

# FISHERIES RESEARCH BOARD OF CANADA

## MANUSCRIPT REPORT SERIES (TECHNOLOGICAL)

No. 60

### TITLE

Status of Marine Oil Research at the Technological  
Station, Halifax, N.S., as of January 1960, with  
recommendations for its continuance

### AUTHORSHIP

Dr. F. A. Vandenneuvel  
Technological Station, Halifax, 1947-1960  
(Slightly abridged by N. M. Carter from a special  
report to the Chairman prepared by Dr. Vandenneuvel  
on his resignation from the Board's staff, January 1960)

### Establishment

Headquarters  
Ottawa

Dated August 1960

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## FOREWORD

This report on the status of my work on marine oils at the time of my resignation from the Board, and my recommendation of the direction this work should take in the future, comprises two parts.

Part I (pages 1-13) reviews the work accomplished since 1947. Its purpose is to describe the sequence of investigations up to the present, indicating their relation to the programme. In discussing the methods of approach it shows them to be entirely justified in the light of present knowledge on the subject. Past publications are cited and shown to derive logically from the problems under investigation. The nature and extent of unpublished data are given and conditions under which they would become publishable are indicated.

Part II (pages 14-27) enlarges on the problems currently under investigation. The background and past history of these problems are discussed in detail and recommendations as to their future development are given.

Completion of projects A, B, and C by full attainment of their objectives will mean the start of the productive phase of the work insofar as Marine Oils are concerned. From then on the work would consist in applying the methods established to the study of the marine oils proper. The course of this work is thus very clear.

It will be noted that no treatment of the work concerning practical applications is given in Part I, since the background and purpose of this work have already been described elsewhere (Can. Chem. Proc., 41, 40, 1957). This subject is treated in Part II under Project D.

In this report particular attention has been given to include details which could be useful to the future of the work.

Among the facts given which are considered particularly useful are some which may appear to constitute a criticism of facilities for prosecution of the research programme. Their purpose however is to make easier the future of this work by showing the consequences certain policies had on its course. My comments are intended to help rather than criticize.

F. A. Vandenheuvel, Ph.D.

March 1960.

PART I - REVIEW OF FUNDAMENTAL WORK (1947 - 1960)

The fundamental programme of research which I was requested to outline at the onset of my work with the Fisheries Research Board of Canada (Nov. 1947) was designed to provide much-needed information on oils of marine origin. From the point of view of the Fisheries, such information was ultimately to serve as a basis from which to develop practical applications. From the point of view of lipid chemistry, it would provide invaluable help, in fields such as physiology, nutrition, and medicine, by throwing light on the properties of unsaturated fatty acids, components of the marine oils which are also characteristic of lipids occupying key positions in important biological functions, such as, for instance, the phospholipids and lipoproteins.

The outline of the programme read as follows:

- Project A - Isolation of all constituent fatty acids of the marine oils in the original structural state and in a degree of purity compatible with the unequivocal attainment of the following objectives.
- Project B - Systematic study of the physical, chemical and structural properties of the isolated constituent fatty acids.
- Project C - Establishment of methods of analysis designed to give a complete, detailed, and accurate composition of the marine oils.

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PROJECT A - ISOLATION

In the overall programme, Project A, dealing with isolation, is by far the most difficult to achieve. Detailed examination of the methods used in attempts of this sort by laboratories in various countries reveals that they consist of complicated combinations involving many different techniques, such as soap fractionation, solvent crystallization, distillation, chromatography, and others. All this effort generally yields a few grams or milligrams of material of doubtful purity. This is a situation quite openly admitted by the most distinguished researchers in the field (18). Furthermore, while the considerable loss of material during these complicated processes leaves no clue as to the relative abundance of the isolated constituent in the original oil, there is in most cases no certainty as to the material isolated being truly representative of an original structure. The most recent findings in this field confirm my own early opinion that this state of affairs is due almost entirely to the complexity, inefficiency, and aggressiveness of the methods of isolation used.

From the onset of my work with the Board, I had based a method of approach to this problem on observations made in the course of my earlier work (London University thesis, 1937) which are partially described in an early publication (2).

Consider the diagram (Fig. 1) where the fatty acid constituents of typical marine oils are represented by squares of an area proportional to their relative abundance in this oil. In this diagram, the acids are arranged in columns, representing the carbon series corresponding to the number of carbon atoms at the head of the column. In each column, they are placed in sequence of increasing unsaturation. Thus, the first row in this diagram represents the saturated acids, the second row the monoethenoid acids, and so on to the last row, which includes the acids having six double bonds.

This seems the maximum allowed by nature, a fact also predictable, to a certain extent, from structural consideration (2).

A combination of two physical properties, namely, the vapour pressure and the solubility, should, when applied to the methyl esters of these acids, be all that would be required in a simple, two-step operation, to bring about their total resolution. This is based on the observation, that a difference exists in the vapour pressures of esters belonging to the various carbon series. Since very little difference in volatility exists among the members of the same series, the whole mixture could be separated into eight fractions, each representative of a distinct carbon series, provided a suitable means of fractional distillation existed.

In the next step, each of these fractions could be resolved by using some method based on the differences in solubility which are known to exist between the members of the series.

This simple scheme has formed the basis of my method of approach to the problem.

Fractional distillation. Admittedly, the realization of this scheme depends upon securing two indispensable tools. The first is a method of fractional distillation not only efficient enough to ensure the separation of the carbon series, but also one presenting a low degree of "heat hazard". The facility displayed by the highly unsaturated esters for undergoing structural changes under the influence of heat excludes the most refined variants of the conventional-type still, even when operated at the lowest vacua. There was a time when great hopes were entertained concerning a spinning-band variant of the Podbielniak still operated at 1-micron pressure. The role of the latter piece of equipment, insofar as marine oils are concerned, was eventually reduced to the rectification of concentrate obtained through other means, by

# FATTY ACID COMPOSITION OF HERRING OIL

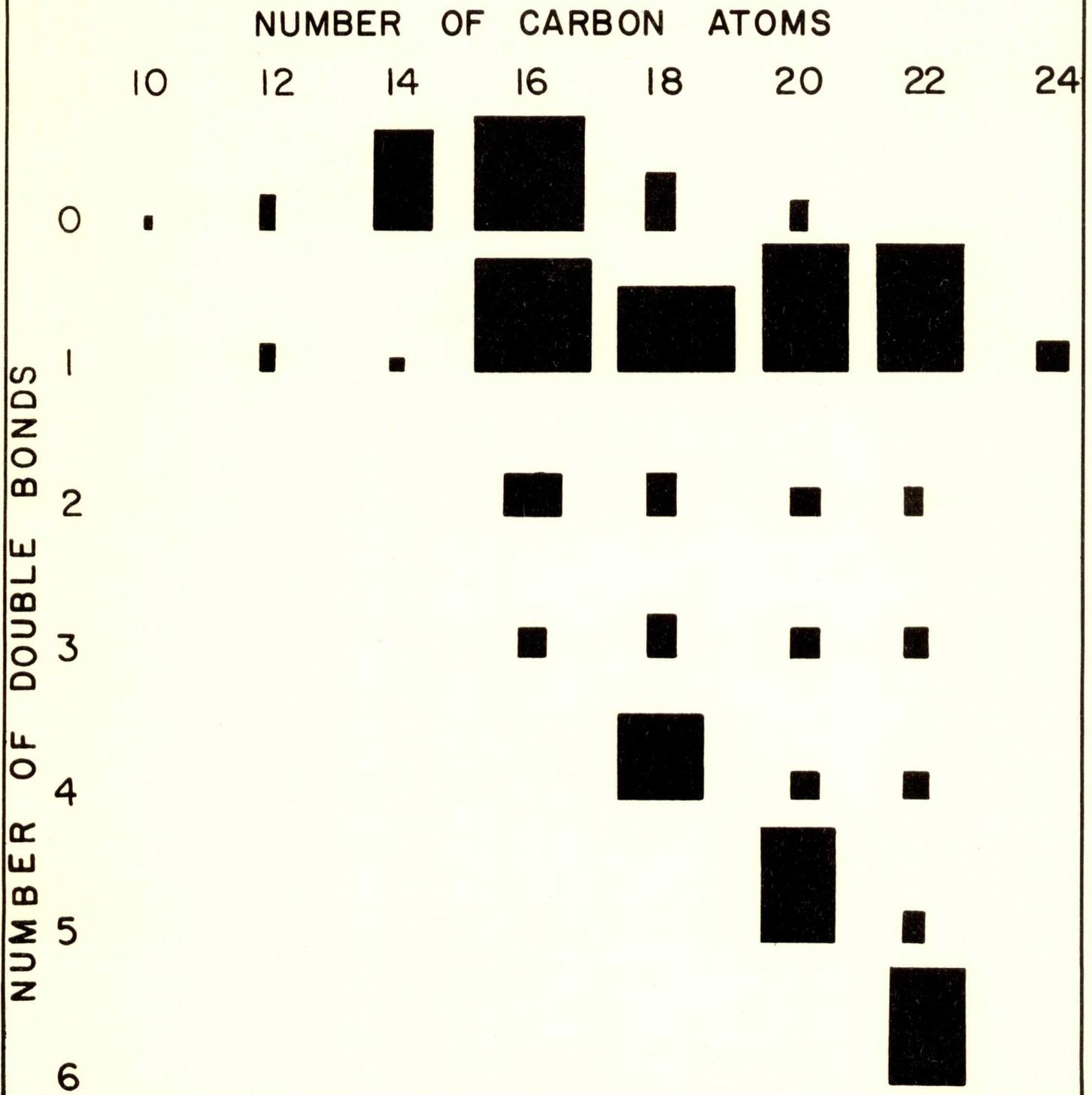


Figure 1.

those who claimed that due to the relatively lower operating temperature, this method could be used safely for such a purpose. Recent evidence, given by Privett (19), shows clearly that even in such limited application the method is unsafe and leads to appreciable amounts of cyclic monomers contaminating the distillate.

Clearly, the situation existing at the onset of the present work has remained essentially the same in regard to the use of conventional-type vacuum fractional distillation for the purpose at hand. If anything, an early conviction that such means is unsuitable has been fully confirmed.

This justifies efforts made from the onset of this work to find a solution in molecular distillation. This procedure offers far more promise in this direction, as evidenced by the findings in my early work (2) as well as that of others. The fact that continuous thin-film molecular distillation affords a means of fractional distillation with a "heat hazard" at least 1,000,000 times smaller (20) than in the most refined variant of conventional vacuum distillation, is enough to justify a thorough investigation of its possibilities in effecting the separation of the fragile highly unsaturated fatty esters.

Before concluding with a brief outline of the work carried on in this direction, I must add that I have kept an open mind to all other possible means of achieving the desired separation. Recently I had hoped that an extrapolation of the current gas--liquid chromatography apparatus might offer a simpler, yet adequate solution. An apparatus, based on this principle, was constructed at the Station and found successful in effecting the separation. However, it must be operated under such conditions as to preclude any possibility of the structural integrity of unsaturated fatty esters being preserved. The operational conditions indicate a "heat hazard" over 1,000 times that of the best type of conventional vacuum still available. Hopes continue to be entertained for effecting certain modifications to bring the "heat hazard" down to the desired level of safety, but such hopes are admittedly very slim.

There is no indication, therefore, that efforts to develop a still based on molecular distillation should be discontinued. The problem in developing a still of this sort can be described as follows: Single-stage molecular distillation, as it is commonly known, is only somewhat more efficient than single-stage ordinary distillation as exemplified by the simple retort still. In view of the difficult problem of separation presented by the carbon series, a molecular still comprising several stages is needed. An early estimation of the number of stages required to obtain a 98% purity in a single pass showed it to be 10, assuming a fairly high reflux ratio, and assuming an overall efficiency not lower than that of a single-stage apparatus.

The construction of a 10-stage molecular still was decided upon quite early (1948). I finished drawing the first plans for this apparatus in the spring of 1949 and construction started in the summer of the same year. For reasons given in a separate account the still could only be tried for the first time in late 1957. Trials ended in 1958. They revealed structural defects tending to decrease the efficiency to an undesirable level. It was then decided to redesign the still completely in order to take into account all developments in high-vacuum technique which had taken place since 1948 and, of course, to remedy the defects which had been observed. Although I completed the new plans a few months later, lack of proper machine shop equipment prevented the construction of the new still until November 1959. It has been progressing ever since.

The design and construction of an instrument of this sort involves the following: very strict tolerance in mechanical parts; complex heat-exchange situation; electrical, electronic, fluid circulation, and control problems, as well as a severe restriction in suitable materials. Increasing the difficulties is, of course, the necessity of co-ordinating all solutions with high-vacuum requirements as well as in relation to each other.

Of all the difficulties encountered, however, by far the greatest has been the inadequacy of facilities available at the Station in meeting the demands imposed by the work itself.

Our work on the fundamental mechanism of molecular distillation resulted in a theory based upon the performance data of a single-stage apparatus. This, in turn, provided a basis from which the performance of a multi-stage apparatus could be predicted and the conditions for an efficient operation could be determined. The greater part of this work is described in a thesis "A New Approach to Fractionating Molecular Distillation" by R. G. Ackman (now with this Station), Dalhousie University, 1952. The conclusions arrived at in this work still await the complement of performance data from the multi-stage still to form a subject worthy of publication.

#### Fractionation of the carbon series

The other necessary condition for the complete resolution of the marine oil esters is the use of a method, based on solubility differences, capable of separating all members of the same carbon series.

Reversed-phase partition chromatography was found to be most satisfactory when opposed to other methods based on solubility differences such as countercurrent liquid fractionation, and solvent fractional crystallization.

Early exploratory experiments made in 1952 had shown that the chromatography of esters, as opposed to that of acids, suffered from a considerable disadvantage in that no direct continuous detection of extremely small amounts of esters, such as occur in a chromatographic eluate, was available. The only means of establishing a chromatogram entailed the classical procedure of evaporating small fractions to dryness and weighing the residues. This most inconvenient and time-consuming procedure is rendered even more complicated by the necessity of protecting all fractions, at all times, from atmospheric oxidation. Such inconvenience rendered obvious the necessity for a direct continuous method of recording eluate composition.

Continuous-recording differential refractometry appeared to offer the answer. Its application to the problem at hand required an apparatus considerably more sensitive, yet as stable as that of Zaukelies and Frost (21), and required additional features not found in this early apparatus. After some time, I finally managed to have my drawings for a suitable instrument accepted by the Physics Department workshop at Dalhousie University, from which it finally materialized in 1955. After a period of tests and modifications, started in 1956, and lasting over 2 years, the apparatus eventually functioned perfectly and has been used ever since the spring of 1958. A paper describing this apparatus has been submitted for publication (17). The refractometer permitted the establishment of the required procedure on model synthetic mixtures of esters, mainly of the C<sub>18</sub> series. Further evidence acquired about the relative solubilities in other series has indicated that the procedure is applicable to all series without modification except for the separation of the least-unsaturated members of the C<sub>20</sub> and C<sub>22</sub> series. Our present work is planned for securing all data capable of helping in a projected extrapolation designed for the treatment of gram quantities of material. A paper on the total separation and estimation of methyl esters by reverse-phase chromatography is in preparation.

The construction and operation of the 10-stage molecular still is thus the only serious outstanding problem delaying the completion of Project A.

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## PROJECT B

The systematic studies of properties require, aside from the completion of Project A, a thorough knowledge of the methods and techniques to be used in such studies. Acquiring this knowledge constituted the purpose of our work for several years. The methods were investigated using as models fatty esters obtained from lipid sources other than marine oils. The preparation of these esters in the purest possible form was in itself a time-consuming and often-repeated task.

The chemical properties investigated were of two kinds. Among those involving reactions of the double bonds, catalytic hydrogenation and alkali isomerization were most extensively studied from the point of view of kinetics. From this work, what constituted data and conclusions of general interest was published (7, 9, 10, 12, 13, 14). In these publications theories concerning the hydrogenation of mono- and poly-unsaturated esters, cis--trans isomerization, and the selectivity were proposed.

Part of the data also formed material for three M.Sc. theses:

- "Selective Hydrogenation" by J. D. Nickerson, Dalhousie University, 1950
- "The Hydrogenation of Fatty Acids and Esters" by A. B. Cahn, Acadia University, 1951.
- "The Alkali Isomerization of Linoleic and Linolenic Acids", by G. H. Richardson, Dalhousie University, 1952.

Chemical properties related to the carboxyl group in fatty acids and derivatives were studied extensively in a 5-year period of work on the synthesis of long-chain di-ethenoic acids. The purpose of this study was to find a procedure for the total synthesis of these acids that would allow C<sub>14</sub> labelling in any position of the molecule where it might be required, and particularly labelling of the carbon atoms at the level of the double bonds. Such labelled acids would help in solving problems regarding the reactions of the double bond.

In the course of this work, data of general interest were recorded. That part of the data concerned with the early, exploratory phase of the work is described in a M.Sc. thesis "Contribution to the synthesis of Unsaturated Fatty Acids" by Frederick B. Fisher, Acadia University, 1953. In one case, additional work has made such data complete and worthy of publication (4). To study such synthesis procedures systematically would require considerable time. The work on synthesis itself, in spite of promising results (22) had to be shelved temporarily in order to pursue the more pressing problem of carbon-series resolution. Consequently, this work ceased when the recording differential refractometer became available (1955).

Work was also carried out in order to establish better techniques for the structural determination of the unsaturated acids. This determination supposes that a procedure for oxidative degradation, yielding fission products truly representative of the structure studied, be available. Establishing such a method, on the other hand, requires an excellent method of analysis of fission products, for example, mono- and di-carboxylic acids. Our first effort therefore was to establish a method of analysis for these acids. The difficulty in this case is due to the wide range of acids to be expected from marine oil acid oxidation (up to C<sub>12</sub>). The method developed was published (8).

It was applied immediately to the study of a number of variants of the permanganate method of oxidative degradation,

using pure unsaturated acids or esters as models. This investigation revealed a gross inadequacy of such methods. Appreciable amounts of abnormal fission products which could be very misleading in establishing an unknown constitution were found to occur. This fact has now been recognized by all scientists in the field.

At about this time, the advent of gas--liquid chromatography, a more expedient and practical method of analyzing fission products, decided the delaying of further investigation on degradation methods until this new technique had become available to us. As soon as this occurred, these studies were resumed, and we undertook a study of ozonolysis. Again the standard and generally recognized ozonolysis procedures were found to be inadequate for the same reason as described above for the permanganate methods, and our conclusions matched those of other workers on the subject, some of whom have come to reject ozonolysis altogether (23). Work on improving the ozonolysis procedure was undertaken. Our findings indicated that one particular modification of the method offers a considerable improvement over any other studied. Further work on poly-unsaturated acids and esters is still needed to firmly establish its general application. If this proves to be true, the guesswork and uncertainties in establishing the constitution of marine oil acids will disappear. Gas--liquid chromatography has proved an invaluable tool in this study and has come to be regarded as the most convenient method of analysis of the fission products. We must also conclude from this study of oxidative degradation methods that a considerable amount of published work on the constitution of marine oil unsaturated acids is of very questionable value.

To conclude the present review of the work carried out in preparation of the studies implied by Project B, our work on the methods of physical constants determination should be mentioned briefly. Observations were made concerning an interesting relationship between the refractive index and the degree of unsaturation of pure, unaltered natural acids and esters, which is of value in detecting heat-altered products (5). The accurate, unequivocal determination of refractive indices of unsaturated fatty esters is dependent upon the amount of gas a specimen contains, as indicated by our unpublished work on the solubility of gases in fatty acids and their derivatives.

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#### PROJECT C - ANALYTICAL INVESTIGATIONS

Investigation of analytical methods were of two kinds - those concerning the marine oils as a whole, and those concerning a detailed analysis of these oils and that of the constituent fatty acids themselves.

Analytical methods of general application to fats and oils were screened by assaying their particular merits in marine oil analysis. In this way, those which were most effective in our particular work could be selected. This investigation has

been re-opened from time to time to verify the claims of newer methods published in the field and so to keep up to date our stock of analytical tools. Those involve the determination of iodine value, hydrogenation value, saponification number, acid number, average molecular weight, acetyl value, free glycerol, mono- and di-glyceride content, and so on, among the chemical constants; and colour, viscosity, specific gravity, refractive index, and so on, among the physical constants.

Our own contribution in this field concerns the determination of hydrogen values (7).  
concerned

Analytical methods with detailed composition of marine oils have formed the object of a continuous series of investigations. Among these, ultraviolet absorption before and after alkali isomerization received a good deal of attention. A new procedure evolved in our laboratory, published in 1953 (9) has been used to examine a considerable number of oils and oil fractions. The data obtained, which have guided us in many problems in marine oil utilization projects, are not at present completely decipherable in terms of composition. This must await application of the same technique to pure samples of the marine oil constituent acids, following which application the method can be standardized for the examination of whole oils or complex mixtures of fatty acids, a work similar to one already described for oils and fats of marine origin or origin other than marine.

We found evidence, in examining by this method herring oil samples throughout the biological cycle, of the possible use for this method in identifying oils through characteristic features of their isomerization absorption spectra. This development must also await standardization through examination of pure constituents in order to become effective.

A method was developed to accurately determine the saturated and unsaturated moieties in each carbon series of the marine oils. This method involved the partition chromatography of the marine oil acids, first after their complete hydrogenation, and second after elimination of the unsaturated constituents. Based on our published method for the total resolution of saturated fatty acids from C<sub>10</sub> to C<sub>24</sub> (11) this method was used extensively to determine the composition of several marine oils.

Exploratory work on paper chromatography of fatty esters and acids revealed the serious limitations of this method. Less accurate than partition chromatography, it required a preliminary separation of the carbon series for which there is, even now, no method available.

Gas-liquid chromatography was found by far the most precise and practical method. Ever since an apparatus was acquired (about 3 years ago) considerable effort has been made in order to keep abreast of all technical improvements, of interest to our work, which have taken place in the fastest development of a technique that science has ever known. Considerable work

was involved in standardizing our instrument for ever more promising types of column packing evolved in specialized laboratories. It appears, however, that insofar as our problem is concerned the ultimate has been reached in column efficiency for the separation of unsaturated esters. Here the limitations which have manifested themselves concern an overlapping of bands corresponding to esters belonging to consecutive carbon series, seriously affecting the estimation of constituents and limiting the usefulness of chromatograms obtained from whole mixtures. The method would, of course, become excellent, provided a preliminary separation of the carbon series be effected. Here again is evidenced the need for a method capable of achieving this result without altering the structure of the unsaturated acids, and consequently presents further argument in favour of the multi-stage molecular still.

To conclude I will mention a paper recently accepted for publication by "Analytical Chemistry", concerning anomalies observed in the gas-liquid chromatography of the lower di-acids, mainly malonic acid, on polyester columns (16). The facts observed are important in interpreting results from oxidative degradation of marine oil and other natural unsaturated acids, most of which do contain the methylene-interrupted type of unsaturation leading to the formation of malonic acid upon ozonolysis.

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## CONCLUSIONS

It is hoped that the above review of the work accomplished has shown the following facts.

- A - Isolation of the marine oil constituent fatty acids, under Project A of this programme, depends on the availability of the multi-stage molecular still. Construction of the second and final model of the still has made good progress since November 1959. The other necessary condition for this isolation has been fulfilled with the chromatographic separation of ester mixtures homogenous as to the carbon series.
- B - Extensive work in preparation of a systematic study of marine oil fatty acid properties, under Project B, has been accomplished. Undertaking of this systematic study requires completion of Project A.
- C - Several methods of analysis applicable to the marine oils have been evolved. The complete and accurate analysis of all marine oil constituent acids is, like the isolation problem, dependent upon the realization of two conditions. The first, the separation of the carbon series, also requires the use of the multi-stage molecular still. The second, the resolution of ester mixtures homogenous as to the carbon series, has been achieved in two different ways:
  - (a) by gas-liquid chromatography on poly-ester columns, and
  - (b) by partition chromatography.

- D - The publications resulting from the above work could hardly be expected to directly concern the marine oils proper, since productive work on these oils must necessarily await completion of Project A. Many of these publications reflect, however, the nature of the work carried out so far. It is a work of preparation, building of tools, and elaboration of methods in view of the productive work ahead. In recent years, two members of our team have concentrated their efforts on a type of work which is not in itself conducive to the production of new contributions, such as, for example, the nevertheless important work of acquiring the techniques of gas--liquid chromatography. Research on degradative oxidation methods and on partition chromatography of the carbon series has mobilized the efforts of two more staff. These projects have almost reached the stage of completion and publication.
- E - The considerable amount of data, still unpublished, falls into four categories:
- (a) data to be completed by the use of the multi-stage molecular still;
  - (b) data to be interpreted by further studies (example: alkali isomerization);
  - (c) data which could be completed by additional work but are not absolutely necessary to the project (example: synthesis work);
  - (d) data from studies about to be completed.
- F - The molecular still appears even more justifiable as the key to the whole programme.

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PART II: CURRENT PROBLEMS UNDER INVESTIGATION

In this section of the report, Projects A, B and C correspond to those defined in Part I (page 1).

The chart shown in Fig. 2 was issued for the benefit of the members of the Eastern Advisory Committee, Fall meeting 1959. It describes the position of each member of the staff in the Station's Organic Chemistry Department, as it stood from 1958, in relation to the problems treated in the following report.

Figure 2.

EASTERN ADVISORY COMMITTEE MEETING - HALIFAX

October 7-8, 1959

REPORT FROM ORGANIC CHEMISTRY DEPARTMENT

By F.A. Vandenneuvel

A. BASIC STUDIES

i) Isolation of component fatty acids  
analysis

Step 1. - Multi-stage molecular still

Step 2. - Reversed-phase chromatography

ii) Structural determination

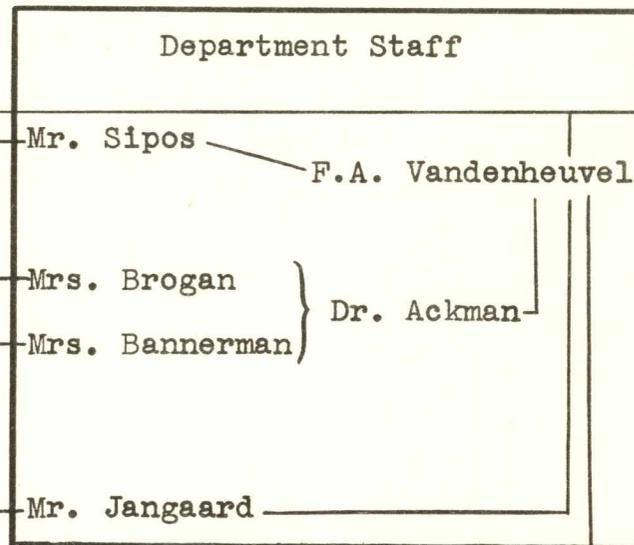
Step 1. - Ozonolysis

Step 2. - Vapor-phase chromatography  
of fragments

B. UTILIZATION OF MARINE OILS

Methanolysis - kinetics, pilot plant

C. MISCELLANEOUS BY-PRODUCTS



Mr. Edmonds  
(Mechanic)

Dr. Guttman

Mr. Power

PROJECT A

Objective - The separation of carbon series.

Problem I - Design, construction and operation of a multi-stage molecular still.

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Part I of the present report has shown the considerable importance of this problem in relation to the whole programme. Without a multi-stage molecular still there is very little hope of achieving the separation of the carbon series and, therefore, the systematic isolation of marine oils constituents in the original state.

Again, if the latter goal is not attained, the projected studies of properties described under Project B cannot take place. Furthermore, unless a new, up-to-date gas chromatography apparatus is purchased, the complete analysis of marine oils will also depend on the separation of the carbon series, i.e., the performance of a multi-stage molecular still. Hopes to achieve this separation without structural alteration of the highly unsaturated esters by using a preparative scale gas chromatography separator are very slim (see Part II, Project A, Problem II).

Efforts in designing, constructing and operating a 10-stage molecular still are therefore completely justified.

When in 1949 the question arose of where and how the multi-stage molecular still would be constructed, my suggestion to have the instrument developed jointly by the Board and by the Eastman Kodak Research Laboratories, then the only organization specialized in high vacuum technique, was discussed. Copies of the correspondence on this subject (January--February 1949) between Dr. S.A. Beatty and Dr. G.B. Reed, Chairman, show clearly that the Board wished to assume the responsibility for carrying out the project without involving outsiders. Assurance was given that all possible effort would be made to cope with the difficulties I had pointed out. My objection that the Station machine shop equipment would be unable to deal adequately with the problem was answered by giving the assurance that the neighbouring research establishments would provide all the facilities required. This was found to be true to a very limited extent only, and soon the Station was faced with the problem of constructing the still by its own devices. At this stage (1950) the solution would have been to equip the Station with the adequate machine shop it sorely needed. Efforts made in this direction were largely limited to the purchase of a new lathe.

The conditions described above largely explain the considerable time elapsed before the first assembling of the parts could be attempted (1956).

It was not until 1958 that the first actual test of the still could be made. This is explained by the incredibly long time required to assemble the still head and obtain high-vacuum tightness. The least modification to a part located inside the condenser would entail up to a 2-month delay before a new test could be made.

Early in 1950 the design of the condenser had been recommended as the best substitute for my initial one. The latter, consisting of a simple all-welded jacket conforming to high-vacuum engineering practice at the time, could not be executed at the Station for lack of specialized welding skill and equipment.

Here was the first instance, of which there have been many since, of the Station limitations in machine shop facilities influencing the course of a project by forcing adoption of a compromise solution resulting in much waste of time, money and energy.

In 1957, before actual try-outs of the first model ever started, the impracticability of the still-head condenser design had been amply demonstrated. At this point it was decided to complete the tests in order to learn as much as possible from the first model before designing a new still head. Having given much thought to this new design before the tests were over, I had come to the conclusion that the most desirable arrangements would be obtained through a particular combination of features. These included parts presenting a number of plane surfaces. A milling machine was necessary in order to obtain the degree of precision required for these parts.

At this time serious efforts were made to obtain a milling machine. Mr. Power had already investigated the possibility of acquiring such a tool which he considered, from the point of view of the engineering department alone, a very desirable complement of the machine shop equipment. Quotations were obtained from various firms and recommendations were made to include in the next budget the cheapest machine available. Including all accessories, the price was about \$2,000.00. This recommendation was not followed. The milling machine never made the budget nor that of any of the succeeding years. As a compromise, a milling attachment for the existing lathe, supposed to be adequate within a very restricted range of part dimensions, was purchased.

This is why in 1958, the try-out period being over, I started actively designing the new still-head taking into account these limitations. Much of my efforts was to no avail however, when it was discovered that even within the restricted range imposed by the milling attachment, the required tolerances could not be met.

This was early in 1959. A large section of the new plans had to be remade. By this time, tired of compromising, I redesigned the parts with only one thought in mind: to ensure proper functioning of the still. Only by the use of a milling

machine could they be made. The perennial round of begging for help in the neighbouring machine shops ended with the following result: the work as a whole could not be undertaken by any; only a working model of one of the parts would be made, as a personal favour, at the Physics Department of Dalhousie University. It was evident, however, that the problem of constructing the rest of the parts could only be solved by the purchase of a milling machine. Eventually the machine arrived in November, 1959.

The construction of the multi-stage molecular still has been progressing satisfactorily every since.

As soon as the working model of the trough was obtained from Dalhousie (April 1959), it was assembled with other parts (rotor, condenser, and a section of the manifold) to constitute the first working model of the basic stage. Ten of these elements will eventually have to be constructed for the still, along with a full-scale manifold. At this time, none of the accessory parts had been constructed. These consist of two types. The first type comprises all accessory parts proper to the basic element, including: (i) the residue scraper, (ii) the temperature-control sensing element, (iii) the liquid distribution arrangement, (iv) the teflon dead-angle shield, (v) the drive, and (vi) the variable-speed arrangement.

The other type comprises all accessories serving a function common to all elements, including (a) and (b) the two end plates of the glass envelope, (c) the glass envelope itself, and (d) the cradle to support it. End plate (a) is fitted with two vacuum-tight multipole electrical outlets (e) a compact high-vacuum rotary gland of 11 elements (f), and a vacuum-tight inlet for the cooling water (g). The other end plate is fitted with a magnetic coil (h), to actuate the reflux dividing arrangement (k). Completing this series of accessories is an external magnetic arrangement (l), operating the liquid distribution system.

A study of accessory parts was undertaken as soon as the first working model of the basic stage could be assembled and could be connected to a variable-speed drive arrangement.

A special study of accessory parts was necessary to ensure the most efficient design before series construction started. After each of these had been tested and suitably modified for completely satisfactory performance, the final plans for the still-head could be drawn.

Among accessory parts, those expected to require considerable investigations were studied first. Thus an excellent working model of the residue scraper (i), one of the temperature-control sensing element (ii), and one of the individual high-vacuum glands for accessory (f), were produced. A satisfactory solution for the variable-speed arrangement (vi) was also found.

At the time of my resignation, study of the liquid distribution arrangement was nearly completed. Among the remaining

accessories, some would not require considerable study and the rest could be obtained by adapting commercial parts to the purpose.

Teflon shield (v), and end plate (a), among the parts still to be studied, may require particularly careful designing.

All plans for parts which had operated successfully were given to Dr. Ackman before my departure. Those parts which had not been completely studied and for which no final plan existed were given to him with instructions concerning the completion of their study. A second copy of plans is in the hands of Mr. R. Edmonds, mechanic.

It is obviously impossible for me to discuss in this report my plans concerning parts which are incomplete or parts not yet studied since in either case the discussion would involve the dimensions of parts which are not in my possession. The remaining work is by far the least critical insofar as the still performance is concerned, and will not require extensive investigation and testing.

The designing of the still-head has been my sole responsibility from the beginning. Dr. Ackman's part in the work concerned with the first model has been mostly that of helping me, from 1956 to 1958, in testing parts and in trying out the still; a much smaller proportion of the work carried out on the second model has involved his participation. This is illustrated by the organizational chart (Fig. 2) indicating the connection of each member of the staff in the Organic Chemistry Department to some specific project under study, as it stood in 1958.

Although Dr. Ackman was kept regularly informed of current progress in the development of the new design and its construction, through my own experience and familiarity with the subject I had visualized certain details and developments which were still insufficiently concrete to formulate without further experimentation by myself. It is therefore very difficult either for me or for him to form an honest opinion as to what further difficulties may be encountered by him in this all-important project.

## PROJECT A

Objective - Separation of the carbon series

Problem II - A preparative-scale gas--liquid chromatography separator

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Felton and Buehler (Anal. Chem., 30: 1163) have recently described the use of inexpensive model-airplane glow plugs as detectors for high temperature gas--liquid chromatography. They also described an electrical circuit of the Wheatstone bridge type for connection to a standard recorder. The simplicity of the proposed system induced us to use it as one of the features in a simple preparative-scale gas--liquid chromatography separator with the hope that this apparatus would provide a substitute for multi-stage distillation in the separation of the carbon series. The general lay-out

of this apparatus is described in Fig. 3 which indicates, without regard to actual dimensions, the main components of the apparatus. Note that the glow plugs, shown as R on this diagram, are connected to the circuit described by Felton and Buehler, a 10-mV full-scale Brown recorder being used to register its output.

At the time I left the laboratory, the following had been achieved.

The equipment had been tested with quantities of mixtures or single compounds, varying between a fraction of a gram and 5 grams. Most of the compounds tried were methyl esters of saturated and unsaturated fatty acids varying from C<sub>2</sub> to C<sub>20</sub>. By adjusting column length and temperature, clean separations were obtained throughout this range. The purity of the compounds separated was checked.

As expected, columns of silicone-coated Chromosorb were more efficient than silicone-coated firebrick. Thus 18 feet of 3/4-inch Chromosorb column was able to separate C<sub>10</sub>, C<sub>12</sub>, C<sub>14</sub>, C<sub>16</sub>, C<sub>20</sub> at 325°C., the rate of flow being about 1 to 1.5 litres per minute. The retention time for C<sub>18</sub> was about an hour under these conditions.

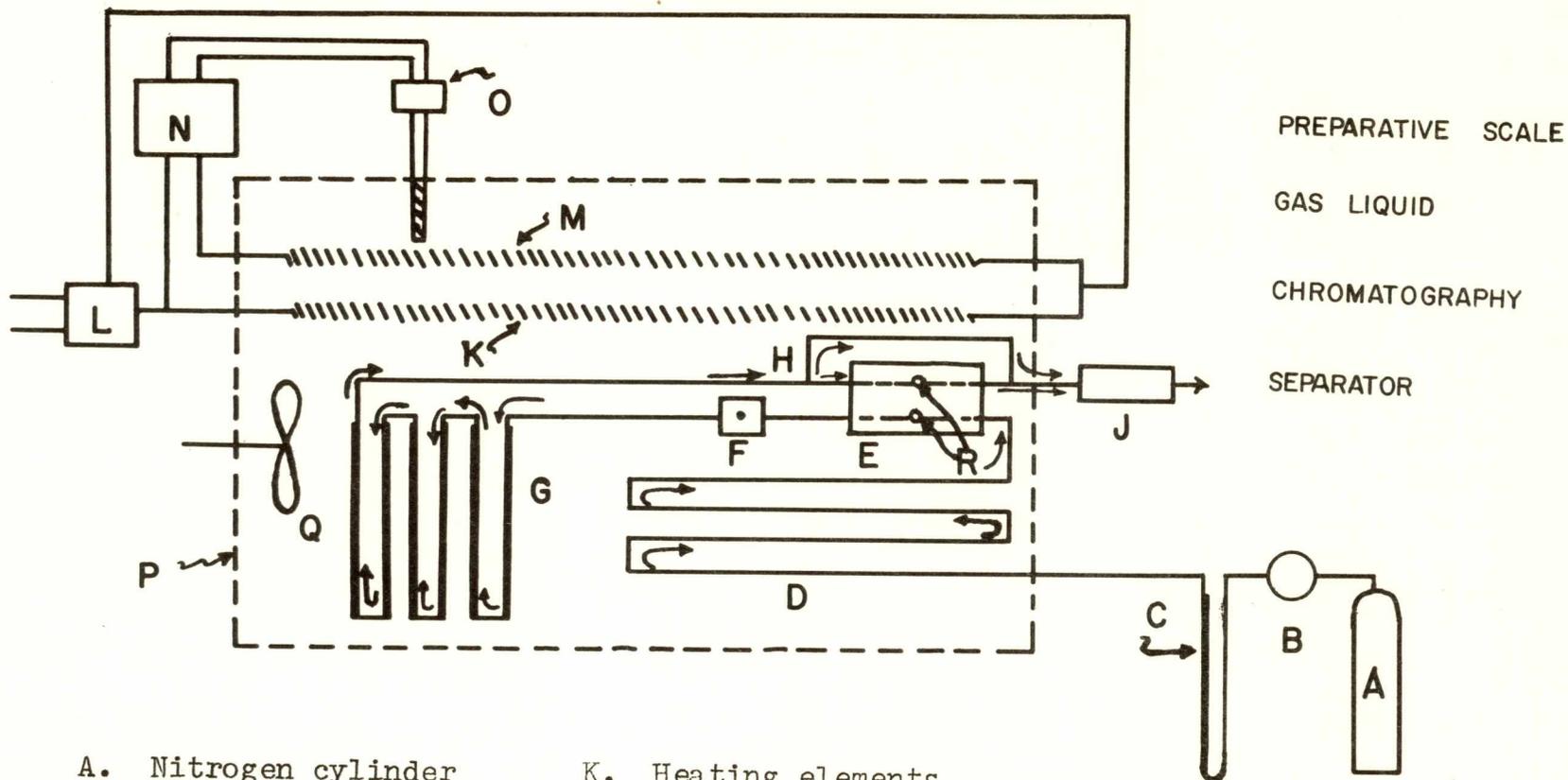
It was noted that the system became unstable beyond a certain flow rate and that a by-pass had to be used to reduce the gas velocity through the detector block on the outlet side. In any case, there seems to be considerable overloading, especially when gram quantities of test material are used. The result is a disproportionation of peak size, the recorded peak for major components being smaller than would be expected. This is not a disadvantage if the equipment is to be used as a fractionator. Correct analytical data can be obtained on the micro-scale apparatus.

As a fractionator in the range 0 to 5 grams, the instrument is definitely superior to the Podbielniak-type laboratory stills. It has, for example, an efficiency of 250 theoretical plates for methyl oleate, which is much better than stills will provide, especially under vacuum. Furthermore, the time required for a separation is incomparably shorter. A forthcoming paper will describe these advantages in detail.

For the purpose of separating carbon series from marine oil esters, however, the above statement must be qualified as follows.

Conditions such as are required at present (C<sub>18</sub> - 1 hour at 325°C.) for the separation of these series are far too drastic. It must be remembered that the conditions used by Privett et al. (J. Am. Oil Chemists' Soc., 36: 443, 1959) in distilling methyl arachidonate were such as to promote the formation of appreciable amounts of cyclic isomers. Although these conditions are not known with considerable accuracy, I learned from a private communication that this distillation took about 12 hours, the still boiler temperature being 180°C. Assuming the usual velocity--temperature relationship of most reactions to hold, the heat hazard would be multiplied by two for each 10-degree rise in

Figure 3.



- |                          |                               |
|--------------------------|-------------------------------|
| A. Nitrogen cylinder     | K. Heating elements           |
| B. Valves and gauges     | L. Powerstat                  |
| C. Flowmeter             | M. Regulating heater          |
| D. Preheating tube       | N. Electronic relay           |
| E. Detector block        | O. Bimetallic thermoregulator |
| F. Injection arrangement | P. Insulated oven             |
| G. Column system         | Q. Fan                        |
| H. By-pass               | R. Glow plugs                 |
| J. Receiver              |                               |

R.L.M.

temperature. A difference of  $145^{\circ}$  ( $325^{\circ} - 180^{\circ}$ ) means a  $2^{14.5}$ -time increase in the velocity of reactions leading to isomeric forms. On the other hand, Privett's distillation was of 12-hour duration as against 1 hour in our apparatus. This means that the chances of forming isomers are  $2^{14.5}/12$  or about two thousand times greater in the preparative-scale apparatus. To reduce the heat hazard to the point where it would compare with that existing in the column-type still used by Privett would mean operating at about  $200^{\circ}\text{C}$ . with a retention time of one hour. These conditions would also be expected to promote the formation of cyclic isomers. It is therefore quite possible that a reasonable degree of safety would only exist at a lower temperature, say  $160^{\circ}\text{C}$ . (1 hour).

The conclusion obtained through the above reasoning should only be taken as an indication of the present conditions being far too drastic for safe handling of highly unsaturated methyl esters, and that every effort should be made to obtain the lowest possible heat hazard.

In any event, the distillation products would have to be checked for possible alteration. It must be remembered that a low level of heat-induced conjugation does not necessarily rule out cyclic or other heat-induced isomers. The efficiency of urea fractionation in removing cyclic isomers would have to be assessed quantitatively.

I believe that a direct demonstration would require submitting to the conditions prevailing in the separation, preferably by passage through the apparatus, of a sample of pure, highly unsaturated fatty ester. Methyl linolenate, the only readily available ester of this class, is probably much more stable than esters with five or six double bonds which are marine oil components. A demonstration using methyl arachidonate would seem desirable.

The most conclusive demonstration, however, would involve highly unsaturated esters from marine oils obtained through multi-stage molecular distillation.

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#### PROJECT A

Objective - Resolution of the carbon series

Problem III - To secure a partition chromatography method of resolution

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As indicated in the 1958 Confidential Annual Report of this Station (Appendix 31) the separation of methyl esters of the same chain length but differing in the degree of unsaturation had been achieved to a point satisfactory for preparative purposes. This constituted the attainment of the first objective in this

study. As indicated in the same report, work was underway to improve the separation. By October 1959, as indicated in my report to the Canadian Committee on Fats and Oils, complete separation had been obtained. It seemed that we could go ahead with the final objective of this investigation, i.e., the construction and operation of a larger column to deal with gram quantities of material.

Unfortunately, from November on, the results leading to a complete separation could no longer be duplicated although seemingly the same standardized procedure was applied. This state of affairs is not uncommonly met in research of this kind. It arises wherever the results depend on a complex combination of factors some of which are decisive to a degree of which the observer is unaware. A thorough analysis of all possible factors involved is then made and the factor or factors responsible for the effect under study must be investigated one at a time. At this point, it is very important to avoid any prejudice concerning the relative importance of these factors.

Such an investigation can be long and tedious. It is entirely justified, however, on the following two counts:

(1) A thorough knowledge of the factors involved and their relative importance can be obtained by this study.

(2) Control of these factors may lead to consistent satisfactory performance. Even better results may be expected.

In the period from November 1959 to January 1960, it had been established that the properties of the columns varied from one preparation to another although the column material was obtained through a seemingly standard procedure. Variations in properties observed with the same preparation were of lesser extent.

Preparation of the column material involves admixture of 6% water by weight to perfectly dry, 100- to 200-mesh crushed firebrick. The moistened material is then suspended in dry iso-octane and an excess of dimethyldichlorosilane is slowly added under continuous stirring. Stirring is continued for 30 minutes after which the product is transferred to a Büchner funnel where it is washed repeatedly with 80 to 82% aqueous ethanol until acid-free. After a final washing with 95% ethanol it is air dried slowly at a temperature below 50°C., then at room temperature in a vacuum desiccator.

This preparation is very similar to that described by Vandenheuvel and Vatcher (Anal. Chem., 28: 838, 1956) for the preparation of silane-treated silicic acid. The same apparatus is used.

After a cursory investigation of the factors (involving the quality of the reagents, time of addition, and various other ones) had failed to give any particular clue to the solution of

this problem, a series of tests was made before undertaking a systematic examination of all factors.

The tests consisted in effecting some drastic change to the components of the basic preparation described above, with the hope that a change in the final properties, clearly indicative of the importance of the modified component, would be obtained.

Thus, successively, firebrick was replaced with Chromosorb; dimethyldichlorosilane by other silane derivatives (monomethyl, trimethyl, diphenyl), and in the preparation of the column iso-octane was replaced by various solvents, including the 2,2,5 isomer of octane. The use of silicone grease in substitution for the silane--water treatment had been planned before I left. These experiments revealed no advantage in any of the substitutions except, possibly, from the 2,2,5-trimethylpentane. The use of silicone grease, at present under investigation, shows some promise. In any case, the results obtained during October 1959 could not be equalled.

This points out the necessity of a systematic investigation centered on the basic preparation being made, without any change in the nature of the reagents. A similar investigation of the column preparation and loading should be made. Further study of the properties of material coated with silicone grease should also be continued if control of the conditions leading to a satisfactory separation with the silane-treated material is found to be impractical.

It would seem premature to discuss the characteristics of the projected larger column since these will largely depend on the findings of the above investigation.

I must point out that the procedure without any modification is very satisfactory for the separation of saturated methyl esters.

This work is therefore entirely suitable for publication on that basis. One, possibly two papers on this subject are now in preparation.

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## PROJECT B

Objective - Unequivocal determination of constituent fatty acid constitution

Problem - To secure an adequate method of oxidative degradation

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The problem defined as above has been under study for some time (see Part I, page 7). Its outcome is of the highest importance for future studies. Due mostly to inadequacy of existing methods, most of the published conclusions regarding polyunsaturated fatty acid constitution are suspect on the following counts:

(i) The oxidative degradation method used is known to yield large amounts of abnormal fission products.

(ii) The products (mono- and di-carboxylic acids) have been "worked up" prior to analysis by procedures that might have altered the relative amounts present initially, possibly to the point where one or more abnormal products have become predominant.

(iii) Relative yields of the products either are not given, or, on account of (ii) above, they no longer represent the composition prior to "working up".

(iv) The analytical method used lacked sensitivity, thus giving a much "cleaner" picture of the product composition than actually exists.

(v) The sample studied was of doubtful purity, or had been obtained through methods of separation known, or suspected, to induce isomerization.

The unequivocal determination of constitution depends therefore on securing a procedure comprising:

(a) A method of oxidative degradation leading to the least possible amount of abnormal products.

(b) A procedure for the "working up" of the products which entails the least possible losses thus leading to the highest possible yields.

(c) An analytical procedure of the highest sensitivity and precision.

Application to pure oleic acid of a procedure derived from that proposed by P. S. Bailey (J. Org. Chem., 22: 1548, 1957; Ind. Eng. Chem., 50: 993, 1958) for the preparation of adipic acid from cyclohexene by ozonolysis in methanol, has resulted in remarkably high yields in both total and normal oxidation products (total yield, 97%; yield in normal products, >95%) when the ozonide was reacted with performic acid. Working up of the products involves separation of monocarboxylic acids from dicarboxylic acids by steam distillation, a procedure we adopted earlier in the same type of work (see Part I, page 7). This was followed by conversion of both fractions to methyl esters prior to analysis by vapour-phase chromatography.

The remarkably low incidence of abnormal products must be explained by the protective action of methanol involved as a combination with the ozonide polymer, at the time the latter is made to react with performic acid.

Encouraging as it is, the procedure must still be shown to be applicable with equal or reasonably good success to poly-unsaturated acids or esters, since these are of the type involved in our studies, and since an increase in abnormal fission incidence is known to occur by other methods when the number of

double bonds increases. Such work was in progress when I left.

It was planned to study successively, the ozonolysis of linoleic, linolenic, and arachidonic acid and their methyl esters. Pure arachidonic acid was recently prepared by Privett at the Hormel Institute who, I believe, would have granted my request for a sample of this acid.

In the meantime it may be advisable to write a short paper on the results obtained in applying this procedure to oleic acid. These in themselves constitute a remarkable progress as regards the oxidative degradation of monoethenoid fatty acids.

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### PROJECT C

Objective - Detailed analysis of marine oils

Problem - Overcoming limitations of the gas--liquid chromatography analyzer

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The gas-chromatography analyzer in use at the Station is out of date. Still very useful for many purposes, it will not deal adequately with mixtures of methyl esters belonging to two or more adjacent series. Overlapping of bands from neighbouring series prevents a complete analysis to be made. This can be overcome by a preliminary separation of the carbon series. It is doubtful that this could be accomplished adequately by the use of the preparative-scale separator (see Project A, II), and a complete analysis may have therefore to await performance of the molecular still.

The recommended solution is the immediate purchase of a new gas--liquid chromatography analyzer (about \$4,000) whereby complete analyses of marine oils could be obtained within a few months.

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### PROJECT D - HERRING OIL UTILIZATION

The purpose of the work on herring oil utilization has been made abundantly clear in past Annual Reports of this Station and one publication. (Annual Report for 1955 Appendix 33; for 1956 Appendix 34; for 1957 Appendix 15; for 1958 Appendix 37; see also "Methyl Esters from Marine Oils" by F. A. Vandenheuvel and P. M. Jangaard, Can. Chem. Proc., 41: 40, 1957). Fractionation of the methyl esters derived from this oil will lead to a diversity of products having specified properties, in contrast with the oil itself, which, on account of a lack of specific properties, is regarded as a low grade, cheap substitute for other oils. Existing problems in marketing the oil should therefore be eliminated.

Understandably, the value of the process will largely depend on its economics. It has been our objective to decrease as much as possible the cost of production of the final methyl ester fractions.

Adaptation of the process to continuous operation would result not only in a very compact and cheap installation, but also in a lowering of labour costs. Two phases of the process, namely, counter-current fractionation and final distillation, do of course lend themselves very easily to continuous operation. The initial methanolysis phase, on the other hand, required an original study in order to bring it to conform to the same pattern. It has been the object of our work for the last 3 years.

Our efforts in this respect have been completely rewarded, having resulted in the design and successful operation of a continuous reactor of remarkable capacity.

The next step is a complete integration of the three phases in one uninterrupted line of production. To obtain this objective is highly desirable for several reasons, the two following ones being particularly important.

The first is that no adequate estimation of production costs can be made without a thorough knowledge of the system functioning as one unit.

The second is that utilization of the final methyl ester fractions must be demonstrated using products obtained from an installation functioning as a complete unit. Prospective users of the process definitely insist on this point.

I may add that presentation of an honestly established production cost estimate is one of the most important points in selling a process to potential users. It requires a thorough knowledge of the process and its resources. Charlatanism in this respect is most easily detectable by technical experts and is particularly apt to arouse suspicion and so compromise the future of a process.

Among the data to be secured for this purpose are those concerned with the by-products. The recoveries of glycerol and cholesterol, which are obviously very important in this particular process, have required studies for their own sake.

That concerning cholesterol has not been completed. Since it is dependent upon the distillation phase of the process, completion was being delayed until the whole installation could be operated as a unit. A larger amount of raw material for the cholesterol study would then be available. Our work therefore has been concentrated on glycerol recovery, with particular reference to yield and quality. One important goal has been reached in this respect by the establishment of a procedure for eliminating methyl esters and fatty acid contamination of the resulting glycerol. Contamination of this kind so far had con-

stituted a strong argument against methanolysis (as opposed to fat-splitting), in that it prevented the production of a C.P. (chemically pure) grade product.

A complete account of the work on continuous methanolysis and glycerol recovery will be given in two forthcoming articles.