brought about by changes in the amount and composition of dietary fats, have been studied by the groups of J. F. Brock (23) and A. Antonis (24). Recent studies have been concerned with the effects of dietary fats upon serum cholesteryl esters, triglycerides, and phospholipides and their component fatty acids, also upon fecal lipides and on bile acid secretion. These studies suggest that the serum cholesterol lowering effect, brought about by unsaturated fatty acid ingestion, may be caused by increased catabolism of sterols. Work on experimental atherosclerosis in rabbits led to an *in vitro* study of the effects of heparin and calcium ions on lipemic sera.

Lung lipides have been isolated from healthy and silicotic guinea pigs since it has been claimed that the increased lung lipides, noted in this disease, are themselves a factor in promoting fibrosis of the lung. The carcinogenicity of the nonsaponifiables from human cancerous and noncancerous livers has been studied. Autoxidation of such extracts occurs rapidly, using the aerobic conditions of isolation applied by previous workers, and this factor may play a role in the contradictory results recorded in the literature.

The first paper of a series entitled "Studies on Fat Metabolism in Kwashiorkor" has recently appeared; it deals with total serum cholesterol. The enlarged fatty liver, which is a characteristic of this disease, had previously been extensively studied by medical research workers in this country. Some interesting work on the changes induced in pooled human sera by treatment with ether has been done by H. B. W. Grieg. Following such treatment, the bulk of the cholesterol is found in the globulins precipitated by 40% saturation with ammonium sulphate whereas, in untreated serum, the bulk of the cholesterol is found in the proteins not precipitated. It is thought that the protein affected by ether treatment is an α_1 globulin. A number of related studies of general interest to biochemists are being currently pursued.

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Advances in Fat Research in Spain During the Last Fifty Years

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IT IS AN HONOR to be invited to describe the results of fat research in Spain, and I am very pleased that my country should be represented in this symposium. I also want to congratulate my American colleagues on having founded, back at the beginning of this century, a specialized scientific society whose work has made valuable contributions to the progress of science and fat technology.

Some general preliminary remarks are in order to interpret correctly Spanish achievements in the field of fat research.

In the first place, the Spanish fat production and processing industry consists of a large number of small factories with small technical staffs and without research laboratories of their own. At present, most of our industrial laboratories limit their work to the analytical control of raw materials and finished products and do not undertake any work of research or development.

As a result, during the period 1908–1941 research on fats may be considered the work of a small number of individuals interested in these problems, working either in private laboratories or at university centers. Only from 1941 on, there began to appear some research centers independent of the university as a result of the foundation of the Superior Council of Scientific Research and other institutions, such as the Oceanographic Institute, the National Council of Agronomic Research, including its provincial experimental farms, and so on. Finally in 1947 the Applied Research Department of the above-mentioned Superior Council of Scientific Research created the Institute of Fats and Their Derivatives with the collaboration of the private fat industry. This center has given impetus to research in the field of oils and fats, and we shall discuss it later on.

The second factor to be considered is the particular nature of the Spanish fat industry, which is mainly directed to the processing of one staple product: olive oil. Spain's annual production of olive oil averages about 350,000 metric tons and represents more than one-third of the world production. Other vegetable oils, such as cottonseed, flaxseed, and castor oils, are produced only on a small scale, and the production

of animal fats, waxes, etc., is also very limited. To talk of fats in Spain nearly always means to talk of olive oil.

Olive oil is, as is generally known, one of the few fats which can be used as food without first being refined. In addition, it is extracted from a fruit, and the various methods of extraction differ completely from those applied to obtain other oils or fats. Thus the Spanish fat industry has not been able to profit much from technical advances in other countries, and conversely our own studies are little known abroad and have contributed only in a small degree to the solution of problems concerning fats that have arisen in all other countries.

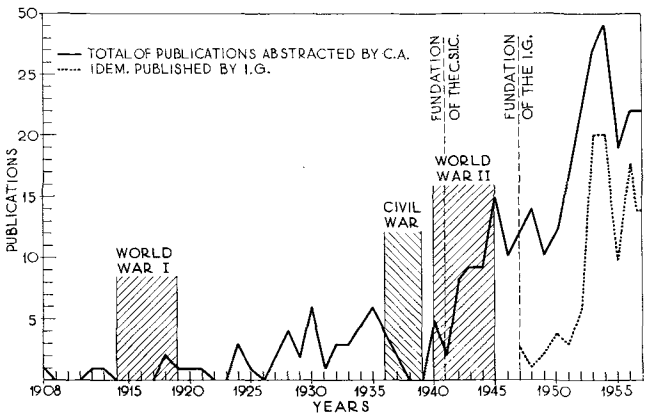

FIG. 1. Publication comparisons.

The Spanish Contribution as Seen in Chemical Abstracts

All this is shown (Figure 1) by plotting the number of extracts from Spanish reviews published by the well-known American periodical, Chemical Abstracts, since 1908. Up to 1943 the number of publications, though increasing slightly, remains very low. Then the effects of the establishment of the Superior Council of Scientific Research began to be noticed by a sudden increase in the number of publications. Since 1950 this number has increased once again, remarkably, as a result of the creation of the Institute of Fats, which is responsible for more than 60% of the publications. The curve shows a decrease for the last two years, but this fact has no real meaning because a fair number of recent publications have not yet been summarized.

The total number of publications during the past 50 years can be estimated at 900 exclusive of patents, which are very numerous in this branch of technology. Our card index records about 1,500 of these.

Contribution to General Chemistry and Fat Analysis

Obviously we cannot make a thorough examination of all these 900 publications. A person with only 15 years' experience in fat research, envisaging facts from a modern view-point, might well neglect some works which in their time showed new ideas and directions and then were superseded as more recent developments within the field were made, developments however which would not have taken place without the help of those prior ideas. Scientific and technical research is now so organized that any new, promising field is promptly invaded by countless research workers so that it is often assigned not to the true pioneer but to the one who has the best equipment at his disposal.

Chemical Composition of Fats and Related Products

An interesting series of papers on the enzymes of some oil-bearing seeds was published in the years 1917 and 1918 by Obdulio Fernandez of the Faculty of Pharmacy of Madrid (1). He and Dr. Mingo studied orange seed oil (2). In a further series Dr. Mingo made a study of custard apple oil (3) and olive kernel oil (4). Papers on other Spanish oil-bearing seeds have been published by Abad and Luna and on esparto wax by Soler and Guzman (5). Within this particular field special mention should be made of the studies of I. Ribas on rye ergot oil (6) and on cork fatty acids (7).

Oils of a number of species of fish have been studied by several Spanish investigators, among whom Santos Ruiz, Montequi, and Otero Aeñlle are prominent (8, 9, 10).

The unsaponifiable matter of various kinds of fish oils and of some vegetable oils like soybeans, cottonseed, and olives has been investigated by Santos Ruiz, Gracian, and Cruz Auñon (11, 12).

Physico-Chemical Studies

Lucio Lascaray is numbered among the Spanish investigators best known abroad for his studies of fats. He has published the results of some of his research in American reviews such as Industrial and Engineering Chemistry (13) and Journal of the American Oil Chemists' Society (14) and has published many papers in the German reviews Seifensieden Zeitung (15) and Fette und Seifen between 1924 and 1952. One of the principal subjects dealt with by this author has been fat hydrolysis, which he has proved to be a reaction taking place homogeneously in the fatty phase. For this reason the reaction velocity is largely influenced by the solubility of water in that phase and can be increased by the application of high pressure or the addition of Twitchell reagents, which are hydratable, ionizable, fat-soluble substances giving up their hydration water and enhancing their activity with the growing degree of hydration. According to Lascaray, the valency of their metallic ions also influences the activity of Twitchell reagents.

Other work in which Lascaray pioneered was the study of the surface of tension of soap solutions. He proved the anomalous variation of surface tension with the concentration of sodium salts of saturated fatty acids and oleic acid (16). Further studies of other investigators have confirmed that this curve is characteristic of the behavior of anionic detergents.

Many other contributions of less importance are the result of Lascaray's work. For instance, he recently developed a sensitive, practical test for the detection of nickel traces in hydrogenated fats, employing dimethyl-glyoxime dissolved in stearic acid; the reaction thus takes place in the fatty phase (17).

The polymerizing effect of electric discharges on some oils was studied by Vian, who gave the process the name of "voltolisis" (18).

Fat Fractionation and Analysis

A series of papers on fractionation with selective solvents was published by Rius *et al.* They deal with the phase diagrams of various components of fats and such solvents as alcohols, furfural, and hydro-alcoholic mixtures (19).

The urea-adducts fractionation technique and the formation of urea adducts with fatty esters have been described by this writer and collaborators in a series of papers which were recently reviewed in a plenary session held at the second Congress of the International Society for Fatscience (20). One of the important features of this study was the formation of urea adducts with mono- and diglycerides and the proof of the anomalous composition of the latter, which is incompatible with the structure commonly assigned to urea-inclusion compounds determined by applying x-ray techniques.

Molecular distillation techniques for the fractionation of fats have been developed in Spain by the technical staff of the Institute Torres Quevedo, using its patents either on a laboratory, semi-industrial, or industrial scale. This investigation was patterned very closely after that of Hickman and co-workers in the U.S.A. Other investigators of the Institute have been working on directed fat *trans*-esterification, combining this technique with molecular distillation.

Numerous Spanish investigators have worked on the standardization and improvement of fat analysis techniques. Among them Medinaveita, Casares, Giral, Father Vitoria, Santos Ruiz, Otero Aeñlle, Montequi, Mongrane, Gracian, R. de la Borbolla, Vioque, and Ranedo are prominent. Also an interesting study on the determination of diene number with p-benzoquinone by Lora Tamayo (21) should be mentioned. Some of these authors have already been cited on account of their research on the composition of some natural fats.

Studies on Olive Oil and Its Technology

Spain's olive oil production dates back to prehistory, and the first writings on olive growing and olive oil extraction appeared in Roman times. Numerous treatises on olive oil dating from the 17th, 18th, and 19th centuries can be found in Spanish libraries. These works, though of small interest in the light of modern knowledge, show our ancestors' ingenuity and capacity for observation which enabled them, with rudimentary means, to state problems and to propose solutions, many of which are still in use.

An instance of the latter is a recommendation found in very old works, according to which the addition of olive leaves to the olives during the milling process preserves the flavor and other organoleptic properties of olive oil. Tests carried out long ago in our Institute showed that the stability of olive oil determined by the AOM method was greater in oils of the same origin if the olives had been milled together with olive leaves. That motivated an investigation in the Institute that recently led to the isolation of a phenolic compound in the olive leaf, the antioxidant power of which is several times greater than that of commercial antioxidants of widespread use, such as N.D.G.A., butylated hydroxyanisole, and alkylgallates, etc.

Spanish papers on olive oil and its technology, especially those prior to the Spanish Civil War, are generally little known abroad because they have been published in various agricultural and commercial reviews of limited circulation.

The number of patented inventions relating to olive oil during the 50 years under consideration is very high, about 1,000. These are largely the fruits of empirical work so that many repetitions will be found and really original ideas are few in number.

An important discovery was made by Acapulco in 1911 (22). This engineer discovered that, when a ground olive paste was brought into contact with a wire screen or netting fine enough to prevent pulp, broken pieces of kernel, and other solid particles present in the paste from passing through, the oil which the pulp contained flowed through whereas the vegetation water, the percentage of which is more than double that of the oil, was retained.

The great advantage of this method lies in the fact that the oil is obtained at low temperature and that no pressure is needed. The application of this method therefore improves the quality and organoleptic properties of the oil. These are of fundamental importance in an edible oil like olive oil, which is directly marketed without further treatment. There are extraction plants of this type in nearly all oil-producing countries, such as Spain, Italy, France, Tunis, and Argentina.

The principles of the Acapulco method and its modifications have been recently studied in the Spanish Institute of Fats. It essentially consists in selective filtration, in which the oil phase passes through the filtering substance without the water phase. The phenomenon is usually explained by assuming that the oil wets the metallic surface and passes through the narrow slits while the water phase is retained in the form of drops like the rain on a waterproof cloth because of its higher interfacial tension with the metal. The studies carried out in our Institute have shown that this interpretation is not correct.

Determination of interfacial tension shows that the cellular juice of olives wets the metallic blades (whatever their nature or shape may be) better than oils do. From this point of view the behavior of oil-vegetation-water systems is quite different from that of pure water and even of various solutions containing organic acids, detergents, etc. It has not been possible to reproduce such surface properties in artificially prepared solutions.

The cellular juice forms a continuous phase in the ground olive paste while the dispersed phase, the oil, is found to a great extent in the form of microscopic globules protected by a membrane of complex lipoproteid structure. They thus become hydrophilic on their surface. The phenomenon must be attributed to the retention of the water phase by the solids coming from the fruit, which are most hydrophilic.

The cellular juice is therefore absorbed by the solids and allows the oil to flow through the slits. The paste surface in contact with the metal of the blades leaves an oil film of practically constant thickness, which is drawn off by the movement of the blades. At the beginning of the extraction practically all the metallic surface is covered by this oily film, and the output of the machine attains its maximum. As the extraction proceeds, the oil content of the paste decreases and so does the fraction of metallic surface brought into contact with the oil and covered with the oil film. On the basis of these facts a mathematic, quan-

titative treatment of the extraction can be made. This phenomenon of selective filtration upon which the Acapulco method is based, is, as a rule, little known outside the olive oil industry and oil-producing countries. I have therefore included it in my report since it is possible that this selective filtration will one day find application in various problems that arise in fields other than that of fats.

Other technical advances achieved by the olive oil industry in Spain during the past 50 years have been made by such prominent investigators as Solis, the inventor of a method for oil extraction by capillarity; Soroa, who studied the effect of pectolitic enzymes in olive oil extraction and was the author of an interesting treatise on this industry; Sagrera and Segura, authors of a continuous oil-extraction method which has attained a certain popularity in the industry.

The Institute of Fats in Seville

The Higher Scientific Research Council of Spain was created in 1941 and is divided into several departments for research into the various branches of knowledge: history, philosophy, jurisprudence, natural science, etc. The department entrusted with the organization of applied research was called Foundation Juan de la Cierva in memory of our illustrious countryman, the inventor of the autogyro, the predecessor of modern helicopters.

This Foundation of Juan de la Cierva, besides coordinating and helping economically the centers of applied research already existing, has created a number of new institutes and research departments in cooperation with the principal branches of industry. There are, among others, the Institute of Coal in Oviedo, of Liquid Fuels in Zaragoza, of Iron and Steel in Madrid, of Building, also in Madrid, of Plant Chemistry in Valencia, etc. The aim was to decentralize and to locate these institutes in the areas of greatest development in the respective industries.

In this way Seville, the center of Spanish production of olive oil and pickled green olives, was the place chosen for the establishment of the Institute of Fats, which started its activities in 1947. Like the others, this institute was given the task of co-ordinating fat research activities, training specialists in this field, and developing research on problems of the greatest interest for the nation. The Spanish industry of fats and pickled green olives was doubtless in great need of the creation of a center of this kind. The commercial value of Spanish fat production may be estimated as amounting, on the average, to 10,000 million pesetas (or about $200,000,000). There was not, before the creation of the Institute, any actual scheme of research and only a limited number of individuals or research teams at universities and technical schools. Contact is maintained with the industries through a number of industrialists, members of the Institute, who form an Assembly, which meets from time to time to discuss matters of current interest. The scientific and administrative activities of the Institute are controlled by a Technical Council, of which outstanding personalities of the fat industry are members.

Every two months the Institute publishes a technical review, Grasas y Aceites, which has attained a large circulation in a short time. It has subscribers in 28 countries and exchanges information with more than 100 reviews dealing with fats, among them, of course, the Journal of the American Oil Chemists' Society.

About 30% of the Institute's activities consists in giving technical advice to industries, making analyses, giving training courses, editing the review, and administering the Institute itself. In the actual research the greatest attention is given, as might be expected, to matters related to olive oil production.

A comprehensive scheme is now being carried out to study the basic physico-chemical principles of the most commonly used extraction methods and to develop new and more simplified techniques that may lead to the design of continuous extraction plants. The study of ground olive pastes has led so far to some interesting conclusions, from which substantial improvements of extraction methods can be expected in the future. It has been found, for instance, that some types of detergents, among which the alkylarylsulfonates can be counted, facilitate the oil separation and break up the oil emulsions produced within the vegetation water. This property is now being investigated systematically in a large number of detergents to determine which show the highest effectiveness and at the same time are not poisonous and do not impair the quality of the oil. A long time will elapse before we can profit from such discoveries, but there is no doubt that they will lead to the development of new industrial techniques that will not in the least affect the quality of the residues and in any case could be applied to the extraction of low-grade oils that must necessarily be subjected to refining.

Another important branch of the Institute's research has been the systematic study of the characteristics of the composition and quality of olive oils coming from different parts of Spain. From this work, if maintained for a sufficient number of years, it is hoped to obtain statistics to show the correlation between the quality of the olive oils and the nature of the climate and soil, of the maturing, the presence or absence of trace elements, etc.

Other work undertaken by the Institute has already been mentioned, for example, the study of the principles of the Acapulco method, the isolation of antioxidants in olive leaves, and research on urea adducts with fatty substances (23).

Finally a field of work, not immediately related to fats but based on the same raw material as olive oil, which in Spain (also, I believe, in America) has great economic importance, is the pickling of green olives for the table. The first scientific work on this process, which has been used in Spain for many years, was made by Professor Cruess of California, who showed plainly the fundamental stages in the process of pickling green olives called "Sevillian-style pickling." We have made in our Fat Institute many studies of this process, which have led to scientific control of the process, thereby considerably reducing the losses.

These researches were directed by Dr. Borbolla, the subdirector of the Institute. A compilation of the work carried out by Borbolla and his collaborators in the pickling of green olives was recently awarded an important prize for research work in Spain (24).

Figure 2 shows a general view of the Institute.

Spanish Bibliography on Fats

We have only quoted those works most characteristic. As to the books published by Spanish authors

FIG. 2. A general view of the Institute of Fats, Seville.

in this period, we shall draw particular attention to the two of Mangrane's, "Analytical and Physiological Chemistry of Oils and Fats" and "Recent Advances in the Chemistry of Oils, Fats, and Their Industrial Derivatives," the first of which has an edition in French; to the book of Santos Ruiz on biochemistry of the lipides; to that of Colom y Blasi on "Industries Derived from Fats," and to that of Soroa on oil technology.

Two specialist reviews on fats are published in Spain, both bimonthly, Grasas y Aceites already mentioned, which is published by our Institute, and the Bulletin of International Olive Culture, published by the Olive Syndicate.

Conclusion

This summary is necessarily a subjective apraisal, and there may be errors or omissions, for which we apologize.

The major point is that Spanish research work in the field of fats (and the same applies to many other fields) has always relied on outstanding figures, who possessed original ideas and developed them within possibilities, which were not indeed always very great. The shortage of material means and the isolation of the few existing research workers made our contribution necessarily small, compared with the work of the rest of the world, which brought about scientific knowledge on fats that we now possess. The almost exclusive concentration on problems connected with olive oil has also greatly limited our scope and also limited the knowledge of Spanish research work abroad.

For some years Spain has been launched on a program of complete renewal, both in this field and in other fields of scientific research, and is at present making a great effort to co-ordinate and increase the material and human resources available for the study of fats.

Too little time has passed for the results of this effort to be evaluated, except in a few isolated cases, but we are working enthusiastically. And if we are able in 25 years to attend the Diamond Jubilee of the illustrious American Oil Chemists' Society, we hope we shall be able to report a greater amount of work and more results of real scientific importance than those to which I have drawn your attention.

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