidal lead he hoped to maintain very low concentrations of lead ions in tissues and to arrive empirically at a level destructive to cancer cells but harmless or relatively harmless to normal cells. This was a surgeon's concept of cancer therapy in the sense that lead was to be used to perform a kind of molecular excision.

Lewis was already experienced in colloid chemistry, indeed one of his earlier papers concerned the electrical charge on colloidal silver.[32] With his associates he undertook the preparation of lead sols for the treatment of patients and he also investigated the fate of administered lead in the body. In addition to this essential support in a clinical investigation, the members of Lewis's team also carried out systematic studies on the chemistry and biochemistry of normal tissues, malignant tissues and chorionic epithelium. Malignant tissues were shown to have a high lecithin/cholesterol ratio compared with normal cells and in this respect tended indeed to resemble chorionic epithelium. The higher ratio was thought to contribute to the greater permeability of malignant tissue towards water-soluble substances. A careful study however showed that no signficant difference in blood pH could be demonstrated between normal and diseased subjects although intravenous lead sol temporarily effected a lowering of blood pH. Lewis found that cancerous cells—like chorionic epithelia—were able under aerobic conditions to produce lactate from glucose whereas normal cells failed to do so. These observations led indirectly to a series of papers on the kinetics of hydrolysis of glucosides (with E. A. Moelwyn Hughes).

The action of pancreatic lipase was found to be accelerated in the presence of blood sera, less so by sera from cancer sufferers than by normal sera. Both colloidal lead and ionic lead had accelerating effects. Lewis also undertook investigations on the physical chemistry of proteins and his views on denaturation excluded hydrolysis of peptide bonds. He also initiated a series of experimental studies on electrophoresis.

* I am indebted to Dr. J. W. Corran for his recollections about the organization and for a list of the papers published. His notes are being lodged with the University Archivist.

The techniques by present-day standards were primitive and it is perhaps not surprising that little in the way of generalization appeared.

There can be no doubt that much of Lewis's post-1920 work would now be designated physical biochemistry. The half-century which has elapsed since the beginning of the research has transformed the subject both from the standpoint of the background of ideas and of technical resources. The work done by Lewis and his group was typical of its period and it perhaps suffered from over-loyalty to the main Blair-Bell concepts. Today the newer knowledge of trace metals, of metallo-enzymes, of lead poisoning and the use of tetra-ethyl lead in motor fuels leaves open many questions and there is a certain irony in the plausible hypothesis that the decline of the Roman Empire was 'due' to lead poisoning from water pipes in the richer households. There are those who are anxious about lead poisoning as an increasing hazard in our time.

The chemical work under Professor Lewis was carried out by Dr. J. W. Corran, Dr. M. Jowett, Dr. H. Miller and Dr. J. Brooks and later by Dr. R. F. Corran. Biochemical work was undertaken by Dr. R. Coope and Mr. J. Patterson. Many senior clinicians were associated with the project and Dr. S. B. Herd, Dr. M. Datnow and Mr. W. R. Williams were among the part-time medical staff.

Photosynthesis in the living plant had been one of Benjamin Moore's main interests, but when he went to London other problems took precedence. E. C. C. Baly, professor of Inorganic Chemistry at Liverpool was like Moore attracted by 'the romance of science' and photosynthesis cast its spell on him too and for a time he was joined by I. M. Heilbron. They followed Moore in accepting the von Baeyer-Willstätter concept that carbonic acid was somehow changed to yield formaldehyde. They appreciated that chlorophylls *a* and *b* were involved and they guessed that carotene ($C_{40}H_{56}$) and xanthophyll ($C_{40}H_{56}O_2$) entered into a 4-component redox system. Much useful work was done on carotenoids but not published. It is now known that the oxygen set free in photosynthesis comes from water and not carbon dioxide and that biosynthesis of sugar occurs by non-photochemical enzymic processes, involving high energy phosphates. The time was not ripe for a successful attack on photosynthesis and although it attracted world-wide attention Baly's work was much criticized on technical grounds. Baly's later work, summarized in his now neglected book, *Photosynthesis* (London, Methuen, 1949), had little relevance to phytochemistry but was really concerned with attempts to reproduce in the laboratory the primitive terrestrial photochemical processes which might have antedated the origin of life.

In 1922 Selig Hecht, a young American physiologist working on vision, wished to measure the absorption spectrum of visual purple as accurately as possible and he came to Baly's department to do so. Baly gave him every facility and Wilberforce allowed him the use of a darkened room in the Physics Department where the low-intensity vision of dark-adapted volunteers could be determined. Hecht extracted the visual pigment from animal retinas and in spite of very great experimental difficulties (arising out of the photolability of the solutions and the need to use strong light) obtained good results. He also made careful measurements on the response of the dark-adapted human eye to different wave-lengths of visible light. This classic investigation[33] proved beyond doubt that the extractable pigment entered into low-

intensity (scotopic) vision. Hecht became a leading figure in the chemical and physiological aspects of vision and one of his pupils, George Wald, made discoveries to be mentioned later (p. 344). The writer was one of Hecht's experimental subjects. Nearly twenty years later he was himself active in establishing the nature of the pigment.

Liverpool's first Professor of Organic Chemistry was Robert Robinson who held the Heath Harrison Chair from 1915–20. His impact on biochemistry came later. He was succeeded by I. M. Heilbron who held the Chair until 1933. While he was at Liverpool some of Heilbron's many research projects had a biochemical side and his sustained interest in sterols began early. In 1922 McCollum had differentiated between vitamins A and D and it soon became probable that the latter could arise by the action of light on an unknown precursor. Cholesterol was a plausible candidate and large batches were subjected to fractional crystallization (Heilbron, Morton and Kamm, 1926),[34] and a small amount of impurity was shown to have a very characteristic ultraviolet absorption spectrum. It was this contaminant that absorbed ultraviolet rays and yielded vitamin D whereas 'pure' cholesterol was transparent and could not be 'activated' (Rosenheim and Webster, 1927[35]). A visit to the laboratory of Windaus at Göttingen allowed Rosenheim to show that ergosterol (from ergot of rye) possessed the same absorption spectrum and gave very active products on irradiation. The natural precursor was later shown to be 7-dehydrocholesterol. Vitamins D_2 and D_3 (ergocalciferol and cholecalciferol) were isolated and the vitamin D story unfolded as the result of intense international effort.

Heilbron carried out sustained research into the constitution of ergosterol, work which has become part of the history of the subject.

After the 1939–45 war much further work on provitamins D_3,[36] on sterol metabolism and on biosynthesis has been done at Liverpool by Goodwin, Glover and their students. Hartles has worked on the mode of action of vitamin D particularly in respect of dentition. Kodicek[37] at Cambridge has recently shown that in the kidney vitamin D_3 is the precursor of a new hormone 1,25-dihydroxycholecalciferol, a substance more biologically potent than the vitamin itself. D. E. M. Lawson, a recent Liverpool research student, has been awarded a Drummond Memorial Prize for his part in this important advance.

Heilbron and Morton turned their attention to vitamin A then a growth-promoting factor of unknown structure. The first task was to concentrate the active material. The only method of monitoring the success of separation methods was the biological assay which was slow, expensive and imprecise. There was however some evidence that the vitamin could be destroyed by ultraviolet light (this work will be referred to later, p. 336). Spectroscopic methods provided a new label. After leaving Liverpool Heilbron continued to work on the structure and synthesis of vitamin A and one of Morton's group, the late A. E. Gillam accompanied him to Manchester where he made his name as a chemical spectroscopist.

Heilbron was succeeded by Alexander Robertson, an Aberdonian who had worked with Robinson and at the London School of Hygiene and Tropical Medicine with Raistrick. Robertson was greatly interested in the chemical constitution of natural products. Among his many fields of activity were studies on fish poisons and natural

insecticides. As an organic chemist Robertson was a brilliant exponent of classical methods and one sensed that he instinctively felt that physical methods almost debased the currency. Many of his degradations and syntheses were elegant in the extreme. He retired early to pursue his hobby of farming. He became Treasurer of the Rothamsted Research Station and this interest in agriculture perhaps brought him nearer to biochemistry than did his organic chemistry.

A difficult problem important alike to medicine, organic chemistry and biochemistry is the origin and nature of melanins. Robertson and his colleagues made a notable contribution to the study of these black, red and brown pigments.[38] They devised methods for preparing 5,6-dihydroxyindoles and discovered that in oxidising these melanin precursors hydrogen peroxide was formed. Polyphenol oxidase hastened the initial uptake of oxygen, yellow intermediates were recognized and were probably dimers derived from transient *o*-quinones from which melanins were formed. Various synthetic melanins were obtained and to judge from recent work (see R. A. Nicolaus, *Melanins,* Paris, Hermann, 1968) this valuable research has stood the test of time very well.

When H. J. Channon* came to Liverpool in 1931 he saw the need to establish an Honours School of Biochemistry in the Faculty of Science as his most urgent task. As a matter of fact an Honours School had been set up just before the 1914–18 war and one person, Dr. Howell Evans, had graduated, but the exigencies of the war period proved decisive and the project lapsed. It is not clear why Ramsden did not revive the School when the University admitted the survivors of five years of scholarship men in 1919–20. Channon however was able gradually to recruit additional members of staff and the new honours course was soon found to be viable. During the period 1933–39 the intake was never great but the ability of the honours students was high and excellent work was done by a good number working for Ph.D.

Channon and his colleagues devoted much attention to the course in biochemistry for medical, dental and veterinary students reading for the Second M.B. and similar examinations and both lectures and practical work were brought up to date.

The story of squalene affords an example of co-operation between organic chemists, biochemists and analysts. In 1917 a well-known London consulting analyst (Chaston Chapman) had been examining fish liver oil samples from Portugal labelled 'cod liver oil'. The fishes from which the oil came were large specimens of *Centrophorus granulosus* and *Scymnus lichia* caught in deep water off Morocco.[39] The liver oils proved rich in a hydrocarbon which Chapman called *spinacene.* Tsujimoto[40] in Japan had however already discovered the hydrocarbon, had given it the name squalene, and had suggested a formula $C_{30}H_{50}$. Chapman's formula ($C_{29}H_{45}$) was wrong and the name squalene was retained because of Tsujimoto's priority. Chapman sent specimens of 'spinacene' to Baly for ultraviolet absorption measurements. The present writer carried out the tests and found no selective absorption despite the unsaturation. The double bonds were not conjugated.

Heilbron's group[41] isolated squalene from the livers of elasmobranch fishes and

* Harold John Channon (b. 1897), C.M.G. Research Worker at University College, London, 1922–27. Cancer Research at University of Leeds 1927–31. Johnston Professor of Biochemistry, University of Liverpool 1931–43. Research Manager, Unilever 1943–55. Member of Advisory Committees on Education in the Colonies 1939–44 and of Colonial Products Council 1956–59.

other sources. They confirmed the formula $C_{30}H_{50}$ and noted that H. J. Channon, then at University College London, had fed squalene to rats and had recorded a consequential doubling of the liver cholesterol content. With T. P. Hilditch and E. D. Kamm[42] squalene was hydrogenated and the $C_{30}H_{62}$ saturated derivative was prepared and characterized. The exact constitution of squalene was not easily established and several degradations were essayed between 1926 and 1930. The full story of the nature and origin of squalene could not possibly have emerged at the time—and indeed had to wait many years until isotopically labelled precursors came into use.

Channon and his associates[43] after carefully noting the Japanese work and that of Heilbron's group, described how rats on a diet containing one per cent of squalene suffered no obvious ill-effects but the liver non-saponifiable fraction rose by a factor of 2 to 6 and the liver cholesterol content was doubled. Channon and Tristram returned to the problem in 1937 and confirmed the earlier work. The paper was however in some respects ahead of its time.

Channon and Marrian[44] had found in the liver lipids of animals what appeared to be a hydrocarbon $C_{45}H_{74}$. Some forty years later a group led by the present writer discovered a family of polyprenyl alcohols[45] $(C_5H_8)_nH.OH$ where n=14–23. The most plentiful of these *dolichols* is $C_{100}H_{163}OH$. To have isolated and characterized such substances in 1926 would have been impossible and there is evidence that the Channon-Marrian compound was impure dolichol.[46]

Channon's interest in lipids came to a focus on the induction of fatty liver in animals. With Wilkinson[47] he showed that rats given a diet containing fat and 2% of cholesterol produced 'cholesterol fatty livers'. Choline in the diet opposed this effect but did not result in a decrease in other lipid constituents. Best, in Toronto, had found that choline 'cured' the fatty liver characterized by excessive amounts of glycerides. As there seemed to be discrepancies between the findings at Toronto and at Liverpool, Channon visited Best's laboratory to find an explanation. Substantial progress was recorded in a long series of papers published between 1935 and 1943, mainly in the *Biochemical Journal*. The Liverpool and Toronto results were found not to be qualitatively at variance; they differed in degree and the protein intake exercised a controlling influence on the amounts of lipids in the animal liver.[48] The importance of protein was manifest in work with Wilkinson and Beeston,[49] and variations in glyceride, phosphatide and sterol contents of liver were demonstrated with Aylward and Wilkinson.[50] Later work was concentrated on attempts to establish which of the constituent aminoacids of proteins were responsible for the lipotropic action.

Fatty infiltration of the liver remains a big problem with many ramifications and there can be no doubt that the work of Channon's group* has a permanent place in the history of research on this topic.

* Among the young lecturers recruited by Channon were Folley, Smith and Loach. S. J. Folley became Head of the Physiology Department at the National Institute for Research in Dairying and Research Professor at the University of Reading. He made noteworthy contributions to the biochemistry and physiology of lactation and was elected F.R.S. in 1951. J. A. B. Smith became Head of the Hannah Dairy Research Institute at Ayr and made many contributions to biochemistry and ruminant nutrition. He was President of the Nutrition Society (1969–71). J. V. Loach was drawn into administration during the war and was for many years afterwards Registrar of the University of Leeds. G. T. Mills, a pupil of Channon, was a lecturer at Liverpool in the early post-war period and has since done substantial research in the U.S.A. and elsewhere.

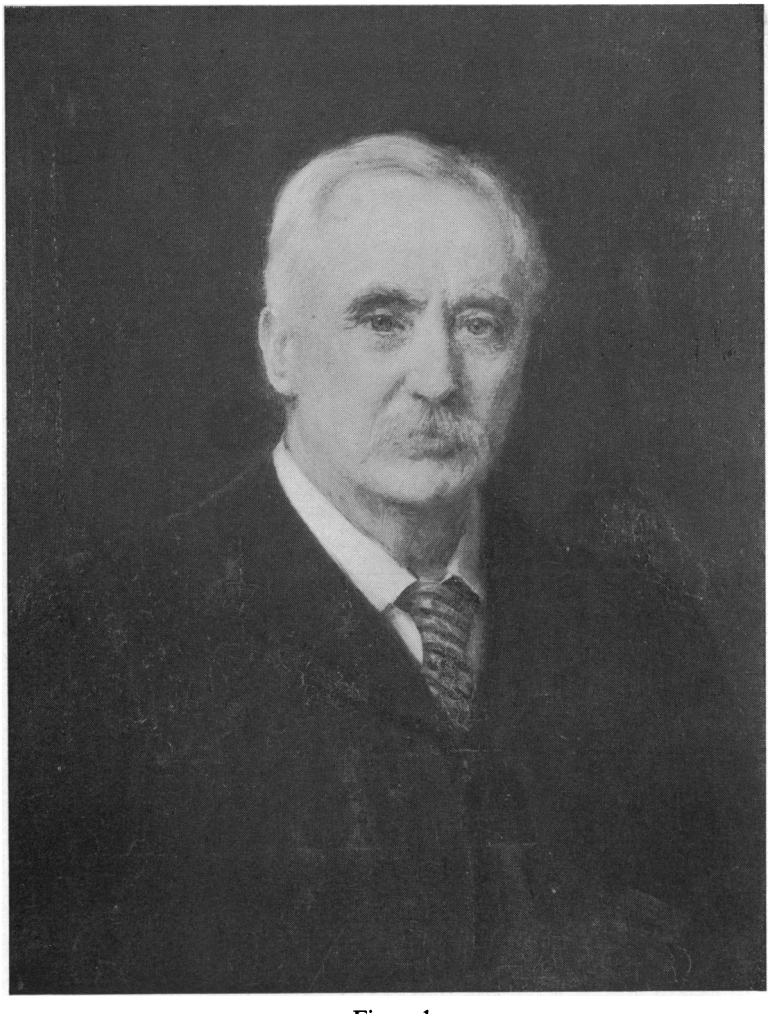

Figure 1
William Johnston. (From a photograph of a painting, kindly supplied by his grandson, Mr. W. S. Johnston of Liphook, Hants.)

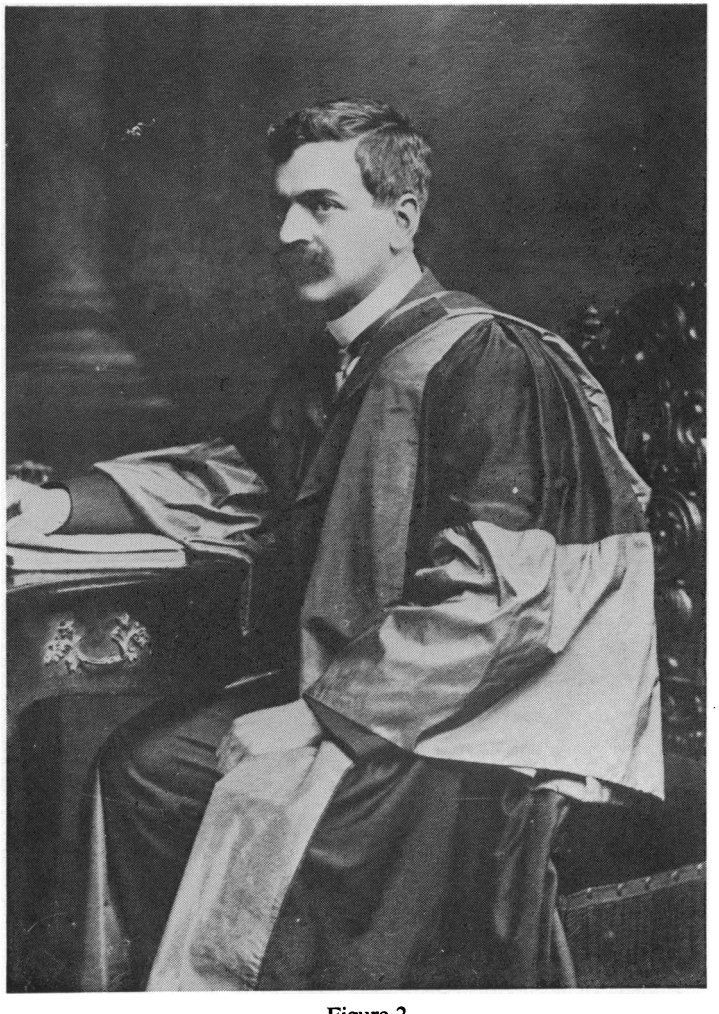

Figure 2
Professor Benjamin Moore, F.R.S.

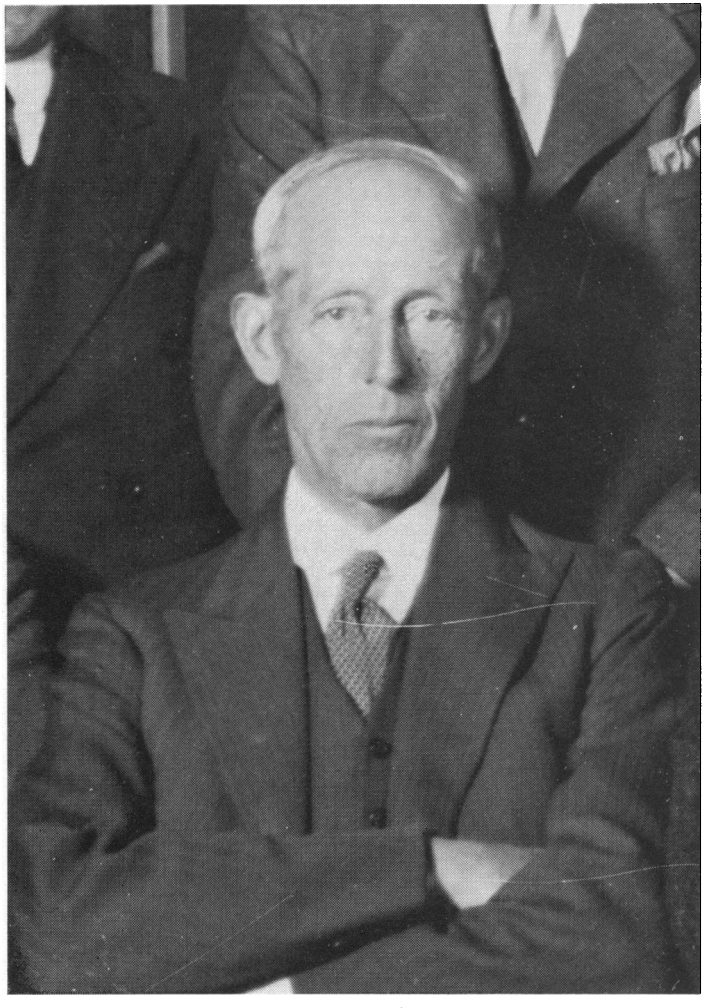

Figure 3
Professor W. Ramsden.

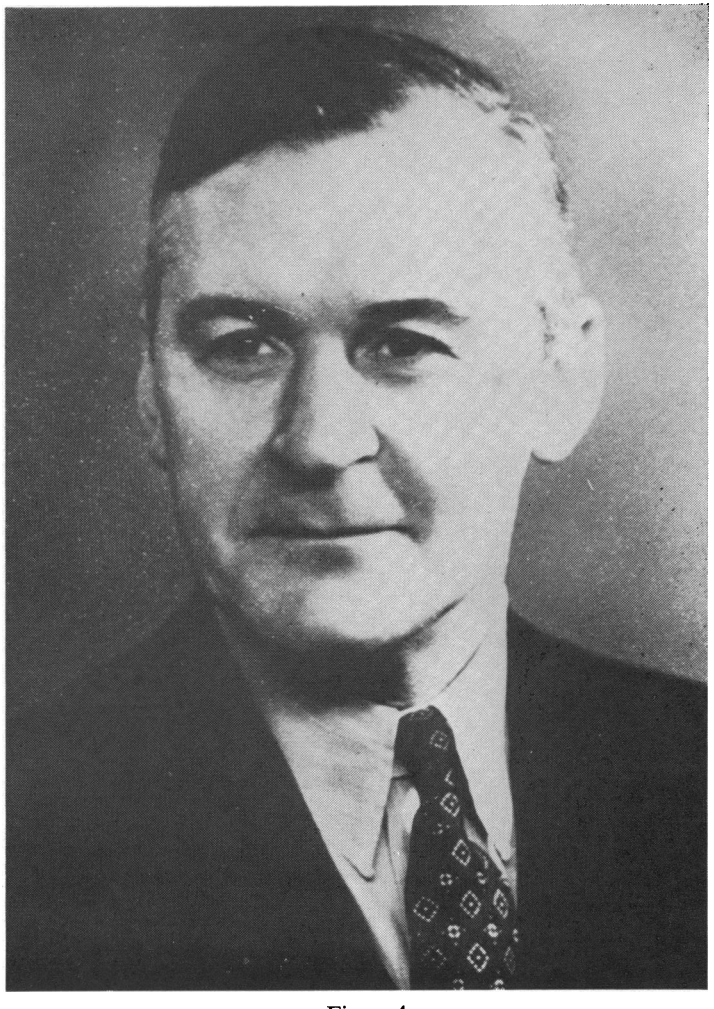

Figure 4
Professor H. J. Channon, C.M.G.

The first Professor of Chemistry at Liverpool was James Campbell Brown and when he died (1910) he left the residue of his estate after his wife's demise (which occurred in 1923) to endow a Chair and Department of Industrial Chemistry. The professor was to conduct research in some selected branch of chemistry that had industrial applications. The Trustees and the University representatives agreed that the first holder should study the chemistry of fats and fatty acids. An appointment to the Chair was made in 1925 and Professor Hilditch* worked at Liverpool with great success from 1926 to his retirement in 1951. When he began, knowledge about the constitution of natural fats was rudimentary but in twenty-five years of sustained effort Hilditch and his students transformed the subject. Apart from the industrial aspects (the soap, margarine and cooking fat outlets) the significance of Hilditch's work to biochemistry was obvious. The biosynthesis of fatty acids in plants and animals, the nutritional roles of saturated and unsaturated fatty acids and the problems of obesity and of atherosclerosis all needed more facts and better analytical methods. Hilditch's first task was to improve analytical methods. He then set out to ascertain the composition of the mixed glycerides in all the principal natural fats. An early collaborator was J. C. Drummond with whom Hilditch carried out work on cod liver oil and other fish liver oils for the Empire Marketing Board.[51] Joint work was done on butter fat with Kon[52] at Reading and some new sources of drying oils were studied for the Colonial Products Research Council.[53]

As time went on many improved analytical methods were introduced but the polyunsaturated acids of drying oils and of fish oils were specially troublesome. The double bonds are there separated by—CH_2—groups and this lack of conjugation is responsible for the absence of selective absorption in the ultraviolet region. Controlled alkali isomerisation however gives rise to strong and characteristic absorption bands in di-, tri-, tetra- and penta-enoic acids. The process is not stoichiometric and it becomes reproducible only when the conditions of concentration, temperature and time of heating are adhered to strictly. With Morton and Riley[54] the method was studied in detail and empirical conditions were established for the spectrophotometric determination of linoleic, linolenic and related acids. The method proved very serviceable for many years when there was no alternative but it has since been replaced by gas chromatographic methods of analysis.[55] About 300 papers appeared during the period 1926–52 and Hilditch's research students included many who have since distinguished themselves in the same or related fields.

In 1928 two Americans were at work in the Spectroscopy Laboratory of Baly's Department. The older man, J. W. Woodrow was a Professor of Physics from Ames, Iowa, and a former Rhodes Scholar. The younger man, W. R. Brode, was a Guggenheim Fellow (later Professor at Ohio State University, scientific adviser to the Secretary of State and a President of the A.A.A.S. and of the American Chemical Society). Brode brought with him valuable 'know-how' from the Bureau of Standards at Washington and while he was at Liverpool new spectroscopic equipment from Adam Hilger was thoroughly tried out. A prototype visual spectrophotometer of the

* Thomas Percy Hilditch (1886–1965) C.B.E., F.R.S. At Joseph Crosfield & Sons, Warrington 1915–26. Campbell Brown Professor of Industrial Chemistry, Liverpool 1926–51. See *Biogr. Mem. Fellows, R. Soc.*, 1966, **12**, 259–89.

Hilger-Nutting type played a notable part in Morton's researches on vitamin A, carotenoids and visual pigments. It is now in the possession of the Biochemical Society for preservation. While he was at Liverpool Woodrow did attractive work on cod liver oil. Brode (with Morton) studied cobalt compounds in a manner which illustrated refinements in spectroscopic techniques and the growing understanding of absorption spectra.

The group led by Morton continued to work in the field of structure-absorption relationships and a parallel interest in biochemical applications developed quickly. At that time there was great interest in vitamin A, it still had to be isolated, characterized and synthesized but the only unequivocal 'label' was its biological effect. The best-known source was cod liver oil but the potencies of different samples were difficult to assess. The fact that the vitamin could be destroyed by ultraviolet irradiation implied that the molecule had its own absorption spectrum. A colour test involving either arsenic trichloride or antimony trichloride seemed promising. The Liverpool group, inspired and assisted by Heilbron, was able to demonstrate that the ultraviolet absorption curve with a maximum at 328 nm,[56] was due to the vitamin and that under suitable conditions the colour test could be made quantitative. A thin film of silver deposited on the outside of a fused silica test tube acts as a light filter and transmits only a narrow band of the spectrum on either side of 330 nm. Vitamin A-containing solutions placed inside the tube show a decrease in selective absorption at 328 nm. on exposure to an ultraviolet light (e.g. from a quartz mercury lamp). Parallel bio-assays carried out by Dr. Katherine Coward and others allowed 'conversion factors' to be calculated.[57] The ultraviolet absorption 'label' played a considerable part in surveying the liver oils of many fish species and in showing that cod liver oil was a poor source compared with, for instance, halibut liver oil. Richer sources allowed the preparation of concentrates and eventually vitamin A was crystallized. The problem attracted world-wide attention and several very strong groups of investigators contributed to the final proof of structure and the total synthesis. As much as perhaps any other aspect of biochemistry, research on this problem alerted analysts and biochemists to the value of ultraviolet spectrophotometry. From being a technique limited to specialists, photoelectric spectrophotometry quickly became indispensable in all biochemical laboratories.

Among the highlights of the Liverpool pre-war contributions to vitamin A studies were (i) improvements in assay procedures which in the end made routine bio-assays obsolete; (ii) the discovery of vitamin A_2 and its distribution in freshwater and marine fishes;[58] (iii) the discovery of unsuspected and large amounts of vitamin A in the lining of the gut and the pyloric caecae of many fish species.[59] Valuable work in this area was done by J. R. Edisbury, J. A. Lovern and others.

At the time of the discovery of vitamin A stores in fish intestines the commercial synthesis of the vitamin seemed only a rather distant prospect. Halibut liver oil was coming into medicinal use and some vitamin A-rich liver oils (tunny, sturgeon, soupfin shark) became available and very small amounts sufficed to 'top up' the potency of batches of cod liver oil. Realization that limited portions of the halibut 'pluck' contained more vitamin A than the liver and were being discarded at sea had obvious implications. Lovern (working at the Torry Research Station, Aberdeen)

belonged to the Scientific Civil Service and I was receiving some financial help from the Medical Research Council. On the advice of Mr. H. E. Potts, a well-known Liverpool patent agent, we took out a provisional patent and passed it on to the Medical Research Council for a decision. After careful consideration the Council decided not to complete, the reason being that vitamin A was a natural compound necessary to man and as such ought not to be patented. No doubt the controversial earlier Steenbock patent, taken out by the Wisconsin Alumini Foundation for vitamin D formation by photochemical action, influenced thinking and opinions were deeply divided on such issues. The stance could not however be sustained and it became clear that methods of obtaining natural products in usable form could promote the general welfare within the protection patent law.

The Liverpool work on vitamin A owed much at the beginning to Heilbron and later Edward Mellanby (Secretary of the Medical Research Council). The Council's grants were in those days very small and it was Mellanby's personal interest in vitamin A which kept him in touch with the advancing chemistry. R. H. Creed (retired 1971) was Morton's technician over a long period and the M.R.C. provided his salary for many years.

When the war broke out in 1939, A. L. Stubbs (with Morton) was required to carry out an urgent secret investigation by the chemical warfare authorities, and T. W. Goodwin was recalled to work on vitamin problems for the Ministry of Food. When J. C. Drummond became Scientific Adviser under Marquis (later Lord Woolton) it was soon seen that margarine was going to be extremely important in a food rationing system. The vitaminization of margarine with calciferol and fish liver oil concentrates provided a convenient and technically practical way of 'insuring' most of the people against specific deficiency, while cod liver oil and orange juice were desirable for infants. The Government decided to provide the concentrates to be incorporated in the margarine by the manufacturers. When the U.S.A. entered the war large amounts of very rich fish liver oils were donated to this country under the Lend-Lease arrangements. All the concentrates and all the margarines made throughout the war were tested at Liverpool in the spectroscopic laboratory. Analytical methods were improved and a Beckman Photoelectric Spectrophotometer (the second to reach this country, the first going to the Shell Laboratories at Thornton) was acquired.

The quantitative human requirement of vitamin A was not at that time known and this was deeply unsatisfactory. Prisoners of war were sent vitaminized chocolate when that was possible but the right amounts of vitamins A and D to add could not be gauged. There was some reason to suppose that—assuming an allied victory—vitamin A deficiency might be a serious problem in occupied Europe. Bio-assays for vitamins A and D had of course been conducted on rats but extrapolation from experimental animals to man was far from easy. The Vitamin A Sub-Committee of the A.F.F. (accessory food factors) committee was encouraged to plan trials on young adults. A number of volunteers, all conscientious objectors to military service, had been used at Sheffield University for an investigation carried out under the supervision of Dr. Kenneth Mellanby on the spread of scabies. With this organization as a beginning arrangements were made to maintain some two dozen volunteers on a diet as near as possible to being devoid of carotene and vitamin A. It was planned to

maintain the volunteers under frequent medical supervision, to test their blood quantitatively for vitamin A at regular intervals and to measure their low intensity vision. A great deal of work was done at Liverpool (by Morton, Goodwin and Creed) on the components of the diet and on the carotene and vitamin A levels in the blood. The analytical techniques for determining blood levels were then rather primitive. The full story of the Sheffield experiment and its participants was recounted after the war in a report[60] edited by H. A. Krebs (then at Sheffield) and Miss E. M. M. Hume of the Lister Institute.

Goodwin and Morton visited Sheffield regularly for about two years. One of the most important results of the investigation was the verification that many people in this country have up to two or three years supply of vitamin A stored in the liver. This was consistent with the work of T. Moore[61] on a large number of specimens of human liver collected at autopsy.

The volunteers consistently showed blood levels of about 1 international unit/ml. of vitamin A (1g. vitamin A=3.3 x 10^6i.u.) and did not show retarded dark adaptation until the blood level was reduced to half. Only a small number of the volunteers became sufficiently vitamin-A deficient for tests to ascertain the daily intake needed to reverse the symptoms, but the findings did lead to an estimate of the requirement which has stood the test of time. The experiment as a whole has its historical significance.*

An interest in animal nutrition was shown in a paper[62] (Matthews† & Morton, 1938) on 'Computation of the cost of foods for livestock'. It contained a nomogram whereby the price per lb. of starch equivalent could be read off; it tackled the problem of allowing financially for the 'concentration effect' of foods with a high protein/starch ratio and it attempted a rough and ready correction of costs in terms of protein quality. An Australian bank decided to circulate the information given in the paper to all its farmer clients. The Ministry of Agriculture in London decided that the copyright of the authors would be infringed unless there was a token financial transaction. We agreed and accordingly, Matthews and I eventually received cheques for half-a-crown each drawn on the Bank of England! At that time the Corporation of Liverpool had a very large number of horses—hunter types for the mounted police and shire horses for carts and lorries. Veterinary officers often had to make quick decisions about costs of food.

Experience had been gained during the war in the conduct of dietary surveys and in the early post-war period Mr. A. V. J. Hinds, Secretary of the United Liverpool Hospitals, invited me as Head of the Biochemistry Department to carry out a dietary survey of the four main teaching hospitals. Complete records were kept of foods entering each hospital over a fixed period together with full information on the

* The writer happened to take the Chair at the meetings of the vitamin A sub-committee which planned the experiments and is glad to pay tribute to the fortitude of the volunteers and the scientific participants. The experiments lasted much longer than was expected and a large team of investigators had to carry out measurements under wartime conditions with unsuitable equipment. One of the volunteers (W Bartley) became a biochemist and was eventually appointed to the Sheffield Chair first held by Sir Hans Krebs.

† H. T. Matthews, FRCVS was then a senior veterinary officer for the Corporation of Liverpool. He later served with the Ministry of Agriculture and became a Reader in Veterinary Preventive Medicine when a new Veterinary School was set up at Cambridge.

numbers of patients and of staff fed, wholly or partially. The staff of the Biochemistry Department and the research students entered with admirable patience and thoroughness into the task of analysing the data. A careful job was done with the aid of tables emanating from the Ministry of Food. The inquiry proved very useful; it illustrated for biochemists and all concerned with hospital administration the realities of large-scale catering on a fixed budget in a post-war period with continued rationing. Today such dietary surveys would not require so much time and manpower, the computer can come to the rescue, although the real problems remain the same.

At one stage the weakest factor in the diets served in the teaching hospital was the supply of animal protein. The Board of Governors enquired how this could be increased *without raising the weekly food bill per person.* At the time margarine (vitaminized and of good quality) was generously subsidised while butter was still very dear. The only possible answer to the query was: 'buy margarine instead of butter and spend the difference on eggs or fish'. When this was reported, Mrs. Bessie Braddock (Mrs. E. M. Braddock, J.P., M.P. Labour, Exchange, Liverpool, 1945–1970) having an aversion to margarine based on childhood association, 'blew her top' at the suggestion and practically vetoed it. Characteristically however she made amends later by demanding an increase in the catering allowance and getting it. A redoubtable Liverpudlian, she would have accepted the wry verdict of the Book of Esdras (*Esdras,* **3**, 12–13, New English Bible). 'Women are strongest but truth conquers all'.

One important school of thought has regarded the 'essential' fatty acids and other polyunsaturated acids as playing a part in the prevention of atheroma.[63] A Liverpool study[64] of the fatty acids esterified with cholesterol showed an inverse relationship between the iodine value (a measure of unsaturation) of plasma cholesterol fatty acids (CFA) and the plasma cholesterol level. The essential fatty acid content of atheromatous tissue was no lower in cases of death from coronary thrombosis than in cases of death from other causes); in neither was it less than in normal healthy aortas.

An investigation on groundnut meals by Lord and Wakelam[65] (1950) of J. Bibby & Sons, Liverpool* had shown that the solubility of the protein fraction was much reduced by using steam freely during the removal of oil solvents. There was evidence of concomitant degradation of protein but the use of dry heat and minimal amounts of steam reduced the damage. Cama and Morton (1951)[66] studied the nutritional changes accompanying different types of processing. Groundnut meal at a 9 per cent protein level failed to maintain normal growth in rats and other symptoms of deficiency occurred. These could be prevented by adding methionine, cystine and biotin to the diet. At a 20 per cent protein level growth was normal. It was found that controlled heating and steaming enhanced the growth-promoting activity of groundnut meal in comparison with either unheated or overheated meals. The lower nutritive value of the unheated meal was due in part to poor availability of sulphur-containing aminoacids and biotin and also in part due to the presence of a heat-labile trypsin

* This provides an opportunity to place on record the fact that for many years the firm made a gift of £250 per annum to supplement the research funds of the Biochemistry Department. Two other Departments were given similar grants.

inhibitor. Supplementation of groundnut meal with caseinogen considerably enhanced the nutritive value.

Another nutritional problem was in 1955 referred to a Panel* on Composition and Nutritive Value of Flour under the Chairmanship of Professor Sir Henry Cohen. Before the war straight-run white flour of 72 per cent extraction was in general use but in relation to whole grain it was known to be a poor source of B vitamins. In July 1940 synthetic thiamine (vitamin B_1) began to be added to white flour and by 1942 two-fifths of the flour was being fortified. The growing shortage of imported wheat made it necessary to increase the extraction rate to 85 per cent with the result that the darker flour, nearer to wholemeal, did not require enrichment. The extraction rate had to be put up to 90 per cent in 1946 for a short time but it was soon reduced to 85 per cent until 1950. A conference convened by the government of the day had in 1945 recommended that flour should not be allowed in future to fall below a certain standard and levels of three nutrients were specified, namely, 0.24 mg. thiamine; 1.6 mg. nicotinic acid and 1.65 mg. iron in 100 g. flour. These nutrient levels were tokens or symbols of quality and the advice given was against enrichment and in favour of relatively high extraction to include nutrients present in the whole grain. When cereals were decontrolled in 1953 the controversy on enrichment had not abated. The government decided to allow white flour to be milled (extraction rate below 80 per cent) provided the product was enriched with the three nutrients to the prescribed minimum levels. But the subsidy was withdrawn and the public could buy a $1\frac{3}{4}$ lb. loaf made from darker flour (80 per cent extraction) at $7\frac{1}{2}d.$ (National bread) or a similar loaf made from enriched white flour at $10\frac{1}{4}d.$ to $1s.$ $0d.$ By 1954–55 subsidised National bread was being made from a flour containing significantly less vitamin B_1 and slightly less nicotinic acid and iron than was present in the 70 per cent extraction flour used to make unsubsidized bread. This was the complicated situation facing the Panel; the 1953 compromise was unworkable and dissension on scientific and nutritional issues was open and serious. Lord Cohen's legendary skill as a chairman had to be fully deployed.

An important factor in the inquiry was the work of Widdowson and McCance in Germany where experiments carried out just after the war on children at Wuppertal, Duisberg and Vohwinkel demonstrated the high nutritive value of wheat in any of the forms customarily consumed by man. Diets in which 75 per cent of the calories were derived from wheat flour and 21 per cent from vegetables and which contained only 8 grams of animal protein a day provided under-nourished children aged 5–15 years with all the nutrients required for a high rate of growth and development. For a period of eighteen months McCance and Widdowson were led to believe that the difference between unenriched low extraction flour and higher extraction flour was less than was expected and probably small enough to be ignored on an otherwise well-balanced diet. The Panel, reviewing all the evidence submitted, saw some need to enrich 70 per cent extraction flour with thiamine and nicotinic acid but not other vitamins of the B-complex. In effect the Panel concluded that white flour suitably enriched with thiamine, nicotinic acid and iron was not significantly different from

* Professor Sir Henry Cohen (Chairman), Professor A. C. Chibnall, Professor J. H. Gaddum, Professor R. A. Morton and Professor L. J. Witts.

higher extraction flours, but that the enrichment of white flour was advantageous.

The Report[67] of the Panel was a nutritional milestone. Some nutritionists felt let down by a Report that seemed to weaken their sense of mission but time has perhaps shown it to have been both realistic and dispassionate.

Another investigation with an important nutritional aspect was made by P. Malpas[85] (1939), it consisted of a study of abortion sequences occurring in a total of about 6,000 pregnancies to come under supervision. Some 115 women had abortion and stillbirth sequences, 84 having had three or more consecutive abortions or stillbirths. The overall percentage of all pregnancies ending in abortion was 18 and in most cases only one abortion occurred, but about one per cent displayed 'the pressure of a recurrent cause. Even after a woman has had three successive abortions the chance of her continuing to term is 27 per cent.' even if nothing is done for her. Out of the 115 subjects it was possible to advance a cause in 66, leaving 42 for whom this was not the case. In assessing therapeutic measures the evidence indicated that the 'spontaneous cure-rate' after two abortions is over 60 per cent and the incidence of casual abortion indicated that no therapy could expect more than 83 per cent success. Nine women with a history of sequential abortion were given wheat germ oil as a source of vitamin E and 25 women with similar histories were given no specific therapy. The 'control' group had a spontaneous cure rate of 36 per cent, not very different from the calculated 27 per cent while the groups given wheat germ oil did no better. Malpas thought 'progestin' therapy was somewhat more successful.

This paper[68] had a considerable impact. It put recurrent abortion in perspective and left only a minority of the cases at all open to a nutritional aetiology. The 'fertility' vitamin E had no demonstrable beneficial effect. There was of course no evidence whatever that in this country pregnant women generally had an 'insufficient' intake of the vitamin; even if tocopherol had been effective the appropriate conclusion would have been that women prone to sequential abortion had a specially high requirement. Injections of organic arsenicals (usually novarsenobillon) proved effective in a number of non-syphilitic sequential aborters showing no discernible cause but they had no effect in other cases. The arsenical effect would need further study before the degree of efficacy could be properly assessed.

R. Tecwyn Williams (Professor of Biochemistry at St. Mary's Hospital Medical School, London) spent a number of highly productive years at Liverpool. He is now accepted as the leading authority on the metabolism of drugs. The first edition of his book on *Detoxication Mechanisms* (London, Chapman & Hall, 1947) contained 288 pages. The second edition (1959) was much larger (796 pages) and the literature continues to grow rapidly. Twenty-five years ago the fate of simple substances like benzene, aniline and bromobenzene was little understood and the appropriate techniques of investigation had to be worked out. Detailed studies on sulphonamides were carried out by Williams' group.*

During the early post-war period Professor Stones, Head of the School of Dental Surgery, decided that a non-clinical group devoting its energies to basic research in the dental field was a highly desirable element within the school. He thought that the

* Among the research students trained in this field were G. A. Garton, R. L. Hartles, J. W. Porteous, D. Robinson, J. N. Smith and B. Spencer, all now occupying posts of responsibility.

growing point was probably biochemistry and sought help to find the right man. After investigation an approach was made to Dr. R. L. Hartles who had worked with Dr. R. T. Williams in the Biochemistry Department on detoxication mechanisms. Hartles understood that the new post would be 'mission-oriented' and that *complete* freedom of choice in his research would scarcely fit the scheme. He accepted the post (1948) and after fifteen years of successful research on many aspects of the biochemistry of teeth he became Professor of Dental Science (1963). A strong research group has been built up and numerous members of the clinical staff of the Dental School have collaborated in the work and have gained postgraduate degrees. Professor Hartles has special responsibility for the teaching of biochemistry to dental students. The number of publications from the group now approaches 100. Studies on the environment of the tooth include work on the metabolic activity of the whole salivary microflora and on selected individual microbial species. New light has been thrown on the relationship between salivary peptides and aminoacids and the metabolism of sugars by micro-organisms. Investigations on the dental plaque have shown that the carious process is greatly influenced by (a) salivary glycoprotein and (b) dextrans and levans synthesized by oral bacteria. A long series of papers on dental caries in the rat indicates that all simple sugars are highly cariogenic compared with starches. Sucrose may be a substrate of special importance because some streptococci in the dental plaque use it as a substrate for the synthesis of dextrans and levans.

The study of dietary variations in calcium, phosphorus and vitamin D in the rat raises complex problems in respect of both teeth and bones. A new emphasis has emerged on the role of citrate in calcium transfer to and from bone. Work is in progress on the non-collagenous proteins of bone and dentine to elucidate relationships they may have with the main collagen matrixe, especially as possible crystallization nuclei in bone and tooth mineral.

Dental biochemistry[69] is thus highly integrated with central aspects of microbiology, nutrition and with the chemistry and enzymology of carbohydrates and proteins.

During the war Goodwin and Morton[70] developed the spectrophotometric method for determining tyrosine and tryptophan in mixtures of aminoacids and in proteins. The procedure, slightly modified, is still in use. Although it could be carried out by photographic spectrophotometry it was much easier to perform when a modern photoelectric instrument became available. Photographic methods of measuring absorption spectra in the visible and ultraviolet were capable of intensity measurements to within $\pm$ 2 per cent in experienced hands but the newer photoelectric instruments which became available during the war gave readings to three significant figures. Morton and Stubbs[71] (1946) used this to extend the scope of spectrophotometric analysis both in the achievement of greater precision and in making corrections for 'irrelevant' absorption due to substances other than the one being measured. The correction procedure assumed that for a narrow spectral range on either side of an absorption peak due to the solute under study the irrelevant absorption of contaminants could be linear (i.e. the relation between wavelength λ and intensity of absorption E as a straight line). If the absorption intensities at three wavelengths

near an absorption peak are accurately known for a pure specimen of the compound to be determined the irrelevant absorption is seen to increase the intensity by a different amount at each of the three selected wavelengths. A general treatment based on simple geometry leads to a neat method of allowing for irrelevant absorption. The procedure was applied to anthracene and in particular to vitamin A in fish liver oils and at once gained acceptance. Cama *et al.*[72] (1961) determined the spectroscopic properties of all-*trans* vitamin A and its acetate in various solvents (using highly purified synthetic products obtained from Dr. O. Isler of Hoffmann La Roche) and derived correction equations for 'eliminating' irrelevant absorption. They also dealt with complications in fish liver oils arising out of the presence of neovitamin A (an isomeric form) and vitamin A_2. The Morton-Stubbs correction procedure was adopted by the pharmacopoeias and by international agencies. In fact vitamin A is a very difficult example; the method has given unambiguous results in many rather simpler problems.

The Johnston Laboratories were put to various uses during the war and the large basement was used by a scientific section of the postal censorship. Professor Channon took up an important research post with Unilever in 1943 and Morton was appointed in 1944 to the Johnston Chair. Professor W. C. M. Lewis, then in charge of Inorganic and Physical Chemistry, allowed the entire equipment of the spectroscopic laboratory to be transferred to the Johnston Laboratories as soon as the basement became available. There was therefore no break in the work and as research students and undergraduates returned from wartime duties further investigations on vitamins went ahead, and indeed were greatly facilitated by the opportunities for animal experimentation inherited from Channon.

The visual pigment extractable from dark-adapted retinas by means of a detergent solution was originally called visual purple but is now known as rhodopsin. It was broken down by Wald to give a protein and a substance *retinene* which gave a characteristic blue colour with antimony trichloride. Retinene was a puzzling substance and when obtained from eyes the amounts were altogether too small for structural studies. It seemed to me however that one possibility only would fit the spectroscopic evidence—namely that retinene was the aldehyde of vitamin A. This was confirmed (Morton 1944;[73] Morton & Goodwin 1944[74]) and the evidence was readily accepted. In the course of the work it was found that when vitamin A alcohol was allowed to stand in light petroleum over solid manganese dioxide a smooth conversion to the aldehyde occurred at room temperature. This remarkable process was subsequently found to apply to a very large number of oxidations of unsaturated alcohols and the reaction gained a permanent place in the armoury of chemists. Retinene, from being isolated at only the microgram level from eyes became available in unlimited amounts.[75]

After the war the Liverpool group isolated pure crystalline retinene and later pure retinene$_2$ derived from vitamin A_2 by the manganese dioxide process. Orally administered retinene could be converted to vitamin A (aldehyde→alcohol) in the rat intestine[76] and this led to a proof that the locus of the conversion of carotene to vitamin A was the lining of the gut. This important finding was confirmed by Goodwin and Gregory[77] using the goat, and Kon's team at Reading extended it to other species.[78]

Among other studies carried out at this time was work on rhodopsin with F. D. Collins. The pigment preparations then made from ox eyes were perhaps as pure as any ever obtained. The interaction of retinene (retinaldehyde) with protein was studied.[79]

In due course the labours of Heilbron, Milas, Isler and others resulted in synthetic vitamin A. *Cis-trans* isomerism was found to occur (Zechmeister) in carotenoids and vitamin A (Oroshnik), this proved very important for vision as was shown in the work of Wald.

The Liverpool work on the chemistry of vision centred on rhodopsin (visual purple) and porphyropsin. Wald had observed in the retinas of freshwater fishes a pigment to which he gave the name *porphyropsin*. It was analogous to rhodopsin but its absorption maximum was displaced from *c.* 500nm. to *c.* 525nm. Under conditions of bleaching which yielded retinene$_1$ from rhodopsin, porphyropsin gave retinene$_2$ with a new colour test peak near 700nm.

Retinene$_2$ was shown to be the aldehyde of vitamin A_2 (3-dehydroretinol). In the course of wartime work at Liverpool on high potency fish liver oils, ling cod, a marine fish from Pacific waters had been found to have the two vitamins A in the ratio A_1/A_2=7–10/1 but they were not at the time separable. Accordingly a concentrate rich in both vitamins was treated with manganese dioxide to give a mixture of aldehydes which could be separated by chromatographic adsorption on alumina. The purified retinene$_2$ was crystallized and reduced (e.g. by lithium aluminium hydride) to pure vitamin A_2 which had already been thought to be 3-dehydrovitamin A. The aldehydes, like vitamins A_1 and A_2 exhibit *cis-trans* isomerism and elegant work was done on this effect by Wald, Hubbard, Oroshnik, Paulin, Zechmeister and others (for summary see Morton & Pitt 1957).[80]

Here the work on rhodopsin owed a great deal to Collins, Love, J. N. Green and Pitt.[81-83] Very good preparations of rhodopsin were made from cattle retinas and the spectroscopic properties of the conjugated protein were established. The pre-war studies of Lythgoe's group at London were carried further and work began on the formidable task of elucidating the sequence of steps in the photochemical break-down of rhodopsin. Collins carried out notable work on the regeneration of rhodopsin. The whole study became very complicated and workers in many countries contributed to the unravelling process, Wald's group at Harvard being outstanding. The suggestion that rhodopsin contained retinaldehyde bound as a Schiff-base was first advanced at Liverpool.

Investigations were carried out in various centres on the distribution of rhodopsins and porphyropsins in many species and Dartnall made good use of a technique of partial bleaching and differential spectroscopy. Morton and Pitt[84] (1970) wrote a lengthy review of aspects of visual pigment research in which they collected the results on fish pigments and assessed the plurality of extractable 'rhodopsins' and 'porphyropsins'. They also assembled the evidence on insect vision and the problems of foveal and parafoveal cone pigments. The time was ripe for a survey of the chemistry and of the intermediates occurring in pigment photo decomposition and the latest work on the early receptor potential, which may provide the best link so far between the electrophysiology of vision and the biochemistry, was reviewed.

The biochemistry of vision is a very large subject with a growing literature already

well over 1,000 recent original papers. At Liverpool it was treated as an aspect of vitamin A research to which distinctive contributions could be made.

Soon after the war it became clear to us that the possibilities at Liverpool of research into the biochemistry of vitamin A and of carotenoids were very great and Goodwin undertook a special responsibility for carotenoids. This later bore fruit in his book *The Comparative Biochemistry of the Carotenoids* (London, Chapman & Hall, 1952), shortly to appear in a second edition. It is not easy to summarize the work done under Goodwin's direction.[85] The studies on distribution of carotenoids have ranged from fungi to marine crustacea and fishes and from insects to plants. Uvarov, as Head of the Anti-Locust Research Centre provided Goodwin with materials for penetrating studies on the occurrence and functions of insect pigments. Possible roles of carotenoids in reproductive processes were investigated using the gonads of limpets and the eggs of lobsters and (with A. A. Wilson) the serum of cows approaching parturition. The fungus *Phycomyces blakesleeanus* was used in a sustained attack on carotenogenesis in which quantitatively minor poly-enes were detected and characterized and the effects of various aminoacids and glucose in the culture media were observed. The work was extended to mutants of *Phycomyces* and to an inhibitory action by diphenylamine. From the fungus the attack was moved to the photosynthetic organisms *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides*. This led naturally to *Euglena gracilis* but the work on marine organisms and insects continued. Among the micro-organisms to which the work was extended were *Chlorobium, Chromatium* sp. and *Mycobacterium phlei*. The discovery of mevalonic acid by Folkers provided a fresh impetus to research on the biosynthesis of carotenoids and the later development in the sterol field of double stereochemical isotopic labelling by Cornforth and Popjak enabled Goodwin and his pupils to raise the study of carotenoid biosynthesis to a new level of sophistication.[86]

Goodwin also took up the study of biosynthesis of vitamin B_2 (riboflavin)[87] in the organism *Eremothecium ashbyii*. In this work[14] C-labelled aminoacids were used and in later work on *Candida flareri* the roles of urea and of purines were explored. Riboflavin biosynthesis raises important issues notably in respect of the source of nitrogen but also the biosynthesis of the aromatic ring. The award by the Biochemical Society of its Ciba Medal to Goodwin testifies to the fact that the major aspects of carotenoid biosynthesis are now clear.

Goodwin was elected to the Chair of Agricultural Biochemistry at the University College of Wales, Aberystwyth. There he soon built up a considerable research school and besides continuing his investigations on carotenoids he contributed to terpenoid biosynthesis in plants and to steroid biosynthesis in fungi. The growth in knowledge concerning carotenoids has been immense; a few years ago less than fifty members of the family were known, by now there are between three hundred and four hundred and some authorities expect the number of naturally occurring carotenoids to reach one thousand. The distribution of carotenoids in nature has been studied with skill by the schools of Goodwin, Liaaen-Jensen and others, and Weedon has contributed greatly to the structure elucidation. A surprising number of variations has been noted and the multiplicity of carotenoids shown to arise from permutations and combinations of a limited number of molecular moieties. A large number of the more

familiar carotenoids (and derived compounds) can now be synthesized thanks to the work of Isler and his group at Basle. Carotenoids obtained by methods first worked out in the context of vitamin A synthesis are now available commercially, and isotopically labelled carotenoids are accessible for research. Moreover the biosynthetic pathways have in the main been established.

It might seem as if the carotenoid story is now within sight of completion and indeed so much has been done that there is an element of truth in this idea. Neverthless the functions of carotenoids are imperfectly understood. There is certainly a subsidiary role in photosynthesis and both protective and precursor roles have been established but much remains to be done. Goodwin and his pupils have been and are in the thick of the fray at Aberystwyth and at Liverpool.

The emphasis on biogenesis and function has led to the adoption of novel and increasingly 'biological' techniques. This was seen in studies on the distribution and formation of quinones in maize and barley shoots and in studies on the biosynthesis of certain water-soluble vitamins.

Goodwin returned to Liverpool as Johnston Professor in 1966 in succession to Morton and was elected F.R.S. in 1968. Among his other activities he has edited multi-author volumes and has organized and edited the *Biochemical Society Symposia* (first edited by R. T. Williams). He plays a prominent part in international biochemistry.

John Glover graduated in Chemistry at Queen's University, Belfast, early in the war and his Musgrave Fellowship was held over until 1946 when he came to the Biochemistry Department at Liverpool to work for a Ph.D. His first studies were on vitamin A, in collaboration with Goodwin and Morton and concerned the mobilization of retinol into blood from liver stores of retinyl esters. There was also a demonstration that retinaldehyde was converted to retinol (vitamin A_1) mainly in the intestinal wall. As the work developed it was thought desirable to obtain fresh tissues from fishes and particularly halibut. Glover sailed from Aberdeen on a trawler to distant northern waters where, despite the bad weather, he managed the necessary dissections and brought his material safely home under alcohol. He later shared in the demonstration that β-carotene is converted to vitamin A in the animal intestine. Soon after he had been appointed assistant lecturer, Glover was awarded a Commonwealth Fellowship which took him to the U.S.A. to work with M. D. Kamen at Washington University, St. Louis. There he gained a knowledge of microbiological methods applied to the photosynthetic organisms *Rhodospirillum rubrum* and acquired experience in the then rather new techniques for studying biogenesis and intermediary metabolism with the aid of radioactive isotope labelling.

On his return to Liverpool almost every type of investigation in the Biochemistry Department profited by Glover's training under Kamen, notable examples being carotenogenesis and the conversion of carotene to vitamin A.

A study of sterols in the intestinal wall of the guinea pig[88] had drawn renewed attention to 7-dehydrocholesterol and to the importance of mucosal cells. Glover undertook a series of studies on the biosynthesis absorption and metabolism of sterols[89] with Colin Green, W. M. F. Leat, D. W. Stainer and I. Mercer. With J. G. Desai he investigated the sterol complexes in bile and intestinal fluid and the role of

phospholipids. This work led to the development of electrophoresis in the department as a method of investigating lipoproteins in blood serum.

Glover continued his work on labelled β-carotene and with E. R. Redfearn advanced an important scheme for the mechanism of transformation into vitamin A *in vivo*. This was developed in several investigations and later reviews.[90]

Glover's expertise was used to good effect in the early work on the biosynthesis of ubiquinone in the rat.[91] With Packter he identified ubiquinone and ubichromenol in *Aspergillus fumigatus*. A new quinone, rhodoquinone, was discovered in *Rhodospirillum rubrum*.[92] The latest advance made by Glover's group is the isolation of a specific retinol (vitamin A) carrier protein in the blood. This is an important achievement.

Glover has published several valuable surveys of physicochemical methods in the service of biochemistry. He was made a professor in the department in 1966.

H. K. King, a Cambridge microbiologist, was trained by Dr. M. Stephenson; he came to Liverpool via Nottingham (where he worked during the war at Boots Pure Drug Company on vitamin B_{12}) and later the University of Edinburgh. Hugh King's scholarship is witnessed by a long series of reviews on advances in many aspects of biochemistry that he contributed to *Science Progress*. He now occupies the Chair of Agricultural Biochemistry at Aberystwyth. At Liverpool King's microbiological researches ranged widely and the following topics may be noted: aminoacid biogenesis and metabolism, substrate-coenzyme affinity, bacterial cell walls, role of pyridoxine and quinones in micro-organisms. King and his students were perhaps the earliest workers to observe the coexistence of ubiquinones and menaquinones (vitamins K_2) in a limited range of bacteria. They went on to study intracellular distributions in appropriate organisms and to fractionate bacterial respiratory systems. This led to the effect of lysozyme on respiration and electron transport and to extensions of the survey of bacterial quinones. Microbiology at Liverpool has also been greatly assisted by Dr. N. G. Carr and Dr. J. M. Turner, both Leeds graduates who had done post-doctoral work in the U.S.A.

The Scientific Advisory Committee* of the British Egg Marketing Board subsidized research at Liverpool on vitamin A in poultry and Dr. J. N. Thompson held one of the Post-doctoral Fellowships from 1962. With G. A. J. Pitt and J. McC. Howell (a lecturer in Veterinary Pathology) and Thompson collaborating, a strong team was set up and important results were obtained.

It has already been found that the responses to vitamin A deprivation of rats and of domestic poultry differed and in particular it was noticed that in chicks the nerve lesions were dominant and indeed death occurred in the young birds before there was loss of appetite or any sign of xerophthalmia (Heaton *et al.* 1955, 1957; Lowe *et al.* 1957*a*)[93] Deprived cockerels showed convulsive seizures followed by very speedy recovery.

The Liverpool group kept male rats on a diet in which vitamin A had been replaced by the corresponding acid. Many authors had claimed that retinoic acid could replace vitamin A in all its known functions except those requiring retinaldehyde for the

* Set up after a preliminary enquiry by A. C. Chibnall. The committee was in existence for 9 years and among the advisers from outside the poultry field were A. S. Parkes, E. C. Amoroso, R. A. Morton and C. H. Waddington. Experts in the field included among others R. F. Gordon, J. Young, T. C. Carter and H. Temperton.

formation of visual pigments. In fact the retinoic acid diet resulted in changes in the testes which could be reversed by a retinol supplement. The lesions were histologically characteristic.

Hens given a retinol-free diet supplemented by retinoic acid or its methyl ester laid eggs at a normal rate. When fertilized, such eggs showed a cessation of embryonic development after two days' incubation. Development was stimulated by injected retinol, retinyl acetate, retinaldehyde and β-carotene but not retinoic acid. In cockerels retinoic acid or its methyl ester maintained testicular development but failed to do so in rodents. The acid failed to maintain vision in birds; this observation is important since it provides the first direct evidence that retinaldehyde is necessary for *photopic* vision in birds with cone retinas. Retinoic acid proved to be very toxic to chick embryos. Vitamin A deficiency in growing and adult birds gave rise to abnormal activity of periosteal bone perhaps causing compression of nerve tissue. The group took advantage of the fact that there is no storage of retinoic acid and by the artifice of withholding the retinoic acid supplement they speedily produced vitamin A deficiency in adult birds until then apparently perfectly healthy. Lesions of the central nervous system were seen and described, especially in ataxic chicks. Changes in bone came after ataxia and brain compression.

Birds reared from hatching on a vitamin A-free diet plus retinoic acid showed no overt signs of deficiency other than a progressive failure of vision. Histological changes in the retina were minimal and vision was rapidly restored after administering retinyl acetate. Males given retinoic acid had normal testes. Abnormality was seen in the embryo and the need for retinol by the chick embryo seems unequivocal.

Rats were kept on a basal A-deficient diet supplemented by retinoic acid. They grew well and were healthy apart from retinal and testicular lesions characteristic of vitamin A-deficiency.

Many able workers have studied the vitamin A-deficiency syndrome. The special contributions made by this group of workers[94] depend to a considerable extent on the differentiation between the effects of retinol and retinoic acid.

In the course of post-war work on vitamin A-deficient rats spectrophotometric studies on unsaponifiable fractions indicated that animal tissues could contain two new substances. They were provisionally designated substance A (SA) and substance C (SC). Each had its characteristic absorption spectrum which was used for monitoring procedures aiming at isolation. Substance A was next found in yeast and distribution studies showed it to occur very widely. After considerable effort it was characterized and named ubiquinone because it was nearly ubiquitous and had the structure of a substituted benzoquinone. It was, however, a very unusual compound in that it possessed a polyprenol side-chain $C_{50}H_{81}$, i.e. $[C_5H_8]_{10}H$. Further research showed that there are several naturally occurring ubiquinones with side-chains of the type $[C_5H_8]nH$ where $n=6$ to 13. A different approach at Madison, Wisconsin, led Lester and Crane to discover ubiquinone independently and they preferred to call it coenzyme Q.

Substance C was less widespread. One of the best sources turned out to be human kidney unless the kidney was diseased. With the co-operation of pathologists about 100 lbs. of human kidney was obtained and worked up in batches. Eventually 125 mg.

of SC was obtained and its structure established. It turned out to be a cyclised derivative of ubiquinone. Soon after the structure was established it proved easy to prepare ubichromenol from ubiquinone.

A quinone from plants, now known as plastoquinone had been discovered some years earlier. It was soon apparent that ubiquinone, plastoquinone and vitamin K had functional as well as structural resemblances. The best-known plastoquinone (PQ-9) had a nonaprenyl ($C_{45}H_{73}$) side-chain and recent work by Liverpool graduates has established that a hydroxyl group can occur in six different positions in the side-chain ($PQC_{1\text{-}6}$). Moreover each of these can occur as an ester ($PQB_{1\text{-}6}$) so that at least 12 derivatives of plastoquinone-9 (PQ–9) occur naturally. The vitamin K_2 family (menaquinones MK-n) also have a variable polyprenyl side-chain. Four tocopherolquinones are related to four tocopherols (forms of vitamin E).

Plastoquinones and phylloquinone (the original vitamin K_1) are present in the chloroplasts, the photosynthetic organelles of green leaves. Ubiquinones are characteristic of mitochondria in both plants and animals. Many micro-organisms contain a menaquinone which is sometimes replaced by ubiquinone; a few species contain both.

Crucial to the main biological roles is the reversible oxidation-reduction quinone $\leftrightharpoons$ quinol process. Mitochondria such as those prepared from liver or heart muscle tissue contain four complexes with separate functions in electron transport involving the production of energy for work. Ubiquinone and cytochrome c act as linking agents and the quinone$\leftrightharpoons$quinol system is an indispensable part of the sequence of operations. Electron transfer and oxidative phosphorylation are central to the complex process of gaining energy in the form of adenosine-triphosphate (ATP) for metabolism. Although oxidative phosphorylation is not even yet fully understood a central biochemical role for ubiquinone is accepted. In micro-organisms a sub-cellular fraction analogous to the mitochondrial fraction of animal tissue contains ubiquinone or menaquinone or both. When the two quinones occur together they appear to catalyse different parts of the electron transport system.

Theories of photosynthesis require two distinct light reactions. There is good evidence of a plastoquinone-plastoquinol pool. The electron flow is driven by absorption of light by two active chlorophyll complexes in a membrane system and plastoquinone is located between the complexes. Flash photolysis (very rapid photochemical change) indicates that a dimer (PQ–PQ) becomes a semiquinone PQ^{-}–PQ^{-}) which splits to give the quinole PQ^{2-} and ordinary PQ.

The literature on biologically active quinones is now enormous and covers many aspects not mentioned here. The electron transport problems are central to biology as a whole. The Liverpool contributions to these studies, particularly in respect of the original synthesis and later the synthesis of labelled quinones have been greatly assisted by a happy collaboration with Hoffman La Roche of Basle and in particular with Dr. O. Isler. A great deal of work on the multiplicity of quinones has been done at Liverpool and at Aberystwyth by Liverpool graduates who took up posts under Professor Goodwin. The field has been surveyed recently.[95]

After the discovery of ubiquinone (Q-10) with its $C_{50}H_{81}$ side-chain a new interest arose in isoprenoid alcohols. It emerged that plastoquinone (PQ-9) had a $C_{45}H_{73}$

side-chain and Rowland's earlier discovery[96] of solanesol in tobacco needed to be exploited. It occurred to me that this alcohol might be present in the fibrous waste left behind after the extraction of nicotine from tobacco dust. In fact the lipid from such waste was found to contain no less than 10 per cent of solanesol which proved a very useful intermediate for syntheses. Solanesol is a homologue of geraniol, farnesol and geranylgeraniol and like them has the all-*trans* structure.

In the course of studies at Liverpool on the minor constituents of sterol-free unsaponifiable matter from animal tissues we had long been puzzled as to the nature of the diluents present in certain fractions that showed little ultraviolet absorption. After working up human kidney in order to isolate ubichromenol an opportunity arose to examine by-product fractions of reasonable size. The outcome was to isolate a polyprenyl alcohol to which the name dolichol was given.[97] Application of classical methods indicated a single compound $C_{100}H_{163}OH$. But when newer methods of separation were applied, the dolichol was found to be a mixture of polyprenols. The dolichol fraction from pig liver contained six alcohols and baker's yeast dolichol had five. Altogether nine members of a series were found ranging from C_{70} to C_{110}.[98] The predominating alcohols from pig kidney were C_{90} to C_{105}. When these alcohols were examined for their mass spectra, infrared absorption and nuclear magnetic resonance it was shown that dolichols have a saturated terminal isoprenoid unit (bearing the hydroxyl group) so that the classical dolichol is $C_{100}H_{163}OH$ rather than the polyprenol $C_{100}H_{161}OH$.

Extension of the research to plants, marine organisms and micro-organisms has opened a new chapter in biochemistry. This is being brilliantly developed by Hemming, Pennock and their students and quite important biosynthetic roles have emerged. Many other fruitful lines of research are under investigation by Drs. Goad, Green, Carr and Turner and the younger members of staff. The adoption of biochemical methods by other Departments is today even more widespread than in the very early days.

The award of a Leverhulme Trust Emeritus Fellowship is acknowledged with thanks.

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