Symposium on Fifty Years of Fat and Oil Research in Selected Countries of the World *

Some Significant Advances in Oils and Fats Research in Great Britain in the Last Fifty Years[1,2]

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IN BRITAIN systematic research into the chemistry of oils and fats began in industry in response to an obvious need for information. The greatest single influence was the introduction of the hydrogenation process. Simultaneously with German development, Normann's discovery was developed in Britain by the firm of Joseph Crosfield and Sons Ltd. By 1910 whale oil had been hardened as a substitute for tallow in soap manufacture, but partially hydrogenated whale oil could not be simply substituted for tallow to give a satisfactory soap. Thus detailed studies of fatty acid composition and of the hydrogenation process were begun. Research workers at Crosfield's subsequently published paper after paper, which may be said to mark the beginning of modern systematic research on fat chemistry.

I shall attempt throughout this review to maintain some historical sequence but occasionally will review some particular work or some individual. Subject matter will be limited to the composition of the natural fats, *i.e.*, of their component acids and glycerides.

The Work of Hilditch and His School

Outstanding among British leaders in research on fats is T. P. Hilditch. He joined the research staff at Crosfield's in 1911. In 1926 he took the chair of industrial chemistry at the University of Liverpool and founded a school of academic research into fat chemistry, the influence of which has been world-wide. Study of the component fatty acids of fats by ester distillation began at Crosfield's in 1912–13, but the first detailed paper giving results obtained with this technique was published in 1925 (1). The latest and most sensitive method of fatty acid analysis, gas-liquid chromatography, was also developed in Britain (2), and fatty acids were the first test substances for a technique since extended to a wide range of compounds. From the earliest days of fractional distillation, from Willstätter flasks to gas-liquid chromatography, is a long stride, both in refinement of technique and in our knowledge of fat chemistry. Thus the academic research workers have paid the debt they so clearly owed to the industry.

At Crosfield's Hilditch was also closely concerned with studies of the hydrogenation process. By 1913 these had demonstrated its selective nature and had enabled Armstrong and Hilditch (3), after a wartime delay, to publish in 1919 a theory which explained a) the selective hydrogenation of esterified linoleic acid in the presence of esterified oleic acid, b) the partial breakdown of selectivity when the free acids were used, and c) the production of elaidic acid and of *iso*-oleic acids during the hydrogenation of oleate (4). This theory, involving the formation of an unstable intermediate between fatty acid and nickel catalyst, paved the way for a more satisfactory understanding of many aspects of catalysis while the value of the practical results to the fat-hardening industry needs no emphasis.

The single most significant factor in the development of oils and fats research in Britain was the chance that sent Hilditch to Crosfield's. The result has been a colossal, direct contribution to our knowledge of fat chemistry and a clearly tremendous, indirect contribution through his numerous students from all parts of the world. It is impossible to summarize the results of his work, the latest edition of his text book (5) being a fitting monument to it. Fatty acid composition of fat after fat was determined until it was possible to show the patterns that run through biological families (6).

Next in volume of output from the Hilditch school were studies of glyceride structure. Although his theory of "even" distribution in seed fats has been criticized recently, more experimental work lies behind it than behind some of the alternative proposals. Its opponents owe a debt to Hilditch for developing techniques applicable to this difficult field, *e.g.*, oxidation by permanganate in acetone or acetic acid to determine fully saturated glycerides, another inheritance from Crosfield days (7). Further, the latest delicate procedure of selective enzymic hydrolysis is confirming one aspect of his conclusions, namely the specific incorporation of saturated and unsaturated acids in particular positions on the glycerol molecule (8, 9). Thus the pioneer work of Hilditch, and the stimulus of his ideas, will have played an outstanding role.

There have been numerous contributions from Hilditch and his colleagues on other aspects of fat chemistry. However his analytical work on the fatty acid composition and glyceride structure of natural fats is the main basis of his outstanding reputation among chemists in this field.

In a fascinating historical lecture (10) Hilditch paid tribute to the happy and helpful environment he entered at Crosfield's. It served as the foundation for much later work with his students. Working in premises ludicrously inadequate by modern standards, all of us in that then young school were not only happy but always encouraged and inspired by the intimate association which Hilditch maintained with each and all of us. A measure of the contribution of Hilditch to fat chemistry in the 26 years he was at

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[1] This paper was prepared as part of the program of the Department of Scientific and Industrial Research.

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* Condensed from the original versions, which were presented at the 50th anniversary meeting of the American Oil Chemists' Society, in New Orleans, La., April 21, 1959.

Liverpool, from 1926 to 1951, can be found in the 74 research students who passed through his department, of whom 30 were from overseas.

Before leaving the subject of component acid determination, I should like to mention a British finding which, through its practical development predominantly in the U.S.A., has had widespread application in this field. In 1931 Morton, working in Liverpool and in collaboration with Hilditch, first reported the characteristic ultraviolet spectra of polyethylenic fatty acids, arising during their liberation by alkali saponification (11). Six years later Moore, working at Cambridge, provided the explanation when he demonstrated that conjugation of the double bonds occurred during saponification of linseed oil (12).

Polymorphism

Studies of the fatty acid composition and glyceride structure of natural fats, as discussed above, depend ultimately on knowledge of the properties of the pure, individual components. Beginning in the early 1920's, work in Britain was laying the foundations for our knowledge of the thermal and x-ray properties of fatty acids and their derivatives. Muller (13) and Shearer (14), working in London under the guidance of W. H. Bragg, Piper (15) in Bristol, also de Broglie in France (16) carried out more or less simultaneously the first x-ray studies of long-chain aliphatic compounds, including fatty acids and soaps.

McBain was engaged at Bristol on his classical work on soap solutions. In 1927 Garner succeeded McBain as Leverhulme professor of physical chemistry and continued at Bristol those studies of the thermal properties of higher fatty acids and similar compounds which he had begun in London (17). Also at Bristol, Francis was attempting to study the mixtures of higher fatty acids obtained by oxidation of waxes. Discussion of their problems led to Piper's x-ray studies, which began with some pure soaps supplied by McBain and were extended to paraffins from Francis' studies. Francis concluded that x-ray studies would help in the identification of his higher fatty acids, and Malkin was appointed in 1925 to assist with the chemical side of the work, *i.e.*, the synthesis of pure acids. A most significant event was the arrival of Malkin at Bristol. Interestingly Malkin's introduction to oils and fats began in the same place as Hilditch's, *i.e.*, Joseph Crosfield and Sons Ltd. By 1930 Malkin had begun on his own long program of studies of the thermal and x-ray properties of pure fatty acids, their simple esters, and their mono-, di-, and triglycerides. He had been mainly responsible for the great development of our knowledge in this field, for which all workers with natural fats owe him a debt of gratitude.

The study of polymorphism in long-chain compounds offered an explanation of certain otherwise puzzling observations, *e.g.*, the well-known alternation, in certain series of compounds, of properties (such as melting point) peculiar to the solid state, between homologues with odd and even numbers of carbon atoms, respectively. This behavior is shown by fatty acids, methyl esters, and the higher-melting polymorphs (β' and β forms) of ethyl esters and glycerides but not by normal paraffins, primary alcohols, and the lower melting (α) forms of ethyl esters and glycerides. Malkin (18) demonstrated that alternation depends on a tilted carbon chain in the unit cell of the crystal and can be attributed to the type of packing of the terminal planes. In general, even-numbered chains have close packing, with higher melting points than the odd-numbered chains with alternate closely- and loosely-packed planes, but occasionally the odd members may be the higher melting, *e.g.*, with the alkyl iodines. In the nonalternating series the chains are vertical to the terminal planes.

Polymorphism facilitates the unequivocal identification of a compound, particularly when x-ray data are allied to thermal data obtained from heating and cooling curves. With fatty acids of 20 or more carbon atoms, separation of homologues becomes progressively more difficult and criteria of purity become more exacting. Melting points approach a limiting value asymptotically, but x-ray long spacings show a constant increment with chain length. Francis, Piper, and Malkin (19) showed that the identity of a very long-chain acid can only be established with certainty when the melting points and long spacings of two crystal forms are known. But it is outstandingly in the study of triglyceride structure in natural fats that thermal and x-ray data obtained on pure synthetic compounds are indispensable. Malkin's pioneer studies in this field began in 1934 (20), and he and his colleagues have contributed an immense amount of detailed information (21). Malkin maintains that, in addition to a vitreous form, solid triglycerides exist in three crystalline modifications (four modifications for some unsaturated mixed glycerides). The melting points of these, combined with their long and short x-ray spacings, provide the necessary "finger-print" for identification.

Recently Chapman, working in the laboratories of Unilever Ltd., has pioneered the application of infrared spectroscopy to the study of polymorphism in 1- and 2-monoglycerides (22), in 1:3-diglycerides (23), and in saturated triglycerides (23, 24). He has shown that such compounds exhibit small but characteristic differences in I. R. spectrum in the liquid state and in the various crystalline states. Compared to x-ray studies, I. R. spectral analysis has the merit of permitting rapid scanning while the sample is being heated or cooled; the use of very thin samples

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is a further advantage because of the low thermal conductivity of fats.

Structure of Unsaturated Fatty Acids

In 1923 Muller (13) in London and Piper (15) in Bristol independently found that the x-ray long spacing of fatty acids increased regularly with the number of CH_2 groups in the chain. Muller noted however that elaidic acid, the only unsaturated acid in his series, had a long spacing some 9 Å greater than that of stearic acid. This difference is equal to about four methylene groups in the saturated acid series. Later the same year Muller and Shearer (14) reported a study of the long spacings of oleic and elaidic, also of erucic and brassidic acids, and compared them with those of stearic and begenic acids. While oleic and erucic had values slightly below those of the saturated acids, elaidic and brassidic had considerably greater values. Consideration of possible chain configurations led to the conclusion that oleic and erucic acids must be the *cis*-isomers. For erucic this was a definite reversal of the current view, and virtually so for oleic acid. This work, although not absolutely conclusive in the absence of data on comparable long-chain compounds of known geometrical configuration, was a great advance on the largely speculative views that preceded it. It was comparable in many respects to, and just as fundamental as, the modern infrared technique, the development of which will be discussed shortly.

In 1925 Armstrong and Hilditch (7) described their new method of oxidation by permanganate in acetone or acetic acid. It had the advantage of simplicity in use and gave a high yield (80–90% of theory) of the di-carboxylic fragment when applied to the ester of a mono-ethenoid acid, such as oleic. The yield of monobasic acid, although smaller (50–60% of theory), was still adequate for characterization. With this method, which has since found extensive application even in the most recent work, the authors showed that the myristoleic and palmitoleic acids each had the double bond in the 9:10 position (25).

Next the work of Farmer on some of the polyunsaturated compounds may be noted. Commencing in 1935, he and his colleagues published a series of studies on unsaturated fatty acids. In the first (26) he characterized the licanic acid of oiticica oil, which was the first acid of its type to be described outside the carotenoids. In the second (27) he reported the structure of parinaric acid, which was a second representative similar to elaeostearic acid; licanic acid was the first.

Mention of parinaric acid leads to two further topics, namely, the value of synthetic work in confirming a structure deduced analytically and the use of infrared spectroscopy to define the geometrical isomerization present in these complex acids. The British contribution to synthetic work comes later in this review, but the subsequent partial establishment of the isomerization in parinaric acid by Ahlers and his colleagues (28) should be discussed now.

Just as ester distillation was first employed because of an industrial need, so was the application of infrared spectroscopy to the study of *cis-trans* isomerism in fatty acids. Ahlers worked at the Paint Research Station, near London, and the industry required more fundamental information on the drying properties of various oils. This Station of the Research Association of British Paint, Colour, and Varnish Manufacturers was established in 1926 under L. A. Jordan, who is still its director. Its staff has made numerous valuable contributions to the knowledge of the chemistry of oils and fats.

Hence three events of outstanding significance to the development of oils and fats research in Britain occurred almost simultaneously, namely, the appointments of Hilditch at Liverpool and of Malkin at Bristol and the establishment of the Paint Research Station. Ahlers' opening paper with Brett and McTaggart (28) in 1953 records the first detailed application of the novel technique to the problem of geometrical isomerism in fatty acids. The spectra from 2.5 to 15 μ were given for 19 fatty acids, all with 18 carbon atoms, saturated, monoethenoid and polyethenoid, conjugated and nonconjugated. Among the more important findings were the association of a sharp band at 10.33 μ with nonconjugated *trans* double bonds, the progressive displacement of the maximum to shorter wavelengths by increasing conjugation, and the simple additive effect of the absorption of each double bond. A less definitive but still useful property was the rather broad band in the 14–16 μ region associated with *cis* unsaturation.

In all previous reports except one from the American paint and varnish industry (29) the postulated structures for elaeostearic acid had included one *cis* bond in the β-form of the acid. Ahlers, in agreement with the American conclusions, decided that the β-acid is all *trans* and that the α-acid has one *cis* bond. The limitations and pitfalls of infrared studies might be noted. Thus Ahlers, like others, concluded that distinction between *cis-trans-trans* and *trans-trans-cis* arrangements was probably impossible by this technique. However his rejection of a *trans-cis-trans* structure for α-elaeostearic acid, although agreeing with the findings of later synthetic work, was based on a false interpretation of the splitting of the *trans* absorption into two bands. His later similar interpretation of the spectrum of punicic acid was disproved by the same synthetic work. The data for parinaric acid were more difficult to interpret but suggested that the β-acid is almost certainly all *trans* while the α-acid contains one, or perhaps two, *cis* bonds.

Farmer and van den Heuvel (30) had confirmed the postulated structure of the punicic acid of pomegranate seed oil as a geometrical isomer of elaeostearic acid. Ahlers concluded (31, 32) that the natural α-form of punicic acid has the *cis-cis-trans* (or *trans-cis-cis*) structure and showed that the trichosanic acid of snake gourd oil is probably identical with punicic acid (33). He was right about the presence of two *cis* bonds, but synthetic work has established his rejected *cis-trans-cis* formula as the correct one. In collaboration with Gunstone, Ahlers concluded that the geometrical isomerism in kamlolenic acid is the same as in elaeostearic acid (34), a view arrived at independently at about the same time by other British workers (35).

Gunstone, a member of the Hilditch school at the end of World War II, has made a considerable contribution to our knowledge of natural unsaturated fatty acids. He became interested in possible isomers of ricinoleic acid since various reports of the occurrence of this acid were based solely on such clues as iodine-, saponification-, and acetyl-values. Examination of the seed oil of *Strophanthus sarmentosus*, which, by analogy with other members of the same genus, should contain an unsaturated hydroxy acid, showed that it actually contains an isomer of ricinoleic acid, in which the double bond and the hydroxyl group are transposed, namely 9-hydroxy-octadec-12-enoic acid (36). Some of his work on kamlolenic acid, another C_{18} hydroxy acid, has been mentioned. There seems to have been a surprising more-or-less simultaneous investigation of this acid in three laboratories, leading to the same conclusions. In India Aggarwal and his colleagues (37), who originally discovered the acid, and in Britain Calderwood and Gunstone in Glasgow (38) and Crombie and Taylor (35) in London all arrived at the presumably correct formula although Aggarwal's choice between two alternatives was by analogy whereas Gunstone's and Crombie's work left no alternative. Study of another suggested hydroxy acid, vernolic acid, led Gunstone to the discovery of the first epoxy acid to be found in a natural fat (39). Simultaneously with Australian workers (40), Gunstone also showed (41, 42) that the so-called "santalbic" acid of seed fats from the genus *Santalum* is *trans*-octadec-11-en-9-ynoic acid, hence identical with the ximenynic acid previously described by S. African workers (43), who collaborated with Ahlers in Britain (44).

Synthesis of Unsaturated Fatty Acids

In the last 10 years there has been a remarkable development in Britain of work on the synthesis of unsaturated higher fatty acids. This recent phase may be said to have begun with the work of Bowman. Having developed his synthetic route in which a double bond is introduced at a chosen point *via* an alkoxy-ketone (45) or alternatively *via* an acyloin synthesized by an improvement (46) on Ruzicka's route, he and his colleagues rapidly synthesized all the more common monoethenoid acids with from 14 to 22 carbon atoms (45, 46, 47). For three of these acids, namely tetradec-9-enoic, eicos-9-enoic, and erucic acids, they were the first recorded syntheses. The acyloin route permits ready separation of the subsequent *cis* and *trans* isomers at the stage of the corresponding *erythro* and *threo* dihydroxy-acids. Then in 1950, just after Bowman began publishing this work, Raphael and Sondheimer, using the alkyl acetylene route with subsequent partial hydrogenation of the di-acetylenic acid, reported the first synthesis of undoubted *cis-cis*-linoleic acid (48).

Having first adapted the Kolbe anodic coupling reaction to the synthesis of long-chain saturated acids (49), Linstead and his colleagues demonstrated its applicability to the synthesis of both ethylenic (50) and acetylenic (51) fatty acids. Apart from the simplicity of the main reaction, there is the outstanding advantage that no geometrical isomerization occurs. The unsaturation is already present in one or other of the chains being coupled. Thus erucic and selacholeic (nervonic) acids were synthesized from oleic acid (50) without producing any *trans* isomers. Synthesis of an acetylenic acid, of course, also permits ready production of an all *cis* ethylenic acid by selective partial hydrogenation, as in Raphael and Sondheimer's synthesis of linoleic acid. Stearolic (51) and tariric (52) acids were synthesized anodically and reduced to oleic and petroselinic acids, respectively. The method has obvious application to the proof of a postulated structure. Thus anodic syntheses of both isomers of octadec-11-enoic acid and comparison with the data of other workers afforded evidence that vaccenic acid is a mixture with *trans*-octadec-11-enoic acid as a major component (53). Similarly anodic production of *threo*-11,12-dihydroxy-eicosanoic acid from the corresponding dihydroxy-stearic acid permitted comparison with the two products of stereospecific oxidations of natural eicos-11-enoic acid. This afforded the first proof of the previous assumption that this acid, like oleic, has the *cis* configuration (54).

TABLE I

Some Key Dates in the History of Oils and Fats Research in Great Britain Since 1909

1912–13	First ester distillations
1923	First x-ray work of fatty acids and derivatives
1925	First publication on ester distillation
1926	Hilditch school founded at Liverpool
1934	Application of x-ray studies to triglycerides
1950	Synthesis of linoleic acid
1952	Introduction of gas-liquid chromatography
1953	Application of I.R. spectroscopy to unsaturated fatty acids
1953	Anodic synthesis extended to unsaturated acids
1956	Application of I.R. spectroscopy to glyceride structure
1957	Total syntheses of elaeostearic and punicic acids

Other British work during the last decade has resulted in the successive syntheses of various natural fatty acids. In 1952 came the first syntheses of tariric and petroselinic acids (55), two years before their anodic preparation. In 1953 isanic acid was first synthesized (56) while in 1954 we had first syntheses of DL-ricinoleic acid, independently from two laboratories (57, 58), and of ximenynic acid (59). The year 1956 was notable for the first synthesis of the solid form of hexabromostearic acid, in effect the synthesis of all *cis* linolenic acid (60). In 1957 the total synthesis of ximenynic acid was achieved (61) (the first synthesis in 1954 having begun from ricinstearolic acid), also the total syntheses of α- and β-elaeostearic acids and punicic acid (62). I have already mentioned that this determined for the first time that punicic acid has the *cis-trans-cis* structure. The latest contribution is the synthesis of both *cis* and *trans* forms of 8-hydroxy-ximenynic acid, confirming the probable *trans* structure of the natural compound (63).

Conclusion

At the beginning of our period of review the chemistry of the natural fats was not only a virtually unexplored field, but it was one that did not attract many research chemists. The same could be said, and was in fact said (64), some 15 years later. The position reached today is so totally different that it must be hard for younger chemists to appreciate the magnitude of the change, which virtually began after the first World War. Apart from the interruption of the second World War, British contributions to the advancing tide of knowledge of fat chemistry have been continuous and have included many of great significance, as I have tried to show by some selected items in Table I. The present symposium affords a picture of how the British work fits into the world-wide pattern, which for most purposes can best be contemplated as an interwoven whole.

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Significant Advances in Fat and Oil Chemistry in France During the Past Fifty Years[1]

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DURING the past 50 years or more, specifically since 1908, significant progress has been made in the chemistry of fats and oils in France in both research and industry. In 1908 the extent of chemical research was extremely modest; a couple of professors studied fats from time to time and very few industries even had a control laboratory at their disposal; those which existed were devoted solely to the commercial control of products. At that time plants simply consisted of oil mills and soap factories that operated mainly on a small scale. Today there are several important governmental research laboratories, and many industrial companies have research as well as control departments. Plants are definitely more important and operate by scientific methods. New industries have been created: cake treatment, detergent industry, lipochemistry, etc.

Progress was slow but became very significant after the second world war. To show the tremendous growth during the last 50 years it would be interesting to quote some statistics. Unfortunately they are quite difficult to compute for the first 25 years since most of the oil and soap plants were small enterprises, which generally utilized local raw products and sold their merchandise in their own area. It is helpful to consider the case of peanut oil in this connection.

Today the most important edible oil in France is imported peanut oil; in 1957 it represented 70% of the fluid edible oils. (The remainder consisted of olive oil, 8%, rape seed oil and others, 22%.) Our principal source of peanuts is Senegal, and we are practically their only customer. Therefore Figure 1, which gives the global exportations of Senegal peanuts, reckoned with hulls, is particularly significant. As can be noted, the production has increased regularly except for the periods of the two world wars when exportations decreased significantly; in 1932 the depression is also very obvious.

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[1] The original paper contained numerous photographs and charts which it is not possible to reproduce. Edited by Waldo C. Ault.