Milne

CONFIDENTIAL

FISHERIES RESEARCH BOARD OF CANADA

ANNUAL REPORT

for

1952

of the

PACIFIC FISHERIES EXPERIMENTAL STATION

NEAL M. CARTER, Director

VANCOUVER, B.C. December, 1952

FISHERIES RESEARCH BOARD OF CANADA

PACIFIC FISHERIES EXPERIMENTAL STATION
VANCOUVER, B.C.

REPORT FOR 1952

Neal M. Carter, Director

This Report covers the period from November 1, 1951, to October 31, 1952.

BUILDING AND EQUIPMENT

With the impression that the present building may have to be used for a while longer than was earlier anticipated, some shortcomings that might otherwise be tolerated were adjusted as far as limitations of structure, space and funds permitted. Many of the changes were necessary to improve working facilities of the present expanded staff and to accommodate already authorized further additions to the staff.

Dr. Tarr was provided with somewhat more space than formerly by assigning to him the late Dr. Bailey's office and laboratory. Fluorescent lighting and a large built-in constant-temperature cabinet unit were installed during the change-over. The laboratory and office vacated by Dr. Tarr were assigned to the new biochemist, Dr. MacLeod, and the work tables were covered with linoleum edged with metal trim. Lenoleum was also laid on the badly worn floors of these two laboratories and the hallways of this second floor of the building. Increased electrical power requirements in the building necessitated the installation of a 400-ampere single-phase service and wiring for a 100-ampere subfeeder to the top floor. The exterior of the building was sand-blasted to remove grime on the brick front and old paint on the other surfaces, then given a waterproofing treatment and two coats of paint (except on the brickwork). A plate glass front window that had been cracked since the Station occupied the building was replaced and an inner layer of pebbled glass was installed to above pedestrian eye level across the front windows to replace the faded green paint originally applied.

Much of the interior alteration work and maintenance was ably performed by Mr. Freeman, Maintenance Supervisor, who also constructed many of the cabinets, pieces of apparatus, accessories to purchased equipment, etc. needed during the year. Welding and other new machine-shop equipment was acquired for his use. Appearance and cleanliness of the building has been well kept up by Mr. Enright, Janitor, and a part time assistant.

Acquisition of equipment during the period under review included:

(a) One Brown Electronic 12-point strip chart recorder for refrigeration and other engineering experiments.

(b) One Equipoise Drafter and additional Leroy templates for mechanical drawing.

(c) Parts and accessories for a refrigerated display cabinet constructed at the Station.

(d) Single-pen recording thermometer with two-zone electric control.

(e) One Distillation Products laboratory centrifugal molecular still for investigations on oils.

(f) One hand-operated hydraulic curb press for work on Central Region fisheries products.

(g) An additional Beckman pH meter Model "G".

(h) One ultraviolet lamp for study of fluorescent materials such as chromatograms and crab skeletal material.

(i) One ultraviolet wavelength accessory set for Beckman DU spectrophotometer.

(j) One strip paper-chromatography apparatus for vitamin B_{12} and similar investigations.

(k) One additional muffle furnace for mineral ash determinations.

(l) One Skil belt sander (workshop).

(m) One electric welding machine (workshop).

(n) Two additional thermoregulators for constant-temperature cabinets and other temperature control work.

(o) One additional head and set of tubes for high-speed centrifuge used in microbiological work.

The American Can Co. through its lease of canning equipment to the Station on a dollar per year per machine basis, exchanged the obsolete can closing machine for a new Canco Model 5 closing machine.

STAFF

A list of staff employed at any time during the twelve-month period ending November 1, 1952, also the staff organization as of that date, immediately follow the present introductory section. The net increase in staff (24 to 26) during the above period is two junior non-scientific members.

Dr. MacLeod (Principal Scientist, biochemistry) replaces the late Dr. Bailey (Senior Scientist, biochemistry); Mr. Fagerlund (Junior Scientist, chemistry) replaces Miss Kristjanson (Senior Research Assistant, chemistry) during continued leave of absence of Mr. Cooke (Associate Scientist, chemistry); Miss Soutar (Asst. Technician Grade 1, chemistry) replaces Miss Walton (Asst. Technician Grade 1, chemistry); Miss Porter (Asst. Technician Grade 1, biochemistry), resigned October 25, has not yet been replaced; Mrs. Howard (Asst. Technician Grade 1, librarian) is assuming the duties of Mr. Holmgren (Technician Grade 1, librarian) who commenced leave of absence; two part-time

Cleaner and Helpers have been added to the staff, one to do evening cleaning, and one to wash glassware.

Mr. Young (Principal Scientist, engineering) continued to be seconded to Headquarters; Mr. Holmgren received leave of absence without salary for educational purposes; Mr. Cooke returned from military service in Korea and resumed duties for about three weeks to write two reports dealing with his earlier investigations, then received educational leave of absence on half salary for undertaking pH.D. work.

Mr. Fagerlund, through his familiarity with Scandinavian languages, has rendered valuable services by translating important scientific articles for the Station.

PUBLICATIONS

A complete list of staff publications that appeared during the period under review follows the list of staff at the end of this introductory section.

The customary four issues of Pacific Progress Reports (Nos. 89-92) were published under the editorship of Dr. Carter. The number of individuals, organizations and firms on the mailing list is 2005 and is steadily increasing. More than one copy goes to many of the addresses.

Various of the publications listed were reprinted in whole or in part by scientific or trade publications, also by abstracting services (e.g. Commercial Fisheries Abstracts, U.S. Fish and Wildlife Service; World Fisheries Abstracts, F.A.O., U.N.; Chemical Abstracts). Some results of this Station's work are thereby quickly drawn to the attention of many interested persons who do not receive the original publications.

Bulletin 89, " Marine Oils with Particular Reference to those of Canada", the 413-page successor to Bulletin 59 of similar title, appeared early in 1952 after several years' preparation under the editorship of the late Dr. Bailey with the assistance of Drs. Carter and Swain.

TRAVEL

In addition to many visits to various fish processing plants on the lower B.C. coast by different members of the staff for investigations purposes and to accompany official visitors desiring to see something of the fishing industries, trips further afield included:

November 1951: Eight members of the staff to an open house held by the U.S. Fish and Wildlife Service Technological Laboratories at Seattle.
Dr. Tarr to Long Beach, Calif., for a three-day visit to various scientific laboratories.

January 1952: Drs. Carter and Swain to the annual meetings of the Board at Ottawa.

February: Dr. Carter to the annual B.C. Natural Resources Conference at Victoria.

March: Eleven members of the staff to the third annual meeting of the Pacific Fisheries Technologists (2½ days) at Seattle.

April: Mr. Lantz to Bellingham, Wash., to visit plants.

June: Dr. Carter to Board Executive meeting at Quebec. Dr. Tarr to meeting of Institute of Food Technologists, Grand Rapids, Mich., where he presented a paper on antibiotics in flesh food preservation. Other eastern points were visited.

July: Mr. Schmidt to whaling plant at Coal Harbour, B.C., to secure whale materials.

August: Mr. Baker accompanied test run of railway refrigerator cars from Prince Rupert to Montreal (Summary No. 12).

September: Mr. Lantz to Alberta, Saskatchewan and Manitoba in connection with Station's assistance to fisheries of the Central Area.

October: Dr. Carter to Board Executive meeting at Ottawa. Mr. Schmidt to Saskatoon to attend the first Western Regional Conference of the Chemical Institute of Canada, thence to Winnipeg in furtherance of the Station's assistance to fisheries of the Central Area.

SCIENTIFIC, INDUSTRIAL AND OTHER RELATIONS

The preceding outline of staff travel, and various Investigators' Summaries, indicate some of the valuable relations formed or maintained. Additional activities included:

Dr. Tarr was appointed for a three-year term commencing January 1952 as an associate editor of "Food Technology"; this involves refereeing certain papers intended for that journal or in "Food Research". Dr. Swain chaired the symposium on marine oils at the February meeting of the Pacific Fisheries Technologists. This annual meeting embraces representatives of government, University and industrial personnel from Alaska to California and the Hawaiian Islands who are interested in advances in the technology of fisheries products and by-products.

Several important visitors from overseas were entertained and familiarized with the work of the Board and the Station, also taken to representative fish processing plants. Members of the staff of the U.S. Fish and Wildlife Fisheries Technological Laboratory at Seattle visited the Station, and Dr. W. Hastings of the University of Washington School of Fisheries brought a group of his students to the Station to acquaint themselves with our lines of work.

Two members of the staff of the Department of Fisheries of East Pakistan, Messrs. S.A. Kabeer and S.U. Qadri, under the auspices of the Colombo Plan spent several months using the Station as a base of operations while familiarizing themselves with administration and operation of west coast fishery industries. They were also given an orientation course in fisheries technology at the Station and participated in some of the investigations being carried on. Mr. S.H. Chowdhury, Supt. of Fisheries, East Pakistan, had been given similar facitities for about two months commencing in November 1951.

Collaboration with the Department of Fisheries. Relations with the Department, both through its Ottawa Headquarters and directly, have been actively maintained. During the period under review, these relations included:

(a) The Station made available at the request of the Department through the Board the facilities mentioned above for Messrs. Chowdhury, Kabeer and Qadri. Assistance was rendered by the Department's Western Division in this connection.

(b) The Station made its facilities available to two of the Department's Central Division Inspectors, Messrs. J.B. Peters and O.B. Rutherford, who during their six weeks' stay were given an orientation course in west coast fisheries technology and inspection and taken on plant visits at coastal points from Seattle to Prince Rupert. The Department's western office and Canned Fish Inspection Laboratory ably assisted.

(c) The Station scientific staff participated in the giving of a two-week refresher course in February to the Department's Western Division Inspectors.

(d) The Station assisted the Department's Vancouver office in the entertainment of Mr. LeMare of the Dept. of Fisheries of Malay, by demonstrating the technological work of the Station and having a staff member accompany the party on a plant visit at Steveston.

(e) Miss M. Allman, Home Economics Demonstrator with the Department's Western Division has been given information and publications relating to the processing and nutritive values of fish as food, and provided with experimental facilities in the Station's demonstration kitchen and cold storage rooms. A jacketed refrigerated display cabinet was planned and constructed partly for her use in demonstrations (Investigators' Summary No. 10).

(f) Dr. Carter and Messrs. Schmidt or Harrison attended three meetings called by the Department's Western Chief Supervisor for discussing regulations concerning dry salting of British Columbia herring with representatives of that industry. Experimental work in collaboration with the Department's Inspectors was followed on several occasions by exchange of advice and data on procedures, and many samples of the products were analyzed for salt and moisture content at the request of the Inspectors, in addition to the Station's own programme on salting (Investigators' Summaries Nos. 21-23).

(g) Mr. Lantz, Mr. Schmidt and Dr. Swain carried out numerous experiments and tests at the Station in response to requests for advice and assistance from the Department's Chief Supervisor for the Central Division and his Inspectors at Prince Albert, Winnipeg, Churchill, etc. These officers were most helpful in assisting in securing samples for this work as well as for the Station's more general programme related to the Central Area fisheries. They also provided much appreciated facilities during the trips made by Mr. Lantz and Mr. Schmidt to discuss problems on the spot. A procedure for conducting tasting panel tests was supplied. Arrangements have been made for Dr. Tarr to visit the Central Division office at Winnipeg for two days shortly to participate in meetings there between the Department's officials and the industry re plant sanitation, preservation of quality, and inspection.

(h) The Department's senior officials at Ottawa, the Chief Supervisors, all western Inspectors, many of the central Inspectors, and some of the eastern Inspectors, regularly receive the Pacific Progress Reports and certain Vancouver Station Industrial Memoranda. Approximately monthly reports on Station activities have been prepared since June for submission to senior Department officials, and since 1950 a page proof of each Pacific Progress Report has been regularly sent to the Information Branch at Ottawa for early use of any material in it desired for "Trade News".

(i) Miscellaneous mutual services rendered between the Department's Vancouver office or Canned Fish Inspection Laboratory and the Station in 1952 also included: Continued arrangement with the Halibut Commission for permission to catch and retain halibut out of season for experimental purposes; discussion on worms in canned herring; discussion on measurement of seine net length while set; suggestions for detection of gasoline fumes aboard Departmental vessels; assistance in court action re "fresh" or "frozen" halibut out of season; translation of Norwegian articles; discussion on measurement of sturgeons; treatment of fish with antioxidants; trimethylamine tests for freshness; direct bacterial counts for quality; analysis of possibly polluted waters (e.g. Investigators' Summaries Nos. 51 and 52); exchange of administrative information, statistics and, on occasion, office supplies and forms.

Relations with other Government Departments, Universities, Industry and the Public have been well maintained. Information and services were exchanged with the local Food and Drugs Laboratory (e.g., rearing of white mice at the Station until October); the National Research Council (e.g. assistance in its refrigerator car experiment, Summary No. 12, our supplying and receiving Canadian Committee on Food Preservation reports and reprints); the Provincial Game Department also Department of Lands and Forests; the B.C. Research Council (e.g. molecular distillation of a sample of chemical in the Station's new still); University of B.C. (e.g. mutual identification of specimens of various materials, placing the Station's lyophilizing equipment at the disposal of a graduate student, continued tests of Station equipment by Chemical Engineering Branch, Summary No. 49); and with other bodies. Relations with the Industry are described in other sections of this Report. Types of information given to the public in the form of personal contacts, correspondence, library service, leaflets and reprints during the year have been too numerous to summarize. The Director gave addresses on the Board's and Station's work to two Service Clubs and to the Agricultural Institute of Canada.

Grateful acknowledgment of all services, samples and other materials supplied gratuitously by Government Departments, institutions, firms and individuals is here expressed.

STAFF OF THE PACIFIC FISHERIES EXPERIMENTAL STATION

SCIENTIFIC STAFF

N. M. Carter, M.A.Sc., Ph.D., F.C.I.C., F.R.G.S., Director.
R. A. MacLeod, M.A., Ph.D., Principal Scientist (from June 30).
H. L. A. Tarr, M.S.A., Ph.D., Senior Scientist (to December 31, 1951)
Principal Scientist (from January 1).
O. C. Young, M.B.E., M.E., Principal Scientist (seconded to Headquarters).
N. E. Cooke, M.A.Sc., F.C.S., Associate Scientist
(on leave of absence for military service to August 14)
(on educational leave of absence from September 5).
J. S. M. Harrison, B.A.Sc., Associate Scientist.
A. W. Lantz, B.Sc., Associate Scientist.
S. W. Roach, B.A.Sc., Associate Scientist.
L. A. Swain, M.A., Ph.D., Associate Scientist.
P. J. Schmidt, B.Sc., Assistant Scientist.
Burnett A. Southcott, B.S.A., Junior Scientist (to September 30)
Assistant Scientist (from October 1).
U. H. M. Fagerlund, M.Sc., Junior Scientist (from February 11).
Helen M. Bissett, B.S.A., Senior Research Assistant.
Svava Kristjanson, B.A., Senior Research Assistant (to December 10, 1951).

TECHNICIAN STAFF

E. G. Baker, Technician, Grade 1.
E. J. Holmgren, B.A., Technician, Grade 1 (Librarian) (to September 10)
(on educational leave of absence from September 11).
Ruth I. Howard, B.Sc., Technician, Grade 1 (Librarian), part time
(from September 8).
Irene M. Porter, Assistant Technician, Grade 1 (to October 25).
Iris R. Smith, Assistant Technician, Grade 1.
M. Joyce Souter, Assistant Technician, Grade 1 (from November 1, 1952).
Joyce B. Walton, Assistant Technician, Grade 1 (to December 31, 1951).

OFFICE AND BUILDING STAFF

J. W. Kilpatrick, M.C., Administrative Officer, Grade 2.
Phyllis Tweedale, Clerk, Grade 3.
Nellie E. McBride, Stenographer, Grade 3.
Lorna Ferguson, Clerk, Grade 2B.
F. C. Freeman, Maintenance Supervisor, Grade 3.
P. E. Enright, Caretaker, Grade 2.
Waike M. Kuusk, Cleaner & Helper, part time (from February 1 to October 12).

TEMPORARY STAFF

Marian Jurda, Cleaner & Helper, part time (from October 6 to 27).
Margot Rieger, Cleaner & Helper, part time (from October 1).
Barbara Taylor, Cleaner & Helper, part time (from November 1).

ORGANIZATION OF STAFF OF THE PACIFIC FISHERIES EXPERIMENTAL STATION

(26 members as of Nov. 1, 1952: 11 scientific; 10 non-scientific; 1 seconded to Headquarters; 2 on leave of absence; 2 temporary)

ADMINISTRATIONAL

Director	Dr. N. M. Carter
Executive Assistant (Admin. Officer, Grade 2)	Mr. J. W. Kilpatrick
Secretary and Clerk (Clerk Grade 3)	Miss P. Tweedale
Stenographer (Stenographer Grade 3)	Mrs. N. E. McBride
Executive Clerk (Clerk Grade 2B)	Miss L. Ferguson

INVESTIGATIONAL STAFF

(1) Bacteriology and Microbiology:

Principal Scientist	Dr. H. L. A. Tarr
Assistant Scientist	Miss B. A. Southcott, B.S.A.
Senior Research Assistant	Miss H. M. Bissett, B.S.A.

(2) Biochemistry:

Principal Scientist	Dr. R. A. MacLeod

(3) Chemistry:

Associate Scientist	Dr. L. A. Swain
(Associate Scientist (On educational leave of absence)	Mr. N. E. Cooke, M.A.Sc.)
Assistant Scientist	Mr. P. J. Schmidt, B.Sc.
Junior Scientist	Mr. U. H. M. Fagerlund, M.Sc.
Assistant Technician (Grade 1)	Miss I. R. Smith
Assistant Technician (Grade 1)	Miss M. J. Souter

(4) Engineering:

(Principal Research Engineer (Seconded to Headquarters Unit)	Mr. O. C. Young, M.E.)
Associate Scientist	Mr. A. W. Lantz, B.Sc.
Associate Scientist	Mr. S. W. Roach, B.A.Sc.
Associate Scientist	Mr. J. S. M. Harrison, B.A.Sc.
Technician (Grade 1)	Mr. E. G. Baker

LIBRARY

(Librarian (Technician, Grade 1) (On educational leave of absence)	Mr. E. J. Holmgren, B.A.)
Librarian (Technician, Grade 1, part time)	Mrs. R. I. Howard, B.Sc.

MAINTENANCE

Maintenance Supervisor (Grade 3)	Mr. F. C. Freeman
Caretaker (Grade 2)	Mr. P. E. Enright
Cleaner & Helper (part time, temporary)	Mrs. M. Rieger
Cleaner & Helper (part time, temporary)	Mrs. B. Taylor

PUBLICATIONS AND REPORTS OF THE PACIFIC FISHERIES EXPERIMENTAL STATION NOV. 1, 1951-OCT. 31, 1952

NOTE: For continuity of these lists in the Station's processed Annual Reports, titles of publications and reports that appeared too late in 1951 to be inserted in last year's Report are included here. Such titles were added in the list given in the Board's printed 1951 Annual Report.

1. Bailey, B.E. (editor), N.M. Carter and L.A. Swain (assistant editors). Marine oils with particular reference to those of Canada. Bull. Fish. Res. Bd. Can., No. 89, 413 pp.

2. Biely, Jacob, B.E. March and H.L.A. Tarr. The nutritive value of fish meal and condensed fish solubles. III. Effect of heating fat-containing and hexane-extracted meal. Prog. Rept. Pac., No. 89, pp. 79-81. (Dec. 1951).

3. ——— The nutritive value of fish meal and condensed fish solubles. IV. A comparison of herring meals made by drying herring press cake commercially and experimentally at a lower temperature. Prog. Rept. Pac., No. 90, pp. 10-13.

4. ——— The nutritive value of fish meal and condensed fish solubles. VI. An unidentified growth factor(s) in fish meal. Prog. Rept. Pac., No. 92, pp. 10-13.

5. ——— The effect of drying temperature on the folic acid content of herring meal. Science, Vol. 116, No. 3010, pp. 249-250.

Bissett, H.M. (See No. 19.)

Carter, N.M. (See No. 1.)

6. Fagerlund, Ulf, and L.A. Swain. Alcohol in a marine tube worm. Prog. Rept. Pac., No. 92, pp. 16-17.

7. Harrison, J.S.M. and S.W. Roach. The application of vacuum in pre-treatment of fish for canning. Prog. Rept. Pac., No. 91, pp. 10-12.

8. ——— Vacuum treatment for canned fish. Canadian Department of Fisheries, Trade News, Vol. 5, No. 1, pp. 8-9.

9. Lantz, A.W. A jacketed display-cabinet for controlling temperature and humidity to retain quality. Prog. Rept. Pac., No. 89, pp. 76-77. (Dec. 1951).

10. ——— Shrimp processing. Prog. Rept. Pac., No. 89, pp. 82-83. (Dec. 1951).

11. ——— Freezing British Columbia fish at sea. II. Prog. Rept. Pac., No. 92, pp. 21-22.

(See also No. 23.)

12. Maxwell, B.E. The distribution of vitamin B_{12}-active substances in some marine invertebrates of British Columbia. J. Fish. Res. Bd. Can., Vol. 9, No. 3, pp. 164-168.

Roach, S.W. (See Nos. 7 and 8.)

13. Schmidt, P.J. Analyses of British Columbia herring. Prog. Rept. Pac., No. 92, pp. 3-5.

Southcott, B.A. (See Nos. 19 and 24.)

Swain, L.A. (See Nos. 1 and 6.)

14. Tarr, H.L.A. Chromatographic separation and microbiological assay of indigenous and added cobalamins in crude animal protein materials. Canadian Journal of Technology, Vol. 30, Nos. 10-11, pp. 265-279.

15. Microbiological assay of vitamin B_{12} in crude materials. Federation Proceedings (of Fed. Amer. Soc. Experimental Biology), Vol. 11, No. 1, part 1, pp. 297-298.

16. Antibiotics in mink feed. The National Fur News, Vol. 24, No. 7, pp. 12-13, 28.

17. The nutritive value of fish meal and condensed fish solubles. V. The vitamin B_{12} content of herring meals. Prog. Rept. Pac., No. 90, pp. 14-15.

18. Cause of the browning of certain heat-processed fish products. Prog. Rept. Pac., No. 92, pp. 23-24.

(See also Nos. 2,3,4,5,19,24,25 and 26.)

19. Tarr, H.L.A., B.A. Southcott and H.M. Bissett. Experimental preservation of flesh foods with antibiotics. Food Technology, Vol. 6, No. 9, pp. 363-366.

SUBMITTED FOR PUBLICATION

20. Cooke, N.E. Utilization of fish waste. A study of the economics of the separation of Pacific salmon offal. (For Bull. Fish. Res. Bd. Can.)

21. A chemical engineering report on the economic possibility of the large-scale recovery of by-products produced from salmon viscera. (For Pac. Fish. Exptl. Sta. Indus. Mem.)

22. Khan, M.M.R. Studies on the "lipoxidase" in the flesh of British Columbia herring. (To J. Fish. Res. Bd. Can.) (1951).

23. Lantz, A.W. Some aspects of fisheries research in relation to refrigeration. (To Refrigerating Engineering.)

24. Southcott, B.A. and H.L.A. Tarr. The vitamin B_{12} content of certain fishery materials. (To J. Fish. Res. Bd. Can.)

25. Tarr, H.L.A, The action of hydroxylamine on bacteria. (To J. Fish. Res. Bd. Can.)

26. Ribose and the Maillard reaction in fish muscle. (To Nature.)

27. Young, O.C. Some applications of the jacket principle in cold rooms in Canada. (To Canadian Refrigeration Journal.)

INVESTIGATIONS

This review does not attempt to cover all the varied investigations, technical services, etc. that took place during the twelve-month period since the previous report was prepared. The style and length of this review were governed by the intention of having it conform, with a minimum of alteration, to the requirements for this Station's contribution to the Board's printed Annual Report. Because the space allotted for this contribution is just about half that allotted in last year's printed Report, there has been curtailment of some of the background and detail. To make much of this detail available as a proper report to the Board, and for record purposes, the inclusion of a large number of Investigators' Summaries has been resumed after experimentally discontinuing this practice last year.

The indexes at the back of this report may be of assistance in correlating the information in this review and in the Investigators' Summaries following it.

Nutritional Requirements of Bacteria Associated with Fish and Fish Spoilage

Although numerous investigations have been concerned with the kinds and numbers of bacteria associated with fish and fish spoilage, little or nothing is known of the nutritional requirements of the bacteria involved. Since the more we know of our adversaries the better we are able to deal with them, a study of the growth requirements of this economically important group of organisms has been initiated.

Studies were begun using ten organisms isolated from fresh lingcod. Knowledge of the cultural characteristics of these organisms indicated that they were probably all different, though no attempt was made to classify them bacteriologically.

To establish first just how fastidious the organisms are, three media of increasing degrees of complexity were devised. The first medium, designated "simple medium", consisted of inorganic salts (including ammonium sulphate), asparagine and glucose. The second, or "lactic medium", contained among other things eighteen amino acids and nine vitamins, in addition to inorganic salts and glucose. The third, or "complex" medium, was a simple medium fortified with enzymatic halibut hydrolysate and yeast extract.

One organism would not grow on any of these media, three grew on the simple, nine on the complex, and only two on the lactic medium. Lack of growth in the last named medium was found, on further investigation, to be due to the presence of acetate rather than to the absence of an unidentified growth factor in this chemically defined medium.

With the information made available by the results described above, a new basal medium was devised containing glucose, ammonium sulphate, trace metals, a mixture of eighteen amino acids, a mixture of nine vitamins, and adenine, guanine and uracil. All of the organisms but one grow on this medium. The further addition of enzymatic halibut muscle hydrolysate and yeast extract either alone or together improved growth in only one or two cases. It is therefore evident that all of the organisms at present under investigation,

with one possible exception, are capable of growing on media containing known nutritional essentials. It is now a question of determining which components of the basal medium are required for growth by each of the above organisms. Three organisms have been found not to grow if the mixture of eighteen amino acids is omitted. One organism will not grow if the mixture of nine vitamins is absent. Omitting vitamins one at a time from the mixture in the latter case reveals biotin as the required compound. Curiously, when amino acids are omitted one at a time, no requirement for an individual amino acid can be demonstrated for those organisms which will not grow without the mixture present. In addition, in the latter cases, no single amino acid when added to the medium will permit growth equivalent to the mixture. A further interesting observation has been made concerning the amino acid requirements of one of the organisms. Although most of the organisms now being studied are inhibited by the presence of acetate in the medium, one organism not only fails to be inhibited by this simple organic salt containing only two carbon atoms, but its requirement for the mixture of eighteen amino acids can be completely replaced by acetate.

The results obtained so far indicate that the nutritional requirements of the marine micro-organisms are surprisingly complex. Further study should reveal information of considerable biochemical significance.

Antibiotics in Fish Preservation

Previous findings have been extended and verified and a scientific paper has been published in which a detailed account of the work has been given. Of the fifteen antibiotics studied, only aureomycin, terramycin and chloromycetin have shown really valuable bacteriostatic properties in fish flesh, and rimocidin has proved a useful deterrent to yeast growth.

In view of the above findings the stability of aureomycin and terramycin in flesh foods is being investigated. It was found convenient to employ a pad-plate assay procedure, and using this, the addition of aureomycin to fish flesh followed by immediate extraction with acid acetone and assay resulted in recoveries which were somewhat higher than expected. When flesh samples which contained 2 and 3 parts per million of added aureomycin hydrochloride were stored for 4 days at 40°F., there was no appreciable loss in assayable aureomycin, and similar results were obtained with terramycin. It thus appears that flesh does not contain appreciable amounts of enzymes or other factors which inactivate these antibiotics microbiologically. When flesh containing similar amounts of aureomycin was heated at 212°F. there was a progressive loss in antibiotic activity, and after 30 minutes less than 10% of the initial activity remained. That this destruction is probably not due to substances present in the flesh is apparent from the fact that aqueous aureomycin solutions are themselves rapidly inactivated under similar conditions of heating, and that this inactivation is more rapid at pH 6.7 than at pH 4.5. There has been some suggestion that aureomycin is destroyed slowly in flesh stored at temperatures of about 50°F., and an investigation is now in progress to determine whether this is due to bacterial action.

Test for Freshness

As a result of a request by the Department of Fisheries that a recently published Japanese method of testing freshness of fish be examined,

the mercuric chloride reagent therein described was tried on the acidified aqueous extract of the flesh of several species of British Columbia marine fishes, stored for various lengths of time. The test was not satisfactory.

Freezing British Columbia Fish at Sea

During the year investigations into freezing and refrigerating fish at sea other than by brine have continued with the help of the experimentally refrigerated commercial fishing vessel "Tauranga" described in last year's report. Nine lots of fish have been tested by the panel tasters. Three of these tests were with halibut refrigerated in various ways, three were with other flatfishes like brill and rock sole, and one was with tuna canned from a portion of a frozen catch. The two other tests were made on commercially canned smoked fish and on frozen salmon, as a service to the processors. The results of these tests were published during the year in Pacific Progress Reports Nos. 91 (p.20) and 92 (pp.21-22).

The panel tasting of fish from the "Tauranga" has been very satisfactory. The some 245 tasters have been co-operative, keenly interested, and remarkably consistent in their returns. Appreciation is due to the Fishermen's Co-operative Federation for filleting of the brill and sole.

An investigation into the use of brine freezing on board ship has been started this year. The need for freezing at sea has been discussed in other reports of this Station's activities. The problem is to find a method suitable to the type of craft, the species of fish and the eventual form in which the fish is marketed.

Brine freezing has many advantages, some of which are of great value in freezing at sea. Speed is its greatest and best known virtue. Our work and the work of others has shown this speed to be about as follows for sodium chloride brine at approximately its eutectic temperature ($-6^{\circ}F.$):

$$T = r^2$$

Where T = time in hours, r is half the least dimension (in inches) at the greatest cross section.

Refrigerating efficiency is another important advantage for freezing at sea. Brine freezing permits the use of higher refrigerant temperatures which in turn allow a lower ratio of horse power to refrigeration tonnage and a lower ratio of compressor volume to tonnage. Aside from fuel consumption savings this results in substantial reduction in the weight and bulk of both the compressor and its motive power unit.

Another advantage for ship application is the reduction of weight and space of fixed equipment. Refrigerant evaporators are smaller for brine freezing because of their high heat transfer. The space occupied by freezer shelves for air systems cannot be utilized fully for other than its original purpose, whereas brine tanks can be utilized fully for storage space on both inward and outward trips.

However, brine freezing is not without its disadvantages. One is salt penetration and its resultant effects. Salt penetration is not serious in so far as taste is concerned as there is little difficulty in holding the

penetration within tolerable taste limits. Another difficulty, that of holding a glaze, which was of serious consequence twenty-five years ago when brine freezing was rather extensively applied, has vanished with the improvement in commercial cold storage temperatures. At that time -15°F. was the accepted storage temperature. The modern practice is to hold storage rooms at 0° to -10°F. These temperatures are low enough to freeze glazes of high salt content. The effect of salt penetration on deteriorative changes during storage is little known and will bear further investigation. It is hoped that improved storage temperatures will also eliminate or assist in overcoming this problem, along with the use of antioxidants to minimize oxidative rancidity of fats which is sometimes accelerated by the presence of salt.

The speed of freezing which has previously been classed as an advantage in brine freezing at the same time presents a serious problem for ship application. Any increase in the speed of freezing calls for a corresponding increase in compressor capacity or, failing that, a rise in brine temperature. Our solution to this problem has been to employ eutectic sodium chloride ice to store refrigeration, during the periods of low refrigeration demand, for use when needed at the start of freezing. Also by using eutectic ice it is possible to hold the brine in close proximity to the eutectic temperature, at which point, theoretically, there would be no salt penetration.

The first work was to build a slush ice freezer. It was hoped that the fish could be frozen in eutectic ice slush. Unfortunately, this device was a failure because of the extreme hardness of the eutectic sodium chloride ice which, contrary to expectation, could not be dislodged from the surface on which it was frozen. This work was abandoned as it was apparent that equipment required to accomplish this would be too heavy for the type of vessels considered.

The second approach to the problem employs the accumulation of eutectic ice on the refrigerant evaporator surface, and high agitation to reduce the difference in temperature between the brine and eutectic ice. An experimental apparatus has been constructed for the purpose of obtaining some of the fundamental data on this system of freezing. These data include freezing rate, salt penetration, heat transfer, coefficient of evaporators, and agitation requirements. The apparatus consists of a Freon-12 condensing unit, stainless steel tank, and a flooded evaporator.

Preliminary tests have shown that freezing in the apparatus can be accomplished at close to the eutectic temperature, and that salt penetration is slight.

Crab Processing

A problem raised this year was one of providing suitable means for transporting caught live crab to port. It is a problem common to the Queen Charlotte Islands, the west coast of Vancouver Island, and to Prince Rupert areas. Processors feel the crab raised by the trawling fleet should if possible be utilized, and expressed concern over the apparent waste. It was suggested that one or two boats be delegated to experiment with butchering the crab on board, leaving the sectioned halves with legs attached to be iced

and stored aboard until landed. Upon arrival at the processing unit, the crab sections are cooked, chilled and packed into containers for freezing. Samples of the product are being sent to this Station for examination. To assist the processors further, a series of experiments has been made to determine the best equipment and method for freezing crab sections in the shell. In freezing a master carton containing twelve 20-oz. packages or 15 lb. of crab at -12°F. to -18°F., the mean temperature in the packaged product was reduced to 12°F. in 6 hours. When the 20-oz. packages were spread between two metal plates to prevent expansion during freezing and were frozen by air blast at -10°F. to -18°F., the mean internal temperature was 0°F. to -5°F. in 4 hours. Permission to conduct these freezing experiments at the Vancouver plant of the British Columbia Packers Ltd. is acknowledged.

Antioxidants for Frozen Fish Flesh

In continuation of last year's work further tests have been made on the red spring salmon samples which were stored at 14°F., -4°F., and at about -18°F. The results have substantiated those obtained in previous tests in showing that, although the antioxidants used (ascorbic acid and a mixture of ascorbic acid and carrageen) delayed the onset of oxidative rancidity in treated samples, the post mortem age of the fish did not appear to influence rancidity development in either treated or untreated fish. Further tests have been conducted with the object of determining whether the antioxidant butylated hydroxyanisole, the use of which is permitted in certain edible fats, exerts any protective action in retarding rancidity in fish flesh. This substance alone, or in a synergistic mixture with propyl gallate and citric acid, did not appear to be as effective as ethyl gallate, and was considerably less effective than ascorbic acid in delaying development of oxidative rancidity under the experimental conditions.

Other Refrigeration Investigations

This year as in previous years efforts have been made to extend the application of the jacketed system of refrigerating which has been growing in favour as a result of our work on cold storage units and the mechanically refrigerated car. Preliminary plans were drawn for an extension to a cold storage. The principle employed is the jacketing of four entire floors, with resultant economies in mechanical equipment and insulation and an increase in net storage space.

Plans were also drawn up for a refrigerated scow to transport ice and fresh, frozen, and cured fish. Two separately jacketed compartments are planned, each of which can be held at any desired temperature.

Last year an experimental "jacketed" showcase for the retail display of fresh fish was constructed here by the Station. Tests showed considerable improvement in maintaining the true and apparent freshness of fish. Because of this success, a commercial model was built this year chiefly through donation of material and services by interested companies. Demonstrations by the Station and the Western Home Economics Branch of the Department of Fisheries are planned with a view to adoption of this equipment commercially.

This year's work on railroad refrigeration was confined to sending one member of the staff to participate in a road test conducted by the two

Canadian transcontinental railways and the National Research Council. The types of equipment tested were: (a) a standard overhead-bunker car cooled with ice and salt; (b) a standard overhead-bunker car cooled with ice, salt, and ammonium nitrate; (c) a finned overhead-bunker car cooled with ice, salt, and ammonium nitrate; (d) a standard overhead-bunker car cooled with Dry Ice; (e) a mechanically refrigerated car.

Insulation studies during the year included compaction tests on several types of insulating materials subjected to thousands of jarring impacts while installed in experimental sections of a cold storage wall; the efficiencies of plywood and other sheet products as vapour barriers; increased efficiencies of insulation by alteration of certain features of structural design.

Canning

Efforts to produce an improved pack of canned herring were continued this year. Past work has shown that the chief problem is to remove sufficient moisture from the fish while in the cans prior to closing. The method developed last year and described this year in Pacific Progress Reports No.91, pp.10-12, whereby excess moisture is removed by the application of vacuum, has proven most effective; the remaining problem appears to be the development of a satisfactory tomato sauce since practically all canned herring produced in British Columbia is packed in tomato sauce. The sauce used at present by herring canners consists of a mixture of tomato paste and tomato puree with brine added to provide salt and the desired consistency for filling. In commercial practice this sauce when added to standard herring (precooked in an exhaust box) mixes with the excess moisture from the fish to produce a large quantity of thin brown liquid which is most undesirable. When the herring is more thoroughly dehydrated by high-temperature precooking or by the vacuum method prior to adding this sauce, the result is still not satisfactory since during processing the excess water in the sauce is absorbed by the fish, softening and discolouring the flesh.

An ideal sauce should combine the following qualities: it should have a predominantly tomato flavour with some spices added; it should be thin enough to make dispensing easy but should thicken during processing; and it should remain red in colour after processing. To produce a sauce with the above qualities it was found necessary to use a high grade, vacuum-concentrated tomato paste to provide the desired colour and tomato flavour. Vinegar and spices were combined to provide a spiced liquor for adding in the desired quantity for flavouring. To achieve the necessary texture corn starch and water in the proportions necessary to provide an easily dispensed sauce which thickens during processing were used. A sauce made up this way was found to yield an excellent product when added to herring which had been previously treated in this Station's vacuum equipped retort to reduce the moisture content to the desired level.

Some trials were made with a commercial Borg "spun pack" machine designed for the removal of undesired liquid from albacore and similar fish flesh which has been pre-heated in water at about 210°F. until the temperature in the flesh has reached about 150°F. A considerable portion of liquid could be removed, but there was a tendency for the flesh to darken noticeably on canning. Also, some modifications appeared desirable to avoid the loss of fish particles that occurs, and to eliminate the surface markings and tendency for "curd" to form.

The Maillard Reaction in Relation to Canned and Other Products

Work on the Maillard, or non-enzymic browning, reaction in fishery materials has been resumed. This reaction is of basic importance in that it accounts for most of the darkening or discoloration of white-fleshed fish which may occur during canning, for discoloration of dried or dehydrated fish, and may also have an undesirable effect on flavour. Adaptation of the technique of paper chromatography has made it possible to make rapid headway with the problem. In the Annual Report for 1951 (page 157) it was mentioned that pentose sugars appeared to be present in significant amounts in flesh and extracts of skipjack tuna. It has now been shown for the first time that the flesh of many varieties of fish, if examined post mortem, contains appreciable quantities of the pentose sugar D-ribose. Moreover it has been found that in the muscle of living fish no free ribose is present, and that it arises through degradation of the natural nucleic acid or its derivatives which are present in this muscle. The enzymic systems concerned with the liberation of ribose are being studied in detail, and evidence for the existence of ribonucleosideases has been accumulated. The total (free and combined) ribose content of different fish muscles examined post mortem has been shown to vary from 0.1 to 0.3%. Though free glucose is also present in variable amounts in post mortem fish muscle, experiments have indicated that in the concentrations found it is rarely, if ever, an important factor in determining non-enzymic browning, for about five times as much of this sugar as of ribose is required to occasion appreciably the same extent of discoloration under comparable conditions. A spectrophotometric technique has been developed by means of which the degree of browning can be determined qualitatively, and using this technique it has been shown that the extent of browning correlates well with the free ribose content of fish muscle. From the practical standpoint two general techniques for preventing browning suggest themselves as a result of the above findings. These are: (1) removal of the ribose after it has formed, and (2) heat processing the fish before the ribose has formed. These methods will be investigated. A short report concerning this work appeared during the year in Pacific Progress Reports No.92, pp.23-24.

Fish Curing

Pages 157-159 of last year's Report described a modified process of salting herring as developed at this Station, and stated that a commercial trial of the process was made. Reports from consignees in the Orient later indicated that the herring so processed were as well received as those processed in the regular manner. Delay in the commencement of herring fishing near Vancouver this winter has so far not allowed opportunity for a further commercial trial of the modified method this year.

At the request of the Department of Fisheries and the industry, an examination was made of the changes in water and in salt contents of herring during commercial dry salting. These changes were followed by analyses of daily samples over a thirteen-day period. The results showed a lack of uniformity in the product. A laboratory experiment was made in which herring from the same catch were submerged in a brine maintained at saturation (100° salinometer). In three days the water and salt contents had reached the same satisfactory values as was reached by dry salting in seven days. Further

details of these experiments were published during the year in Pacific Progress Reports No.92, pp.3-5. The assistance of the British Columbia Packers Ltd. for providing samples of herring salted to various stages is appreciated.

During the 1951 herring season, the Department of Fisheries was requested by Formosa to certify that shipments of dry salt herring to that destination contain not more than an average of 40 lb. of free salt per 400-lb. box of fish. This Station was requested to recommend a procedure which could produce such a product. It was found necessary to allow for the weight of free salt adhering to the fish after dry salting, and to estimate the weight of salt which dissolves and drains from the box during shipment. The adhering salt was readily removed for measurement by shaking the fish in a fish-net. Experimentation led to the conclusion that 55 lb. of salt could safely be added to a box of fish previously freed of adhering salt. Such a box would not contain more than 40 lb. of free salt after shipment.

Analyses of samples from various lots of commercial dry salt herring for their content of salt, oil, and moisture were made for the information of the Department of Fisheries Inspectors and the processors. The average result was 11.3% salt, 16.8% oil, and 50.0% moisture, within a small range of individual values.

Biological Feeding Value of Herring Meal, Fish Solubles, and Related Products

Last year experiments were carried out which indicated that the fat present in an air-flow-dried herring meal was so altered when the meal was heated in a rotating drum for 2 hours at 300°F. that it retarded chick growth. During the current year another experiment carried out along similar lines has verified these findings. When the fat from meals heated as above was added to similarly heated meals from which the fat had been extracted before heating, these last named meals caused some impairment in chick growth. In order that additional data may be obtained concerning the subject of possible impairment of the nutritive value of fat in herring meal during storage, samples of both fat-containing and fat-extracted ideal herring meals are being stored at 98° and 70°F. for at least 6 months, when they will be fed to test chicks.

In previous experiments wherein ideal meals had been found somewhat superior to commercial meals in promoting chick growth, the results had not been strictly comparable because the meals had not been prepared from the same lot of raw material. This weakness was remedied during the present season; through the help of the fishing industry, ideal, normal commercial, and seriously overheated commercial flame-dried meals were prepared from the same lot of herring. Using these meals the previous findings have been fully substantiated. Moreover, it has been found that the cause of the lower nutritive value of the flame-dried meals is apparently not an impairment in the availability of essential amino acids in the protein, but a deficiency in one of the vitamins of the B complex, namely folic acid. Supplementation of these commercial meals with folic acid makes their nutritive value equivalent to that of ideal meals in chick rations. Microbiological assays have shown that commercial herring meals are deficient in folic acid, while ideal meals have an adequate amount.

A number of experiments to determine the nutritive value of herring meal, condensed fish solubles, and certain related products have been carried out. In these trials fish meal has been fed at levels which are normally used in poultry starter rations, namely 2.5 to 6.0%. In these experiments it has been found that a mixture of herring meal and soya bean meal when incorporated into a normal starter ration promotes better chick growth than does either when used alone. The addition of herring meal (2.5 or 5.0% in different experiments) to corn—soya rations used for chicks or turkey poults was found to promote growth better when used in conjunction with an antibiotic such as penicillin or aureomycin than in its absence. The further addition of all known vitamins of the B complex to these rations was without effect. These results extend and support the findings recently reported in certain other laboratories that fish meal contains an as yet uncharacterized growth factor or factors. In further work several special products have been prepared from the same herring: namely, whole blended fish, an autolysate, and two bacterial digests. The effect of addition of these, and also of herring meal and condensed fish solubles, on growth of chicks fed a typical corn soymeal type of ration has been studied. The levels fed were 2.5 or 5.0% of the ration. In no instance was any of these products more effective than ordinary commercial herring meal in promoting chick growth, and, in one of two experiments, only the herring meal promoted growth. It was concluded that no special benefit appears to be derived from use of these special liquefied products in chick rations. They offer, however, a means of utilizing coarse fish and fish processing wastes under circumstances where production of meal is not feasible. Acid autolysates are fairly readily prepared and, in cool places, are fairly stable. They were made by use of mineral acids such as hydrochloric, or with organic acids such as formic, though the latter class are more expressive. Additional chick feeding experiments during the year have yielded results which indicate that prolonged storage of commercial fish meals may result in some deterioration in nutritive value.

Microbiological Activity of Fish By-products

There are persistent rumours from various sources to the effect that fishery products, in particular whale solubles, are of considerable benefit in ruminant feeding. Since whale solubles have not been shown to be an outstandingly good source of known nutritional essentials, the opinion has been expressed that the effects purportedly observed are due to stimulation of the rumen flora by unknown factors in the whale solubles. A representative of a private American laboratory concerned with fishery products has stated recently that evidence has been obtained in his laboratory which he believes supports the above hypothesis. This investigator claims to have observed that fishery products when added to a suitable medium greatly stimulate yeasts to utilize inorganic nitrogen salts and urea. He infers from this that if yeasts can be stimulated to utilize inorganic nitrogen and urea, so also can the rumen population, thereby permitting a higher percentage of less costly simple nitrogen compounds to be included in the diet of ruminants. His results with yeasts are apparently being used to support a patent application to cover the inclusion of fishery products in prepared cattlefeeds.

In view of the report referred to above, it was of interest to determine whether the presence of a factor or factors peculiar to fishery products could be demonstrated which would affect the growth of yeasts. In addition, if yeast-growth-promoting activity were observed and could be correlated with the chick-growth-promoting action of fishery products, a convenient assay for the chick factor would be at hand.

Two strains of yeast were chosen for this study, Saccharomyces cerevisiae, a typical baker's yeast, and Saccharomyces carlsbergensis 4228, a brewery yeast. The components of the basal medium used in this investigation were the same as are normally included in standard yeast assay media. The nitrogen and vitamin content of the medium was increased over that normally employed in assay media to minimize the possibility that any stimulation from supplements could be due to their ability to supply known nutritional essentials. To this medium, various fishery by-products were added. Supplements included two different samples of whale solubles, one from a local fishing company, the other from a supply on hand from the South Polar region; a sample of herring solubles, one of stickwater, and one of enzymatically hydrolyzed halibut muscle.

Although fishery products are claimed only to increase the utilization of inorganic nitrogen by yeasts, such an effect would ordinarily result in an increased cell yield if products suitable for ruminant nutrition were to be formed. For this reason, cell yield rather than inorganic nitrogen utilization was determined in these experiments.

Since the medium described above is capable of supporting the growth of yeasts, the effect of an unknown growth factor could only be expected either to increase the speed of attainment of maximum growth or to affect the extent of growth of the organisms. To check the first possibility, cell yield was determined before maximum growth was achieved, that is, after 24 hours of incubation. The second possibility was investigated by permitting growth to reach a maximum by incubating for 45 to 48 hours under aerobic conditions, before determining cell yield.

Results of a number of experiments using both strains of yeast revealed no really significant effect of fishery products on the growth of yeasts under aerobic conditions. The same conclusion was drawn from a series of anaerobic experiments with both yeasts, though the results under these conditions were much more erratic.

The results referred to above do not support the conclusion that a growth factor peculiar to fishery products affects the growth of yeasts, and are thus at variance with those purportedly obtained by the American investigators. This variance may of course be due to a difference in the strains of yeast used. The effect of this discrepancy, however, on the validity of the hypothesis that since fishery products will enhance the growth of a strain of yeasts, they will enhance the growth of the rumen flora, is obvious. If the phenomenon reported cannot be reproduced in organisms so closely related as different strains of yeast, then without direct experimental evidence it is difficult to justify the conclusion that fishery products will stimulate such a widely different group of organisms (few if any of which are yeasts) as are found inhabiting the rumen.

Studies Concerned with Cobalamins (forms of vitamin B_{12})

The term "cobalamin" is being used to an ever increasing extent to replace "vitamin B_{12}", for it is becoming more apparent that not only are there many closely related forms of the true vitamin, but that other forms exist which behave quite differently biologically and are somewhat different chemically. In view of the very great irregularities experienced in cobalamin

assay values under somewhat different conditions, a thorough investigation of the assay of the vitamin in crude animal protein preparations has been completed (Canadian Journal of Technology, Vol.30, p.265, 1952). This study has involved the analysis of both raw and heated crude fishery materials, the addition of known amounts of different cobalamins (cyano- and hydroxo-cobalamins) to these, and their recovery. Microbiological assays have been made directly using aqueous extracts, and also with aqueous eluates of the chromatographically separated vitamins. The assay method used was a tube turbidimetric procedure in which both cyano- and hydroxo-cobalamins yielded identical assay values. With this method a very large number of analyses have been made of industrial fish meals, fish solubles and other materials. It has been found that industrially produced British Columbia herring meals are a good and relatively consistent source of the vitamin, and that condensed fish solubles are an equally good but somewhat less consistent source.

In connection with the assay of cobalamins in crude materials and in microbiological fermentation liquors made from fishery wastes, the cup-plate and pad-plate procedures using an Escherichia coli mutant have been studied. This general method, if applicable, would be more rapid than conventional tube assays because of the wide range of cobalamin concentrations which can be covered without the necessity of making dilutions. Unfortunately application of this technique to crude fish products such as press liquids has not been encouraging. Results obtained using different treatments such as the well known use of cyanide or bisulphite have not agreed, and the exhibition zones have often been hazy and indistinct. Further tests with the pad-plate method for assay of purer solutions of both pseudo and true cobalamins are being made.

The discovery that the clam, as an example of marine invertebrate, was a rich source of vitamin B_{12}-active materials was made in the laboratory over three years ago. This was followed by an investigation, the results of which were referred to in last year's Annual Report, of the cobalamin content of a variety of other marine invertebrates. With the discovery of the existence of a class of cobalamins which differ from the true cobalamins chemically and by the fact that they are active in promoting bacterial but not animal growth, this work was resumed. Samples of this new class of cobalamins (pseudo-cobalamins and vitamin B_{12f}) have been obtained through the courtesy of industrial and university laboratories, and the most satisfactory chromatographic (bioautographic) methods of separating them are being studied. When these methods have been well standardized a thorough study of the distribution of different cobalamins in many kinds of fishery materials will be made.

The work on the microbiological formation of cobalamins described in last year's Annual Report has been continued from the standpoint of finding whether a relationship between type of nutritional substrate and yield exists. A rotary shaking machine has been employed in these studies since its use has facilitated the study of a larger variety of substrates than was possible by the aeration technique. In all experiments the media contained two parts per million of added cobalt. The cobalamin yields obtained with shake cultures are almost invariably lower than those obtained by aeration. In a number of experiments no relation between substrate and cobalamin yield was obtained. Cobalamin in small yields was obtained by growing Streptomyces species in mixed inorganic salt solutions containing either glycine or asparagine as sole carbon and nitrogen source. The addition of known fragments of the cobalamin molecule (1,2-diamino-4,5-dimethylbenzene, and 5,6-dimethylbenziminazole) did

not influence the cobalamin yields obtained using crude fish materials or inorganic salt solutions containing asparagine as substrates. It was concluded that improvement of cobalamin yield from Streptomyces species by modification of the substrates will probably be a very difficult matter.

Fish Meal Analysis

Four methods of determination of water were compared, using samples of halibut flesh and salmon viscera as test materials. The most satisfactory of these methods was to mix the ground material with sand and heat at 115°C. for 3½ hours. The "Mikro-pulverizer" hammer mill used for preparation of samples on which moisture determinations are to be made was shown to remove water from the material under examination during the grinding. This was corrected by substitution of a different type of cutter in the instrument.

Conversion of certain coarse fish and fish processing wastes into meal by inexpensive apparatus devised at this Station for use in the Central Region fisheries was described in last year's Report (pp.170-171). This project was discontinued this year with the demonstration that simple jack- or curb-presses could produce a press cake capable of being dried under suitable atmospheric conditions to a moisture content of 8 to 10% satisfactory for storage. These results will appear in Progress Reports No.93 during the year.

Oxidized oil in fish meal is difficult or impossible to extract. It was shown with a fish meal oxidized to two different extents that repeated refluxing with acetone and hydrochloric acid and decanting of the solution gave a yield of oil more nearly that obtained from the original meal, than when the meal was Soxhlet-extracted with acetone. By both methods the oil content of the meal decreased as the extent of oxidation increased. In both cases "oil" was the material extracted by ether from the acetone extract.

Composition of Fish Oils

In present day world markets fish oils have sunk to a low position because of the rapid post-war development and expansion of vegetable oil production, and because of aversion to the "fishiness" of fish oils. The production of synthetic vitamin A has had serious consequences to the fish liver oil industry. These facts make necessary a fresh approach to the fish oil situation in regard to world commerce.

The presence of vitamin A in fish liver oils was an almost unique occurrence of vitamin A which caused a demand for these oils. What is needed now is something else unique in natural occurrence to again make fish oils a desired commodity. That something would seem to be the long-chain highly unsaturated fatty acids which occur primarily in marine animal oils. The constituent fatty acids of the fats and oils from plant seeds and fruits usually have up to 22 carbon atoms per molecule, with up to three centres of unsaturation, although some of the fatty acids in the seed fats of legumes have 24 carbon atoms and the seed fat of a tropical rosaceous tree is unique in having four centres of unsaturation. On the other hand, marine animal oils having fatty acids with up to 26 carbon atoms and up to seven centres of unsaturation have been reported. Interesting uses for these marine oils are possible.

The fatty acid compositions of Atlantic and western Pacific fish oils have been studied in detail. A study of the composition of the marine animal oils available in the eastern Pacific was therefore a logical procedure. Work in this direction was commenced at this Station in 1937 with an examination of pilchard oil, but was interrupted primarily by studies on vitamin A, and later by continuing failure of pilchards to appear in commercial quantities off the British Columbia coast. Such work has therefore this year been revived with an examination of a sample of commercial herring oil from fish caught in January off the northern British Columbia coast. It is proposed to undertake analyses of other marine oils of commercial interest, selected according to the species or parts of the fish from which they are prepared, and the locality and season in which the fish are caught. Following closely upon this survey should be studies upon the utilization of these long-chain unsaturated fatty acids, the distribution of which is so restricted in Nature.

After some delay through mechanical imperfections in the Station's new molecular still, analysis was completed on the fatty acid composition of this sample of oil. The fatty acids of the oil were converted to their methyl esters, which were then fractionated into groups of increasing unsaturation by crystallizations at -25°C. and -75°C. Facilities were not available for as complete a separation as is possible. The fractions so obtained were then fractionated by molecular distillation, a procedure which avoids lengthy exposure of the esters to adverse conditions. Palmitic acid was found to be the saturated fatty acid present in highest concentration, and C_{20} acids to be the most common series of unsaturated fatty acids. The presence of a C_{24} unsaturated acid was indicated. Such an acid has been reported in a Japanese oil stated to be herring, but not in Atlantic herring oil.

The phenomena associated with the separation of stearine from herring oil were examined by centrifuging herring oil during its slow cooling, so that crystals of stearine were removed as soon as they were formed. This had been attempted at this Station fifteen years ago but the rate of cooling available at that time had been too rapid, and a gel was the product. With the improved facilities now available, crystals of stearine formed and were separated readily at 21°F. However, the iodine value of the cold-cleared oil was raised only a little — in one case from 130 (original oil) to 145, at which point only 4% of the sample was liquid. It would appear that the various triglycerides in British Columbia herring oil do not show much variation in unsaturation.

Following a request from industry for a simple and accurate method for the determination of peroxide value of fish oils, an investigation of different published methods was started. One of these has been studied in detail and improvements in the procedure have been made, but it still gives too much variation in results. A second published method, which is a simplified modification of still another method, is now being examined.

Whale Liver Oils

Whale liver oil contains a high concentration of vitamin A and also varying proportions of kitol, the presence of which causes a very modified absorption curve in the spectrophotometric assay used for vitamin A determinations. Assays two years ago showed almost no extraneous absorption in

vitamin A curves obtained from several whale liver oils prepared in the laboratory by ether extraction, although commercially prepared whale liver oils showed marked extraneous absorption.

This season certain liver samples were frozen immediately after removal from the whale, and vitamin A determinations were made as soon as possible thereafter. All contained exceptionally high concentrations of vitamin A. The finback and sperm whale liver oils this time showed extraneous absorption, but there was almost none in the blue whale liver oil. Considerable variations in extraneous absorption have been reported elsewhere for finback and blue whale liver oils. The absence of kitol in all the samples examined two years ago was apparently coincidental. It was of interest to note that the liver samples on thawing did not have the disagreeable odour normally associated with whale livers.

Miscellaneous

"Bute Inlet wax" was further examined this year. Vacuum distillation of the methyl ester fractions prepared after lead salt separation of its fatty acids indicated these acids were one-third myristic acid, one-third palmitic acid, and the remaining third consisted of saturated and mono-unsaturated fatty acids up to and including C_{24}. The possibility that pollen from the lodge-pole pine (which is reported to grow in profusion behind Bute Inlet and very little elsewhere on the British Columbia coast) is the origin of this wax was investigated by storage experiments extending over 5 months with pollen from the same species of tree, growing on Lulu Island, B.C. The results did not seem to substantiate this hypothesis, and the source of Bute Inlet wax remains a mystery.

An unusual odour, suggestive of capryl alcohol, was noted last year in the tube worm Eudistylia vancouveri during a survey conducted at this Station on vitamin B_{12} distribution in marine forms. During the period of low tides this year more worms were collected and a liquid containing the odour was isolated in 0.05% yield by steam distillation of minced worms. This liquid consisted essentially of one or more alcohols of low molecular weight. The small quantity of substance available precluded its further identification.

At the request of the Department of Fisheries analyses were made on samples of water from a metal-pickling effluent emptying eventually into Burrard Inlet near Vancouver. These indicated a complete neutralization of the hydrochloric acid in the effluent during passage through the sump into which it entered.

Crab skeletal materials can normally be detected in cooked crab meat by the bluish fluorescence they cause in ultraviolet light, the use of which is now regular commercial procedure. Unfortunately, as was reported last year, during the winter months the flesh also shows a fluorescence, making difficult the removal of shell scraps. Work on isolation of the material causing this fluorescence was started too late this spring to have available a sufficient quantity of material for examination. Tests by chromatographic analysis showed the presence of several fluorescing materials in alcohol- and acidified water-extracts of flesh.

Some work was done at the request of the Pacific Biological Station on the separation of herring eggs from the vegetation on which they are deposited, in relation to bird predation studies. The eggs were bathed in various alkaline solutions at two different temperatures, without success. The suggestion was later made to use the enzyme hyaluronidase, which met with some success. This is the enzyme occurring in snake venom that causes rapid breakdown of connective materials between the cells in animal tissues.

Abnormally high salmon mortalities again occurred in the Kitsault River of northern British Columbia, a river on which a smelter is located. Last year, cyanide and sulphide could not be detected. Further examination was made at the request of the Department of Fisheries of samples of the river water collected by a Biologist of the Department. Water from above the smelter was clear; from points below it, somewhat turbid. The samples were slightly alkaline and contained some calcium. No cyanide or sulphide, or other indication of pollution, could be detected. Toxic effluents, if present, were of such a type as not to give indication of their presence by the tests applied.

The average annual quota of materials submitted to the Station in the hope they might be ambergris again materialized this year. Jellyfish preponderated. However, an opportunity of examining a lot of true ambergris resulted in our taking colour and black-and-white photographs of the appearance of the material in bulk, as well as of its finer structure and inclusions.

Technical and informational services rendered during the year to the Department of Fisheries, the fish processing and allied industries, educational institutions, trade journals and the press, the public, and to visiting scientists under the auspices of international organizations, have been too numerous and varied to summarize here. These services ranged from giving a course in Canadian fisheries technological methods to a representative from Pakistan through design of cold storage plants, to the possibility of competing with the tropical production of trepang by drying British Columbia sea cucumbers for export.

SUMMARY NO. 1

A STUDY OF THE NUTRITIONAL REQUIREMENTS OF MARINE MICRO-ORGANISMS

R.A. MacLeod

Although numerous investigations have been concerned with the kinds and numbers of bacteria associated with fish and fish spoilage, little or nothing is known about the nutritional requirements of the bacteria involved. Since the more we know about our adversaries the better we are able to deal with them, a study of the growth requirements of this economically important group of organisms has been initiated.

Studies were begun using ten organisms which had been isolated from fresh lingcod by Miss Southcott under the direction of Dr. Tarr. Knowledge of the cultural characteristics of these organisms indicated that they were probably all different, though no attempt was made to classify them bacteriologically. The organisms are distinguished from one another by an arbitrarily assigned number.

The organisms were carried at 4°C. on Bacto nutrient agar slants. Since all of the organisms are capable of growing in Bacto nutrient broth, this medium was used initially to grow the inoculum cultures. Inoculum cultures were centrifuged, washed once and suspended in water. One or, if growth was light, two drops of the suspension were used to inoculate the various media.

To establish first just how fastidious the organisms are, three media of increasing degrees of complexity were devised. The first medium, designated "simple medium", consisted of inorganic salts including ammonium sulphate, asparagine and glucose. The second medium, referred to as the "lactic medium", was patterned after ones normally employed in microbiological assay procedures using lactic acid bacteria as test organisms and contained in addition to inorganic salts and glucose, 18 amino acids and 9 vitamins. The third, or "complex medium", was composed of the simple medium fortified with an enzymatic hydrolysate of halibut muscle and yeast extract.

One organism would not grow on any of these media. Three organisms grew on the simple medium, nine on the complex and only two on the lactic medium. Since the lactic medium contains almost all of the growth factors so far known to be required by bacteria, growth in the complex medium but not in the lactic medium could mean either that some unknown growth factor supplied by the complex medium was needed for growth by these organisms or that something in the lactic medium was capable of inhibiting growth. The latter possibility was tested first by adding lactic medium to the complex medium. In all cases where growth did not take place in the lactic medium, the lactic medium was found to be inhibitory when added to the complex medium. Tests of the various components of the lactic medium on the complex medium revealed that the component of the former primarily responsible for growth inhibition was acetate.

With the information made available by the results described above, a new basal medium was devised containing glucose, ammonium sulphate, trace metals, a mixture of 18 amino acids, a mixture of 9 vitamins, and adenine, guanine and uracil. All of the organisms but one grow on this medium. The further addition of enzymatic halibut muscle hydrolysate and yeast extract either alone or together improves growth in only one or two cases. It is

therefore evident that all of the organisms at present under investigation with one possible exception are capable of growing on media containing known nutritional essentials. It is now a question of determining which components of the basal medium are required for growth by each of the above organisms. Three organisms have been found not to grow if the mixture of 18 amino acids is omitted. One organism will not grow if the mixture of 9 vitamins is omitted. Removing vitamins one at a time from the mixture in the latter case has shown that biotin is the required compound. Curiously, when amino acids are omitted one at a time from the mixture of 18, no requirement for an individual amino acid can be demonstrated for those organisms which will not grow without the mixture present. In addition, no single amino acid when added to the medium will give growth equivalent to the mixture. A further interesting observation has been made concerning the amino acid requirements of one of the organisms. Although most of the organisms now being studied are inhibited by the presence of acetate in the medium, one organism not only is not inhibited by this two-carbon-atom compound, but its requirement for the mixture of 18 amino acids can be completely replaced by acetate.

The results obtained so far indicate that the nutritional requirements of the marine micro-organisms are surprisingly complex. Further study should reveal information of considerable biochemical significance.

I wish to acknowledge the receipt of the following rare and at present commercially unobtainable chemicals for use in nutrition studies with micro-organisms. All were supplied gratis by the individuals or companies concerned:

Pyridoxal phosphate
Pyridoxamine phosphate
supplied by Dr. Carl Folkers of Merck & Co., Rahway, N.J.

Pantethine
supplied by Dr. Esmond E. Snell, University of Texas.

Leucovorin
supplied by Dr. Harry P. Broquist, Lederle Laboratories, Pearl River, N.Y.

Protogen
supplied by Dr. E. Patterson, Lederle Laboratories, Pearl River, N.Y.

SUMMARY NO. 2

EXPERIMENTAL PRESERVATION OF FLESH MATERIALS WITH ANTIBIOTICS

H.L.A. Tarr
Miss B.A. Southcott
Miss H.M. Bissett

This follows as a continuation of previous work (Summary No. A9, 1947 Station Annual Report; Summary No. 11, 1950 Station Annual Report, and page 12 of 1951 Station Annual Report). A paper was read at the Institute of Food Technologists annual meeting in Michigan in which the work was described in detail, and this was published in Food Technology during the year (No. 19 in List of Publications).

The preservation of fish flesh and several beef samples at 0°C. to 21°C. was investigated. The antibiotics were mixed into the minced flesh, and the rate of spoilage was followed by making direct bacterial counts. In this way the following antibiotics were studied: streptomycin, penicillin, subtilin, polymixin B, circulin, neomycin, bacitracin, gramicidin, metholyl gramicidin, tyrothricin, rimocidin, terramycin, aureomycin and chloromycetin. Aureomycin, terramycin and chloromycetin, in the order named, proved the most effective inhibitors of growth of the natural mixed bacterial flora at temperatures between 0°C. and 21°C., while rimocidin inhibited yeast growth. Aureomycin caused marked inhibition of spoilage in 0.5 to 2.0 µg. per gram (0.5 to 2.0 parts per million) concentration when incorporated in minced flesh. It was equally effective when applied by immersing steaks in solutions containing the antibiotic in 5 or 10 µg. per ml. (5 or 10 p.p.m.) concentration. The remaining twelve antibiotics when used in 10 or 50 µg. per gram concentration either exerted a less intense bacteriostatic action or were without effect.

This work is being continued in order to determine stability of aureomycin and terramycin in fish flesh due to heating and certain other treatments, and the specific action of these antibiotics on bacteria concerned with fish spoilage. (See Summary No. 2).

SUMMARY NO. 3

THE STABILITY OF ANTIBIOTICS IN FLESH FOODS — Miss H.M. Bissett

The possibility of preventing bacterial spoilage of flesh foods with the addition of antibiotics has been studied to a limited extent in recent years. The first work was done in this laboratory, using penicillic acid, penicillin and streptomycin. It is only recently that work has been carried out on raw flesh with the newer antibiotics.

Experiments this year at this Station (Summary No. 2) showed that, of 15 antibiotics investigated, only aureomycin, terramycin and chloromycetin inhibited bacterial growth to an extent where they might be useful as preservatives. Of these, aureomycin was by far the most effective. It seemed desirable to determine what happened to these antibiotics when they were added to foods: whether they were eventually broken down by bacterial action during storage or whether they remained intact during storage but were broken down during cooking. Aureomycin and terramycin were selected for this study.

(a) Effect of storage at 40°F. on the stability of aureomycin.

Confidential unpublished data obtained through workers in the animal nutrition laboratories at Ohio State University showed that in meat treated with aureomycin in concentrations of 0.5 to 2 p.p.m., the aureomycin disappeared almost entirely on storage at 10°C. for periods of 6 days.

In the present work coho salmon and beef were ground and samples were treated with 2 and 5 p.p.m. of aureomycin. The samples were incubated at 40°F. (the usual storage temperature of meat and fish) for four days. They were then assayed by a pad-plate method to determine the amount of antibiotic remaining in each sample. (This method was kindly described by the Lederle Laboratories.) Each test was repeated at least twice. The results (Table 1) show that aureomycin is not destroyed in meat and fish during storage under the conditions

TABLE I. The effect of storage on the stability of aureomycin in fish flesh stored at 40°F. (recoveries shown are in p.p.m.).

Storage period (days)	0		4	
Aureomycin added (p.p.m.)	5.0	2.0	5.0	2.0
Salmon (coho)	6.3	2.4	5.2	2.1
Beef	6.0	2.25	5.6	2.6

specified above. Experiments are being carried out using the storage temperatures used by the Ohio workers. The aureomycin inactivation which they describe may be due to bacterial decomposition of the antibiotic molecule at the comparatively high storage temperature they used. The experiment using terramycin gave results similar to those when using aureomycin, though the assay procedure was not as reliable as with the latter.

(b) Effect of heat on aureomycin (i) in flesh
(ii) in aqueous solution at different pH values.

Samples of ground flesh were treated with 5 p.p.m. of aureomycin and heated in boiling water for periods of 0,10,15,20 and 30 minutes then assayed by the pad-plate method to determine the residual antibiotic. The aureomycin was progressively destroyed (Table II) with almost complete destruction at 30 minutes. This experiment indicates that aureomycin would be largely destroyed under normal cooking temperatures. A similar result was obtained with terramycin.

TABLE II. The effect of heating on the stability of 5 p.p.m. of added aureomycin in fish flesh (recoveries shown are in p.p.m.).

Heating period (minutes)	0	10	15	20	30
Salmon (coho)	5.1	2.4	1.5	0.6	0.45
Lingcod	4.8	1.8	1.8	-	0.3

The results of the above experiment suggested the desirability of determining the stability of aureomycin in aqueous solution, and also the effect of pH on stability. Two series of aqueous solutions were prepared by diluting standard solution of aureomycin hydrochloride with distilled water and buffer to give pH values of 4.5 and 6.7 respectively and a concentration of 5 p.p.m. of aureomycin. These pH values were chosen because the aqueous aureomycin hydrochloride solution used had a pH of 4.5 and the fish sample a pH of about 6.7. Samples of the two series were then heated for periods of

TABLE III. The effect of heating and pH value on the stability of a 5 p.p.m. aqueous solution of aureomycin (recoveries shown are in p.p.m.).

Heating period (minutes)	0	10	15	20	30
At pH 4.5	2.1	1.1	1.0	0.45	0.3
At pH 6.7	0.3	0	0	0	0

0,10,15,20 and 30 minutes. The results (Table III) show that aureomycin is progressively destroyed by heat at pH 4.5, and is destroyed entirely in 10 minutes at pH 6.7. The low control value seems to indicate that a change in pH causes a change in the aureomycin molecule which markedly reduces its bacteriostatic action toward the assay organism, Bacillus cereus.

SUMMARY NO. 4

TEST FOR FRESHNESS OF FISH

Ulf Fagerlund
P.J. Schmidt
S.A. Kabeer

Mr. H. Dempsey, Chief Supervisor of Fisheries, Winnipeg, requested an investigation of a Japanese method for measuring freshness of fish, a brief abstract of which in a trade journal had come to his attention. (The original article in Bulletin No. 1 of the Tokai Regional Fisheries Research Laboratory, December 1950, page 128, had been noted earlier at this Station.) In this method freshness is estimated by the amount of precipitation produced in a watery extract of the flesh by a mercuric chloride reagent.

The method was tried on lingcod, halibut and salmon at different stages of spoilage. It was found to be inconsistent and unsatisfactory.

Later, while two of Mr. Dempsey's staff were being given an orientation course in fisheries technology at this Station during the summer, this method was explained to them, and they were enabled to familiarize themselves with several other methods that have been recommended as tests for the freshness of fish.

SUMMARY NO. 5

FREEZING B.C. FISH AT SEA, AND USE OF TASTING PANEL — A.W. Lantz

The investigations into freezing B.C. fish at sea with samples treated in various ways submitted to a panel of tasters have continued during 1952. A total of 9 tests has been made. The tasting panel was also used on two occasions upon requests from industrial processors, as mentioned in a news item in Progress Reports No. 91, page 20. One of these tested chum salmon which had been in storage at Vancouver Ice and Cold Storage Co., and the other compared two packs of canned sardine-type herring produced by Millerd & Co. Ltd.

Three tests with halibut were made between March and May:

Date	Storage period	Preference for fish frozen at sea	Preference for fish on ice, frozen after landing	No preference
March 19/52	3 months	30.4%	40.3%	28%
April 28/52	4 "	31.4%	42.6%	26%
May 9/52	1 week	31.8%	41.6%	26.6%

The consistency of the tasters has been remarkable. Observations have indicated that the majority of the tasters discriminate between the "frozen at sea" and iced at sea" samples making their selection on "moisture" content. The fresh halibut was unfrozen fish having been packed in refrigerated ice and stored 3 to 5 days aboard the "Tauranga". It was frozen at -15°C. at this Station and stored until panel tested.

Four tests were made with other flatfish. The first two tests were with "fresh iced" and "frozen at sea", brill.

Date	Preferred frozen fish	Preferred fresh on ice	No preference
June 19/52	44.2%	35.8%	20%
Aug. 22/52	39.4%	25.0%	35.6%

No reason has been established for the preference for frozen brill.

Two later tests were made with iced fish.

Aug. 25/52. Brill dressed before storage aboard the "Tauranga" and stored in ice was tested against fish stored in ice on the "Tauranga" and dressed after landing.

Results: 37.2% preferred the fish dressed aboard ship.
28.1% preferred the fish dressed after landing.
34.7% could detect no difference.

For the Sept. 26 test, brill that was rinsed before storage in ice aboard the "Tauranga" was compared with brill that was scrubbed before storage in ice.

Results: 36.0% preferred the rinsed.
31.0% preferred the scrubbed.
33.0% could detect no difference.

It appears from this test to be impractical to scrub fish prior to packing in ice.

These tests complete the series using "blast freezing" methods aboard the "Tauranga".

Further details of these tests, together with preliminary results of a tasting panel test on tuna canned commercially after being landed from the "Tauranga" as compared with commercially canned B.C. tuna from another source, were published during the year in Progress Reports No. 92 (No. 11 in List of Publications).

SUMMARY NO. 6

A FISH PROCESSING UNIT — A.W. Lantz

A request for recommendations concerning any alterations necessary to improve refrigeration in a commercial plant during peak production was received. It was suggested to the organization that plant drawings would be required consisting of the following: ice tank, ice storage rooms, sharp freezers, cold storage rooms and engine room, showing the detailed refrigeration lines. The necessary detailed drawings and analyses were made. No actual tests were made on room insulation or structure since this had been fully reported in Summary No. 26, 1945 Station Annual Report.

The following recommendations were made:

Ice Tank. That ice production be reduced to normal capacity for which equipment was designed, and the number of ice storage rooms be increased. That surplus ice manufactured during slack periods be used to supplement quantity of ice produced during peak periods. That the liquid and suction lines be enlarged to take care of the accelerated ice production required for the peak load.

Ice Storage Rooms. Calculations indicated that the coils were sufficient to hold the rooms at 25°F. Suggested installing additional coils to lower storage temperatures to at least 0°F.

Sharp Freezers. The present sharp freezer is too small to permit additional coils for freezing 30,000 lb. in 16 hours. It was suggested that the existing shelf coils be equipped with two axial-type fans. Installation of forced air circulation in the freezers will increase the capacity.

Cold Storage Rooms. The present coils are adequate to maintain 0°F. to 10°F. Additional coils would be required to maintain the room temperatures at -10°F.

Engine Room. The total refrigeration load of this plant is approximately 60 tons and this load is too high for the compressor capacity. An additional compressor was recommended.

SUMMARY NO. 7

CRAB PROCESSING — A.W. Lantz

A problem raised this year was one of providing suitable means for transporting caught live crab to port. It is a problem common to the Queen Charlotte Islands, west coast of Vancouver Island, and Prince Rupert areas. Processors feel the crab raised by the trawling fleet should if possible be utilized, and expressed concern over the apparent waste. It was suggested that one or two boats be delegated to experiment with butchering the crab on board by the method described in Progress Reports No. 87, June 1951, page 34, the sectioned halves with legs attached to be iced and stored aboard until landed.

Upon arrival at the processing unit, the crab sections are cooked, chilled and packed into containers for freezing. Samples of the product are being sent to this Station for examination.

To assist the crab "processors" further, a series of experiments has been made to determine the best equipment and method for freezing crab sections in the shell. In freezing a master carton containing twelve 20-oz. packages of 15 lb. of crab at -12°F. to -18°F., the mean temperature in the packaged product was reduced to 12°F. in 6 hours. When the 20-oz. packages were spread between two metal plates to prevent expansion during freezing and frozen by air blast at -10°F. to -18°F., the mean internal temperature was 0°F. to -5°F. in 4 hours. Equipment for blast freezing 4000 lb. of crabmeat, 750 lb. of crab sections in the shell, and 400 lb. of 6-oz. packages of meats per day has been designed for Queen Charlotte Canneries.

We gratefully acknowledge the assistance of British Columbia Packers Limited who permitted us to conduct these freezing experiments in the freezers at the Campbell Avenue plant.

SUMMARY NO. 8

DESIGN FOR A SCOW WITH TWO REFRIGERATED JACKETED COMPARTMENTS — A.W. Lantz

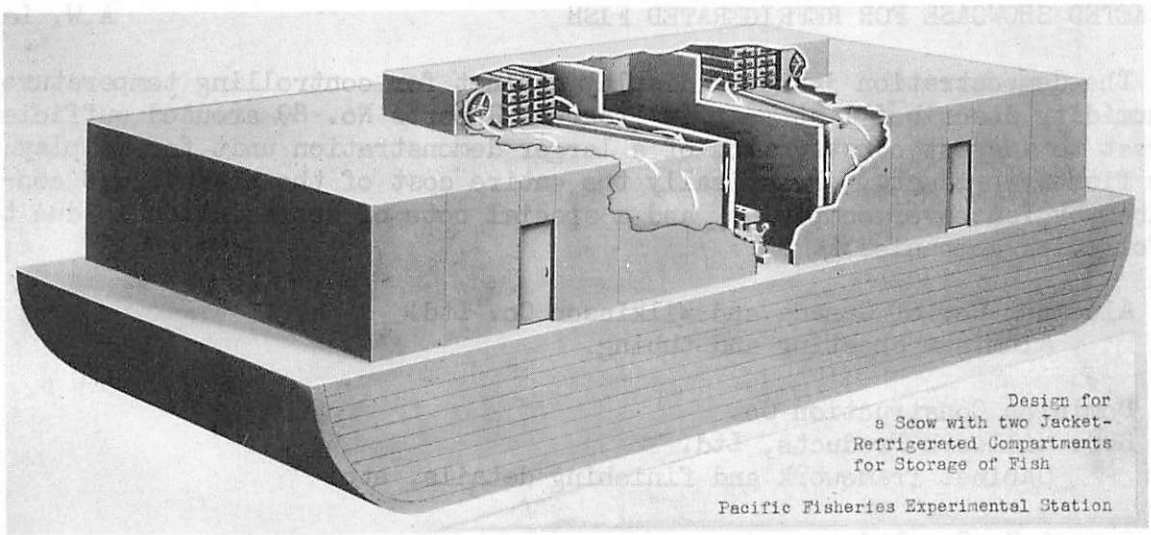
Continuing studies into means of preserving the quality of fish and again approaching the problem of preservation at the source of supply, a scow with two compartments was designed for a combination of purposes:

(1) To supply fish boats at the various fish camps with crushed ice processed from block ice stored aboard the scow in one of the two compartments.

(2) To transport frozen fish and fishing products.

(3) To transport fresh fish from the grounds to processing units.

(4) To transport mild cured and smoked fish; frozen fish and fresh fish to market outlets.



Jacketed-compartment refrigerated scow.

Each jacketed compartment is equipped with a unit blower located on the roof of the refrigerated barge for convenience of operation, ease of inspection, and efficiency. The refrigeration equipment may use either Freon or ammonia and be semi-automatically or automatically controlled. Temperatures in the two compartments may be varied to suit the products stored.

SUMMARY NO. 9

DESIGN FOR COLD STORAGE EXTENSION — A.W. Lantz

During 1952 application of the jacketed system of refrigeration was extended further. As reported in Progress Reports Nos. 77, 88 and 89, the jacketed system was applied to a refrigerated railway car, refrigerated trawler and jacketed display cabinet. More recently the system has been used in the design of a two-compartment refrigerated scow reported in Summary No. 8 and in an extension to a frozen fish storage unit. The latter application was designed to jacket the entire 4 floors of the cold storage section as one unit to conserve equipment and to enlarge storage space. When the design and the extension was requested, consideration was given to a number of problems peculiar to this particular unit. It appeared impractical to jacket the ceiling of a room and the floor of the room above when the product being stored was already frozen.

The principle is simply to place the jacket in those areas exposed to warm surfaces; that is, the ceiling of the building, the exterior walls and the base floor of the storage unit. Each floor or storey is one large storage room 9 ft. high and refrigerated to maintain a temperature of -10°F.

One master air-cooling unit is the only equipment required. This unit situated on the roof of the building forces the cold air down over the warmest sides, i.e., the southern and western exposures, and draws the returning air back up to the coils along the eastern and northern exposed walls.

SUMMARY NO. 10

A JACKETED SHOWCASE FOR REFRIGERATED FISH — A.W. Lantz

The demonstration jacketed display cabinet for controlling temperature and humidity described last year in Progress Reports No. 89 aroused sufficient interest to suggest construction of a larger demonstration unit for display of fresh fishery products. Practically the entire cost of the cabinet was contributed by Vancouver companies, and a special note of appreciation is due to the following companies:

Aluminum Co. of Canada and Wilkinson Co. Ltd.
Aluminum sheeting and tubing.

Dominion Construction Co.
B.C. Millwork Products, Ltd.
Cabinet framework and finishing details, etc.

Drexel F. Co. Ltd.
Supplying and installation of insulation.

Empire Sheet Metal Works, Ltd.
Fabricating ductwork and aluminum liner.

General Refrigeration Engineering, Ltd.
Supplying and installation of compressor unit with blower coils.

McMillan & Bloedel, Ltd.
Sylva-shield and Sylva-cord plywood for exterior finish.

Minneapolis-Honeywell Regulator Co. Ltd.
Temperature indicator and controller.

Pilkington Glass, Ltd.
Fabrication and installation of Triple-Thermopane glass.

Refrigeration Supply, Ltd.
Hardware.

The cabinet will be described and illustrated in Progress Reports No. 93. The Fisheries Department's Pacific Coast Home Economics demonstrator, Miss Mary Allman, is enthusiastic about the possibilities of such a cabinet, and the trade has shown considerable interest.

SUMMARY NO. 11

INSULATION STUDIES — A.W. Lantz

Compacting tests on insulating materials

Westroc, a British Columbia produced batt type insulation, was considered for cold storage construction. Records of tests to determine the stability of the product were not available. Further, there was no information regarding the product retaining its position in enclosed walls under the recommended 10% compression, nor whether the height and consequent weight would have a crushing effect on the insulation at near ground level. Tests were made to determine

whether or not compacting directly proportional to the height of insulated wall could be anticipated. The apparatus developed earlier at this Station and described in Progress Reports No. 67, June 1946, pp. 27-30, was used.

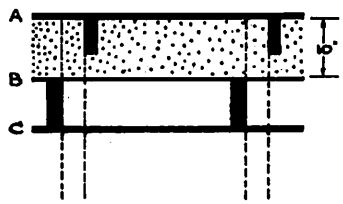
(a) A section of standard wall of 2" x 4" studs at 16" centres with outside sheathing but no inside wall was insulated with Westroc 15" x 48" paper-enclosed batts having a density of 3 lb. per cu. ft. Half of the 2"- and the 3"-thick batts were installed with the paper flanges inserted and stapled on the outer surfaces of the studs and the other half with the flanges inserted and stapled to the inside face of the studs. The only batt in contact with the outer sheathing was the 3" batt with flanges inserted. This wall was subjected to apparatus pounding at the rate of 120 impacts per minute, for a pre-determined period of 72 hours; i.e., 518,400 jarring impacts.

There was no apparent settlement of the batts or separation of the wool from the factory-coated backing paper.

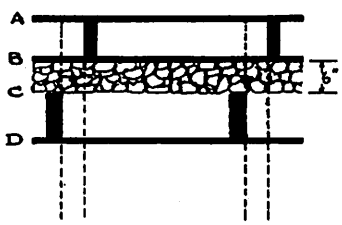
(b) A section of standard cold storage wall composed of 2" x 4" and 2" x 6" studs at 16" centres, with plywood sheathing, was constructed. (See accompanying illustration of proposed wall designs, prepared for discussions on problems concerning insulation.) Insulation to a thickness of 10", as per recommendation for cold storage units, was applied with 10% compression. The 10" thickness consisted of two 4" batts of density 3 lb. per cu. ft. and one 3" batt under compression. The apparatus was operated at 200 impacts per minute for a pre-determined period of 72 hours, giving 864,000 jarring impacts.

There was no apparent change in position and no apparent settlement though the insulation vibrated considerably with each impact.

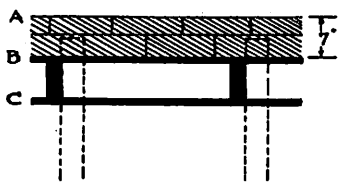
PROPOSED WALL DESIGNS



Loose Fill or Felt Insulation



Cork Insulation



Foamglas Insulation

(c) A wall section similar to (b) above was constructed with two layers of 4" batts and one layer of 3" batts (density 3 lb. per cu. ft.) applied under 10% compression to a total insulated depth of 10" to the upper half of the wall space. The lower section was left empty.

The apparatus was operated at the rate of 200 impacts per minute for a pre-determined period of 72 hours, or approximately 864,000 jarring impacts.

There was no apparent change in position of the applied insulation indicating that the method of supporting insulation batts satisfactorily maintains the position under stress.

Vapour barrier

The method of applying the vapour barrier to cold storage walls has a direct bearing on the efficiency of the wall to withstand moisture. Shiplap has numerous unavoidable cracks to be covered and the applied barrier is punctured by nails during construction. Granted the vapour barrier,

if carefully applied, may be effective; but the fact remains that leaks are possible.

(a) When unsanded plywood was suggested as sheathing to replace shiplap, a vapour barrier of aluminum foil incorporated into the plywood was considered most practical. Sylva-shield was produced in response and appears an excellent product. This material was used in construction of the jacketed fresh fishery products display cabinet described in Summary No. 10.

(b) Plywood plus an externally applied vapour barrier is effective provided the application is satisfactory. Seals of various types were applied along the seams. Perforations in the seals were visible in many of these tests. It was found necessary to apply an emulsion with a brush to the butt joints of the plywood stacked in piles before setting the sheathing in place on the wall. The walls can then be sprayed with a vapour barrier surface coating or may be covered with vapour barrier paper. It is necessary to seal all seams before spraying the wall to assure a vapour-proof wall.

Vapour transmission of:

Shiplap - 450-500 grains of moisture per hour per square foot at atmospheric pressure.

Plywood - 5/16" thick - 4.5 grains of moisture per hour per square foot at atmospheric pressure.

Plywood - 5/16" thick, treated with asphalt emulsion surface coating - 0.4 grains per hour per square foot at atmospheric pressure.

Rigidity — (Results of the U.S. Forest Products Laboratories). Wall with plywood is twice as rigid as wall sheathed with diagonal shiplap.

(c) Some comparisons of costs were made and it appears more economical to use plywood (unsanded) plus an externally applied vapour barrier. Reduction of labour hours in construction offsets the increase in cost of plywood over shiplap.

Increasing efficiency of insulation by alteration to certain features of structural design

(a) It is obvious that the closer any wooden member in a wall comes to occupying the full wall thickness, the smaller is the remaining space available for loose or felt insulation, and the greater are the heat gains into the building, through wood conduction. In the conventional type of frame cold storage building the studs for the exterior sheathing are adjacent to the studs for the interior sheathings. The only available space for insulation between these two sets of studs varies from 2" to 3", depending upon the construction. It is suggested that the upright studs for the exterior wall be staggered, with the studs for the interior wall to provide a maximum depth of insulation and to facilitate application. (See accompanying figure: Loose fill or felt insulation.)

(b) Foamglas, Onazote or similar expanded products.

It was reasoned that cellular expanded materials such as Foamglas, Onazote or Plastazote might be used as a structural material for the exterior walls of cold storage units. The writer felt that Foamglas, a vapour-proof material with a reasonably low coefficient of heat transfer would serve as insulation and vapour barrier as well as forming the exterior wall, in a single application.

The supplying company was approached regarding feasibility of this use of the material. These types of insulation have been prohibitive from a cost viewpoint but if utilized to replace a number of materials, with consequent reduction in labour hours, they can enter the competitive field. (See accompanying figure: Foamglas insulation.)

Further details of these experiments will appear in an illustrated article in Progress Reports No. 93 scheduled for publication before the end of this year.

SUMMARY NO. 12

ASSISTANCE IN RAILWAY REFRIGERATOR CAR EXPERIMENT — E.G. Baker

In the latter part of August and the first week of September the writer accompanied an experimental shipment of frozen fish from Prince Rupert to Toronto and Montreal. The test shipment was carried out by the National Research Council and the Canadian National and Canadian Pacific Railways with assistance from the Fisheries Research Board. As in last year's test trip, the Station was requested to line up the commercial carloads of frozen fish (five carloads in this case) and the writer requested to participate because of his experience with procedures gained in several earlier road tests. The purpose of the trip was to test several types of railroad refrigeration and improvements in present equipment:

(a) Overhead railway refrigerator car with redesigned bunkers with fins. Cooling medium was salt, ice and ammonium nitrate.

(b) Overhead car with standard tanks and fins. Cooling medium, same as (a).

(c) Standard overhead car now in use. Used as a check for (a) and (b). Cooling medium, salt and ice.

(d) Standard overhead car using Dry-Ice for refrigerating.

(e) Mechanical railway refrigerator car designed by Canadian National Railroads and Frigidaire Corporation, based on earlier experiments by this Station, and equipped with a diesel-electric-driven 7½-h.p. refrigerator compressor.

While in Prince Rupert completing arrangements for the test trip, the writer visited all the local fishing companies. At the completion of the trip in Montreal two days were spent at the Research and Development offices of the Canadian National Railway and the Mechanical Department of the Canadian Pacific Railway. The writer also spent three days in Ottawa visiting the Fisheries Research Board Headquarters. During the return to Vancouver, a two-day stopover in Winnipeg was made for the purpose of visiting the Central Station, the Department of Fisheries, and a number of fishing companies and cold storage plants.

SUMMARY NO. 13

ANTIOXIDANTS FOR FROZEN FISH

H.L.A. Tarr
Miss B.A. Southcott
Miss H.M. Bissett

In continuation of previous experiments which have extended over a number of years a recently developed antioxidant, namely butylated hydroxy anisole, has been tested as antioxidant for retarding rancidity in frozen fish. The usual technique employing minced flesh of salmon was used. Some difficulty was experienced in incorporating the butylated hydroxy anisole, or a synergestic mixture (industrial) of this compound with propyl gallate and citric acid, into the fish flesh. With the solution available it was found necessary to make an emulsion using Tween 80. The results obtained on storing red spring salmon samples containing the above, and certain other previously studied antioxidants are given in the accompanying table. As in former experiments, best protection, as judged by slower development of fatty acid peroxides, was obtained in samples containing ascorbic acid. Ethyl gallate proved superior to butylated hydroxy anisole. It would appear doubtful whether, unless use of antioxidants on heated fish materials is anticipated, further tests should be made with butylated hydroxy anisole.

Antioxidant	Peroxide value after 55 days at	
	-10°C.	-20°C.
None	12	2.0
0.02% Butylated hydroxy anisole	13.2	0.97
0.01% " " "	9.0	0.64
0.02% " " " (synergistic mixture)	6.1	0.64
0.01% " " " " "	6.0	0.52
0.02% Ethyl gallate	2.1	0.22
0.04% Ascorbic acid	0.3	0.0
0.04% " " (solution added was adjusted to pH 6.0 with Na_2HPO_4)	0.51	0.0

SUMMARY NO. 14

EFFECT OF POST-MORTEM AGE OF FISH ON RANCIDITY DEVELOPMENT

H.L.A. Tarr
Miss B.A. Southcott
Miss H.M. Bissett

The experiment designed to ascertain the effect of post-mortem age of fish on development of oxidative rancidity during frozen storage in presence and absence of antioxidants has been concluded. Due to a temporary "warm up" in the -25°C. storage room some time after the 202nd day, the samples stored at this temperature gave erratic results, and are not reported. Presumably the ascorbic acid oxidized and was rendered ineffective.

The samples (red spring salmon) were tested after 118 days (as reported in Progress Report No. 88, pp. 67-68, of last year), 202 and 328 days at -10°,

-20°, and (for 202 days storage only) at -25°C. The results indicated that the post-mortem age before freezing (storage for 0, 4 and 6 days in ice) had no important influence on rate of rancidity development, though both a 1% ascorbic acid and a 0.5% Krimko gel + 0.3% ascorbic acid dip delayed the development of fatty acid peroxides. The peroxide values are given in the accompanying table.

Frozen storage temperature	-10°C. (14°F.)		-20°C. (-4°F.)		-25°C. (-13°F.)
Period of frozen storage (days)	202	328	202	328	202
Treatment	Fish stored 0 days in ice before filleting				
Untreated	12.2	not	0.5	1.47	1.8
Dipped in ascorbic acid	5.3		0.19	0.57	1.2
" in ascorbic acid + Krimko gel	2.9	done	0.30	0.38	0.4
	Fish stored 4 days in ice before filleting				
Untreated	10.1	not	0.79	1.7	2.2
Dipped in ascorbic acid	2.1		0.05	0.22	0.51
" in ascorbic acid + Krimko gel	2.9	done	0.0	0.71	0.29
	Fish stored 6 days in ice before filleting				
Untreated	6.5	not	1.8	1.38	2.9
Dipped in ascorbic acid	0.1		0.56	0.1	0.0
" in ascorbic acid + Krimko gel	2.5	done	0.0	0.0	0.0

SUMMARY NO. 15

EXPERIMENTS ON THE BORG PROCESS FOR PARTIAL MOISTURE REMOVAL IN CANNING OF FISH

H.L.A. Tarr
J.S.M. Harrison
S.W. Roach

The following information has accumulated as a result of several trials with the Borg "spun pack" machine, developed in the United States and which has been the subject of some interest among British Columbia fish processors.

A series of initial trials indicated that considerable pressure was required to extract liquid from tuna which had been pre-heated in boiling water (about 210°F.) until it had attained an internal temperature of 140°F. (160°F. in some tests). Further tests were run with skip-jack, albacore and bonito; but unfortunately information gained in these tests was rather inadequate because the fish were not in all cases sufficiently fresh. They did indicate that the Borg machine could extract a considerable quantity of liquid and that the flesh of some species (notably skip-jack and blue-fin tuna) became very dark on canning. The results are given in Table I.

In view of these initial findings a more exact trial was conducted with flesh cut from a fresh (unforzen) albacore. Cans were filled with 7½ oz. of raw flesh and weighed carefully. Lids were placed on each can and three were stored 4 hours at 98°F. and six at 38°F., after the meat had attained these respective temperatures. The filled cans were placed in boiling water (210°F.) and a thermometer was placed in the centre of the meat. The meat in the 98°F. stored cans attained an internal temperature of 140°F. after 8 minutes, while that of the 38°F. cans required 16 minutes to attain the same temperature. After this heating the cans were promptly spun in the Borg extractor with a considerable application of pressure. If only moderate pressure was applied not all the "free" liquid was extracted. The cans were weighed immediately after spinning and the weights are given in Table II. Peanut oil (35 gm.) was added to each can, and they were sealed and retorted for 1 hour.

The percent loss was in general a little higher in group A but this may not be significant. This loss would not seem to be quite adequate because of the free liquid present in the canned product. It is possible that this liquid could be removed by either greater pressure during extraction or by pre-heating until the internal temperature is approximately 160°F. The tuna itself was of excellent quality as far as flavour and colour were concerned.

Another problem which will require solution is the brown discoloration which appears on the exposed part of the surface - i.e., that which is not oil covered. This could possibly be eliminated by reducing time between packing in the cans, pre-cooking, extracting and retorting. This surface discoloration is thought to be due to a thin layer of oxidized fish oil which concentrates on the surface during retorting.

Biochemical work at present in progress indicates that it may be possible to predict which fish are liable to become brown during retorting. (See Summary No. 18).

Further work with the Borg machine will require modification of the extraction head to avoid the loss of fish particles which seems to occur, and to eliminate the surface marking. The problem of eliminating entirely the tendency

for "curd" to form in tuna canned in this manner remains to be solved.

TABLE I. Oct. 3, 1952 tests with different tuna species. These fish were not fresh, and were quite soft. All weights are for convenience in grams (1 ounce = 28 gm.). All except those marked (*) as inverted were steamed in a retort in upright position for 20 minutes at 212°F. This would ensure an internal temperature of about 160°F.

Species	Initial wt.	Net wt.	Pre-cook wt.	Loss	% Loss	Wt. after Borg process	Loss	% Loss	Total loss
Albacore	271	236	240	31/236	13.1	204	16/236	6.8	19.9
	263	228	217	46/228	20.2	202	15/228	6.6	26.8
	283	248	233	50/233	21.4	220	13/233	5.6	27.0
Blue-fin	276	241	240	36/241	14.9	233	7/241	2.9	17.8
	243	207	214	29/207	14.0	195	19/207	9.2	23.2
	285	250	248	37/250	14.7	222	26/250	10.4	25.1*
	259	224	221	38/224	17.0	204	20/224	9.8	26.8**
Skip-jack	269	234	228	41/234	17.5	206	22/234	9.4	26.9
Bonito	258	223	197	61/223	27.3	176	21/223	9.4	36.7

TABLE II. Oct. 10, 1952, tests with fresh albacore. A = Pre-heated 8 min. from 98° to 140°F.; B = Pre-heated 16 min. from 38° to 140°F.

	Initial wt.	Net wt.	Final wt.	Total loss	% Loss	Free liquid (c.c.) after canning
A	244	209	208	36	17.4	-
	246	211	209	37	17.5	12
	250	215	207	43	20	-
B	247	212	202	47	23.3	-
	247	212	200	45	22.4	4
	245	210	200	45	22.4	5
	241	206	199	42	21	-
	245	210	207	38	18.1	-
	246	211	212	34	16	7.5

SUMMARY NO. 16

FLUORESCENCE IN CRAB MEAT — Ulf Fagerlund

An increase during December 1950 in the fluorescence of cooked meat of the Pacific edible crab (Cancer magister) from Boundary Bay, B.C., was reported in last year's Station Annual Report, p. 21. The same observation was made in December 1951, the peak in fluorescence lasting until February this year. Thus the writer, engaged at the Station in February, came too late to be able to observe the peak. However, an investigation was undertaken to eventually determine the source of this fluorescence under ultraviolet light. It interferes in the process of detecting remaining bits of skeletal material by their fluorescence when the cooked meat is irradiated with ultraviolet light, so that these bits may be picked out prior to canning the flesh.

An easily detectable fluorescence was at the time found in fresh cooked crab meat, some parts of the body having a predominant bluish, other parts a yellow, fluorescence.

Preliminary experiments showed that at least part of the yellow fluorescence could be attributed to riboflavin. A fluorimetric assay of the riboflavin content was started, but after a single determination the Beckman spectrophotometer used went out of order and could not be repaired before this investigation had to be discontinued for the season.

Fluorescing materials were extracted from the flesh by boiling it in acidified water or alcohol solutions. Chromatographic analysis of these solutions on "Florisil" columns showed several zones emitting yellow, blue or white-blue light when exposed to ultraviolet radiation. A substance fluorescing with blue light was also extracted from the solutions with light petroleum.

Attempts were made to obtain an extract concentrated enough to make possible an investigation of the chemical nature of the substances, but that would have required considerably greater quantities of crab meat than were then available. The work was therefore temporarily discontinued until December, when the peak in fluorescence is expected to appear again.

SUMMARY NO. 17

THE EFFECT OF FLUORIDATED WATER ON THE CANNING OF FISH — R.A. MacLeod

An opinion concerning the effect of fluoridated water on the canning of fish was expressed for the benefit of Mr. A.T. Muir who was preparing an article on the effect of fluoridated water on the food industry for publication in a trade magazine. According to this opinion, little effect could be expected from the one to two parts per million of fluoride proposed as the addition to the water supply since at least this much and frequently considerably more is normally present in a can of fish when the bones are included.

SUMMARY NO. 18

THE MAILLARD REACTION (NON-ENZYMIC BROWNING) H.L.A. Tarr

Previous summaries regarding this problem, of importance to the canning and other processing of white-fleshed fish, were given in the Station Annual Reports for 1947 (pp. A1-A2), 1948 (Summary No. 25), and 1949 (Summary No. 26); a scientific paper describing the results was published in the Board's Journal in 1950, and a Progress Report on the subject appeared this year (No. 18 in List of Publications). Work on the problem was suspended temporarily until such time as better methods of chemical analyses could be found for determining the causal agents. In the Station Annual Report for 1951 (p. 36) mention was made of the fact that pentoses had been found to occur in tuna flesh and extracts, and this suggested that a pentose might be responsible for browning. During the present year the application of partition chromatography using filter paper has yielded excellent results, and the cause of the browning has been established.

In the previous work cited above it was found that the browning which occurs during the heating of certain white-fleshed fish, as in the usual canning procedures, was due to reactions of the Maillard type, which are brought about by chemical combinations between the various amino groups of the muscle proteins and amino acids with substances of a sugar nature. Attempts to determine the nature of these sugar residues in the muscle failed. The application of the technique referred to above has resulted in their satisfactory concentration and characterization.

Fish muscle tissue is apparently distinct from mammalian muscle in possessing a small quantity of a protein which is linked with nucleic acid (nucleotropomyosin). Nucleic acids are built up of compounds in which various bases (purines or pyrimidines) are linked up with a sugar (ribose). Since ribose is a pentose sugar, and former work had shown that this type of sugar occasions extremely pronounced browning when added to dialyzed muscle which is subsequently heated, it was argued that ribose might be the cause of the browning reaction.

In previous work the degree of browning resulting on heating muscle of white-fleshed fish for a standard time (1 hour in an autoclave at 248°F.(120°C.) had been determined by use of an old Armstrong colorimeter. This instrument was not entirely satisfactory since its use involved superimposing Lovibond glass colour discs (red, yellow and blue) until the right colour was obtained. Determination of browning in the present work has been facilitated by use of a reflectance accessory in conjunction with a Beckman DU model spectrophotometer. For comparative purposes the use of the customary "white" control standard used in reflectance work (magnesium carbonate) proved inadequate, for the reflectance from this material was too high as compared with the muscle samples. A standard was prepared which in its appearance somewhat simulated a hypothetical absolutely "white" fish muscle; this was made by blending 4% high viscosity carboxymethylcellulose plus 2.5% magnesium carbonate with water. When absorption spectra of heated white fish muscle preparations were determined at wavelengths between 400 and 800 millimicrons, it was found that no "peaks" occurred. A wavelength of 500 millimicrons was selected arbitrarily, since excellent comparisons could be made in this region.

Great difficulty was experienced in initial attempts to isolate the sugar component responsible for browning. Usual technique employing trichloracetic acid precipitation of protein, and determination of pentose in alcoholic extracts after precipitation and removal of the barium salts of the sugar phosphate esters in 80% ethanol, yielded inconclusive results. Further attempts to separate the active sugar by drying the muscle in vacuo over sulphuric acid and preparation of an alcohol extract of the dried material followed by chromatographic separation was also unsatisfactory. It was noticed that the muscle preparations became discoloured during drying (yellowish-brown), presumably due to combination of the sugar with amino group, and contained only traces of a sugar corresponding to D-ribose. It was finally found that successful concentration could be obtained in the following manner.

Muscle (1 part) was blended for 1 minute with absolute ethanol (9 parts), the suspension was promptly filtered and 30 ml. of the clear filtrate evaporated to dryness in vacuo over sulphuric acid as rapidly as possible. The dry residue was taken up in 1 ml. of water and the resulting concentrate was applied to Whatman No. 1 papers (0.01 ml. spots at 3-cm. intervals along the starting line). Development was carried out at room temperature for 16 hours with ethyl acetate-water-acetic acid (3:3:1) solvent. The filter papers were dried for one hour at room temperature in an air stream. They were then exposed to ultraviolet light to locate the riboside zones (which, if cut out, would interfere with free ribose determinations). Usually 9 replicate spots were prepared from each extract examined. Ribose was determined in two ways.

(a) Ribose was located on "edge" strip using aniline hydrogen phthalate spray (see below). A strip containing the areas corresponding to ribose was eluted into water (16 ml. for 9 zones), together with a similar strip of the paper which had no riboside zones (control). The eluates were filtered through fine pyrex sintered glass filters to remove fine pieces of filter paper, and duplicate 1, 2 and 3-ml. portions were used for the well-known Mejbaum quantitative orcinol reaction for pentose. That the sugar determined in this way was D-ribose was proven by the fact that pure D-ribose had the same rate of flow on chromatograms, and that mixtures of the muscle extracts with pure D-ribose give only a single well-defined zone on the chromatograms.

(b) The above procedure was found to be very time consuming, and so the following technique was used in most experiments. The filter papers were dried as usual after solvent development, were then sprayed as evenly as possible with aniline hydrogen phthalate reagent and heated for exactly 5 minutes at an average temperature of 105°C. to develop the colour. It was found that this method often developed zones corresponding to D-glucose, D-ribose or to both sugars. The amount of each sugar was determined directly in a Beckman spectrophotometer by means of a "spotometer" (made by Mr. Freeman of the Station staff). The maximum absorption of light of 400 millimicrons wavelength by the coloured zones was found to compare well with the amount of sugar (D-ribose or D-glucose) applied to the chromatograms (1 to 40 µg. in 0.01 ml.). The optimum working range was about 5 to 20 µg.

Using these techniques it has been established definitely that free D-ribose is the important limiting factor in causing non-enzymic browning (Maillard) reactions in fish muscle. With certain fish it has also been shown that heating the muscle occasions a loss (disappearance) of free ribose which corresponds closely with the difference between total ribose and free ribose.

This correlation does not always appear to hold true, but certain factors such as inconsistencies in results obtained in determination of total (combined plus free) D-ribose, and possible hydrolysis of the compounds formed between D-ribose and amino compounds (proteins, etc.), may affect the results. Usually the differences between total D-ribose (0.1 to 0.3% of wet muscle) and free D-ribose (0 - 0.07%) is so great that it is not measurable with any accuracy.

It was found that about five times as much glucose as ribose was required to cause about the same amount of browning when added to white fish flesh (halibut muscle). The amount of browning in heated samples of flesh of some different fish is, within reasonable limits, a function of the amount of free D-ribose present as the accompanying table shows. The values show some "scatter", but this is by no means unexpected, for browning results from a series of complicated chemical changes among which the initial combination of sugar and protein is only one. It would seem possible that, after this initial reaction has occurred, browning may proceed more rapidly in one variety of fish than in another.

Fish	% Free ribose	% Light transmitted at 500 mμ.
Mullet (freshwater fish)	Not measurable	69
Halibut	0.035	63
Albacore (fresh)	0.007	57
Albacore (frozen)	0.03	56
Albacore (frozen, defrosted and held 7 days in ice)	0.029	59
Bonito (frozen, defrosted and held 7 days in ice)	0.18	48
Lemon sole	0.14	40
Skipjack tuna	0.20	37
Lingcod	0.55	33
Red cod	0.25	28

Many of the samples contained D-glucose, some in concentrations of the same order as D-ribose. However, none contained sufficient to account for appreciable browning, for experiments as noted above have shown that glucose is about one-fifth as active as ribose in occasioning browning under comparable conditions.

In order to ascertain the origin of the D-ribose, experiments have been carried out with living fish. Due to inadequacy of local aquarium facilities only sculpins and tom cod were available. Two sculpins and one tom cod were frozen in liquid nitrogen while alive, promptly conveyed to the laboratory and stored at about -25°C. The flesh of these was removed with a chisel as required. The following results have been obtained using these samples.

It was found that there was no demonstrable free ribose, glucose or ribosides in these muscle preparations. However, when samples were defrosted and stored for 2 days at 0°C. (32°F.) free ribose and some glucose was found. Thus the ribose content of sculpin muscle was 600-700 μg./gm. and of the tomcod only about 20 μg./gm. These values did not alter appreciably on

storage at this temperature for an additional 2 days. It was also noted that the defrosted samples, unlike those taken from the live fish, contained free ribosides as determined by irradiation of the chromatograms with ultraviolet light. It is highly probable that practically all of the free ribose arises by post-mortem hydrolysis of the glycoside linkage in one or more of these ribosides. In fact experiments have shown that free ribose is formed when the nucleosides adenosine, guanosine, uridine, and cytidine are added to muscle of sculpins or of lingcod, and the samples incubated at 0°, 4° or 20°C. A mixture of the 4 ribosides increased the ribose content of one sample from 480 to 1300 µg./gm. after 2 days at 0°C. Further work on this problem is in progress using ribose nucleic acid, ribotides and ribosides.

A short preliminary note concerning this work has been accepted for publication in "Nature", in addition to the Progress Report appearing this year as mentioned above.

SUMMARY NO. 19

PREPARATION OF RIBOSE-5-PHOSPHATE AND RIBOSE-3-PHOSPHATE — H.L.A. Tarr

In connection with work on non-enzymic browning described in Summary No. 18 a supply of the above compound was desired.

500 mg. of the disodium salt of adenosine triphosphoric acid (Nutritional Biochemicals) was heated for 11 minutes in a minimal quantity of 1 N HCl at 95 - 98°C., and the solution cooled rapidly after adding 600 mg. of hydrated barium acetate. The pH was adjusted to 8.2 with 30% NaOH, and the solution stored for 30 minutes at 0°C. The precipitate was concentrated by centrifuging, and 80 ml. of absolute ethanol was added to the clear supernatant liquid (20 ml.). After standing overnight at 0°C. the precipitate was centrifuged, washed with a little 80% ethanol, and finally triturated with absolute alcohol. The precipitate was collected on a small sintered glass filter and washed with ether. Yield was 267 mg. of barium ribose-5-phosphate.$5H_2O$ (73% of theory). The ribose content of the preparation by orcinol reaction (Mejbaum) was 52.6% (theory 52.8%). No barium or phosphorus analyses have been made as yet.

Barium ribose-3-phosphate was prepared from yeast adenylic acid (adenosine-3-phosphoric acid) by a method similar to the above. The yield was 1.3 gm. of air-dried white powder (presumably barium ribose-3-phosphate) from 2.5 gm. of the free adenylic acid. This product has not yet been analyzed.

SUMMARY NO. 20

QUANTITATIVE DETERMINATION OF RIBOSIDES AND RIBOTIDES — H.L.A. Tarr

Further to the work reported in the preceding Summaries Nos. 18 and 19, fish muscle does not appear to contain appreciable amounts of deoxyribonucleic acid, therefore no exhaustive attempts to demonstrate 2-deoxyribose or its constituent nucleosides have been made. On the other hand post-mortem fish muscle contains certain ribosides and, apparently, ribotides. In order to determine these a quantitative recovery method suitable for extracts prepared as above (Summary No. 18) has been worked out. It consists of a very short (about 8 hours) filter paper chromatographic separation at 37°C. using a solvent not hitherto used for nucleic acid work. The nucleosides are determined

quantitatively by passing the oil-impregnated filter paper strips through a Beckman DU instrument equipped with a spotometer, the absorption at 260 millimicrons (ultraviolet) being recorded. Further work is being carried out in order to determine ribotides.

SUMMARY NO. 21

FREE SALT IN DRY SALT HERRING — P.J. Schmidt

During the 1951 herring season, Formosa requested that all its purchases of Canadian dry salt herring be certified by the Department of Fisheries to contain not more than an average of 40 lb. of free salt per 400-lb. box of fish. The Department and the industry were faced with several problems. It was not known how much salt would have to be added when packing to ensure that there would not be more than 40 lb. of free salt per box on the average when leaving the B.C. port. Varying amounts of free salt adhered to the fish from the curing process, for which an allowance would be necessary when adding salt during packing. It was known that after packing there was a considerable loss of salt as it dissolved and drained from the box.

Several members of our staff attended meetings with the Department of Fisheries inspectors and the Industry. The writer, at the request of the Department, visited Colonial Packers' dry salt herring plant at Otter Bay, B.C. A few experiments were made. The industrial procedure was witnessed and suggestions were made. Tossing the fish about in a piece of fish net was found to be a satisfactory method of determining experimentally the amount of free salt in a box of fish. Cured herring which had been subjected to a seawater spray to remove adhering salt, were packed with 37 lb. of salt. After 5½ days the free salt that could be recovered from 10 boxes by shaking in the net was as follows: 21, 22, 20, 20, 16, 21, 23, 22, 19 and 24 lb., or an average of 20.9 lb. This meant that there was an average loss of 16.1 lb. When the fish were packed with 75 lb. of salt, the loss was found to be 19 lb. (figure for a single box only). Assuming a loss of 15 lb., the cured fish, free of adhering salt, could be safely packed in 55 lb. of salt.

SUMMARY NO. 22

ANALYSES OF DRY SALT HERRING — P.J. Schmidt

At the request of the Department of Fisheries and B.C. Packers Ltd., an experiment was carried out to follow the progressive change in salt and moisture content of salt herring during the salting process. The results were reported in Progress Reports No. 92, October 1952, pp. 3-5.

The herring were salted in the usual commercial way under the supervision of Mr. P. Sunderland of the B.C. Packers Ltd. Eleven samples of 25 fish were taken over a period of 13 days and analyzed. Satisfactory average moisture and salt contents were reached between the fifth and sixth day. At the same time, 20 lb. of fish from the same catch was salted in an excess of 100° salinometer brine for 6 days. In 3 days these fish had the same moisture and salt contents as the commercial product had in 6 days. Separate analyses were made of fish from the commercial cure which appeared insufficiently salted after 7 days and were definitely of inferior quality. The salt and moisture content differed somewhat from those of the average herring from the same tank but not in the same proportion as the apparent quality.

The analytical results from day to day varied in a somewhat erratic manner and in such a way as cannot be explained by differences in oil content or size of fish. These analyses and others of individual fish have shown that the salting by the usual commercial method may not be uniform. It may be that the fish are so tightly packed in the tank that no salt can get to some. This could be overcome by stirring, which, however, is very impractical on a commercial scale.

SUMMARY NO. 23

ANALYSES OF DRY SALT FISH — P.J. Schmidt

From time to time samples of dry salt herring are submitted for salt and moisture analyses by companies and by Department inspectors. Samples from B.C. Packers Ltd., Nelson Brothers and Colonial Packers were analyzed. The results are recorded below:

Sample	Salt %	Moisture %	Oil %
A	12.6	51.3	15.6
A	11.0	51.6	17.5
B	10.4	51.5	16.8
B	12.6	50.1	17.1
A	11.2	48.0	17.6
C	10.7	49.1	-
B	10.8	48.6	16.1

Todd & Sons, Ltd. submitted a sample of salt cod for moisture analysis. The moisture content was found to be 50.3%.

SUMMARY NO. 24

THE PRESSING AND DRYING OF FRESHWATER FISH FOR FISH MEAL — A.W. Lantz, P.J. Schmidt

This work was started last year in an effort to show how freshwater fish wastes and waste fish could be utilized. A 20-ton hydraulic curb press was pruchased for this and other future work. Pressing and drying data were obtained for whitefish fillet waste, whole mullets and eviscerated and whole tullibee. If the correct procedure is used, satisfactory cakes can be produced from these fish and wastes. For example, press cakes from eviscerated tullibee could be dried at 85°F. and relative humidity of 30% to a moisture content of 8.5% in 5 days. This work has now been terminated and this year's results will be reported during the year in Progress Reports No. 93.

SUMMARY NO. 25

PRESSING AND DRYING OF TUNA WASTE FOR MEAL — A.W. Lantz, P.J. Schmidt

Hart and Howes Co. of Steveston, B.C., requested information on the utilization of tuna waste, a by-product of tuna canning operations. A method of producing meal from this material was desirable without resorting to large-scale reduction equipment. Experiments using an experimental batch-type press built at this Station were carried out. This press was described in Progress

Reports No. 88, pp. 66-67 (Oct. 1951).

The tuna waste was cooked for 5 minutes in water at 160°F. and then pressed while hot to remove some of the oil and water. Cakes ¾ inch thick were produced. Some of the data obtained are recorded below:

Analyses:

Moisture in raw material	59.8 %
Oil " " "	5.41%
Moisture in press cake immediately after pressing	47.2 %
Oil " " " " " "	4.49%

Proportion of raw material removed by pressing:

As oil	3.0 %
As water	34.6 %
As other material (protein, etc.)	9.0 %
Total	46.6 %

Proportion of raw material components removed by pressing:

Oil	55.5 %
Water	57.8 %
Other material (protein, etc.)	25.8 %

Proportion of raw material obtainable as meal having 12% moisture: 32.0 %

The cakes were then allowed to dry. Those dried in the canning room of the Station where temperature varied from 50 to 60°F. and the relative humidity from 60 to 80% reached the equilibrium moisture content of 10.9% in 14 days. Some of the cakes were dried in an air-conditioned tunnel where the temperature was 90°F. and the relative humidity was 30%. These cakes dried to the equilibrium moisture content of 4.5% in 4 days. Time——percent moisture curves which were drawn from the date obtained showed that if cakes with 12% moisture were desirable, they must be dried for 1.7 and 11.4 days in the tunnel and room respectively. The oil content corresponding to 12% moisture was 7.5%.

After drying, the cakes are ground into a meal. Such a meal is of superior quality, as high temperatures during cooking and drying are avoided in this method.

SUMMARY NO. 26

BIOLOGICAL VALUE OF HERRING MEAL AND RELATED PRODUCTS

J. Biely, B.E. March,
(Dept. Poultry Husbandry, U.B.C.)
H.L.A. Tarr

The results obtained in last year's work were summarized on pages 29-33 of the Station Annual Report, and during the present year two Progress Report articles and one scientific paper concerning the results have been published. A number of biological tests have been carried out using chicks or turkey poults

with the following results.

In the 1951 Annual Report an experiment was described in which the data obtained indicated that the fat contained in ideal (air-flow dried) herring meal which was heated for 2 hours at 300°F. was so altered that it retarded chick growth. During the present year this finding has been substantiated in a further chick feeding experiment. It was found that chicks did not grow as rapidly when fed a ration in which ideal meal (containing its normal fat) was heated as described above, as when a similarly treated meal from which most of the fat had been extracted before heating was fed. When the extracted oil from the heated meal was added to a similarly heated, hexane-extracted meal growth impairment of chicks resulted. In order that these experiments may be extended, ideal and normal commercial herring meals have been extracted with hexane and samples of both the unextracted and extracted meals are being stored at 98°F. and at 70°F. These samples will be subjected to chick assays after they have been stored for over 6 months, the experiments being designed to ascertain whether the fat oxidation which has occurred has rendered the fat toxic.

In previous experiments in which ideal meals had been found somewhat superior to commercial meals in promoting chick growth the results had not been strictly comparable because the meals had not been prepared from identical material. This weakness was remedied during the present season, for, through the help of the fishing, air-flow dried (ideal), normal commercial, and seriously overheated commercial flame-dried herring meals were prepared from the same lot of herring in November 1951. Using rations containing 21% protein, and in which the above fish meals constituted the main source of the protein, the results have substantiated and extended those obtained with ideal and normal commercial meals during the 1950-1951 season. Moreover, it was found that supplementation of the normal ration with a number of vitamins of the B complex not usually used in rations of this type caused both the normal and overheated flame-dried meals to support growth of chicks as well as did the ideal meal. This mixed vitamin supplement plus penicillin resulted in even better growth of the chicks, the commercial meals under these conditions proving slightly but not significantly superior to the ideal meal. Very similar results were obtained in another feeding trial in which the fish meals constituted the main source of protein in rations containing 17% instead of 21% protein. A further supply of ideal and commercial herring meals prepared by the industry in late December 1951 became available for feeding trials early in January, and have therefore for convenience been called January 1952 meals. These meals were compared with each other and with the above three November 1951 meals in a feeding trial in which they constituted the sole protein supplement in a ration containing 21% protein but without the addition of the large number of vitamins of the B complex mentioned above. The ideal meals promoted a faster rate of growth of chicks fed on this ration, which bore out the findings in the previous experiments, and extended them to include the newly prepared meals.

Further tests were designed with a view to determining the lack of which of the B vitamins was mainly responsible for the relatively poor growth which occurred with commercial meals. Three commercial meals from the 1950 and 1951 seasons, and a November 1950 and February 1951 ideal meal were fed to chicks using a ration similar to that employed in the above experiments only without the mixed B vitamin supplement, and an identical ration in which folic acid was supplied at the rate of 0.035 gm. per lb. Folic acid improved chick growth obtained with the commercial meals so that it was equal to that obtained with the ideal meals. Since the meals used had been stored for some time at

ordinary temperatures it would appear that their nutritive value was not greatly impaired thereby. However, further experiments mentioned below do suggest that very prolonged storage may cause impairment in the nutritive value of fish meals. Microbiological assays for folic acid have shown that commercial meals contain practically no folic acid while ideal meals contain appreciable amounts of this vitamin. Laboratory heating of ideal fish meal in a drum at 300°F. does not appear to exert the same destructive action toward folic acid as does commercial flame drying. This may be due to retention of moisture during the drum drying procedure. Thus in one experiment the addition of folic acid to the unheated January 1952 ideal meal, or to the same meal which had been heated for 75 minutes at 300°F., had no appreciable effect on its growth-promoting activity for chicks. On the other hand the addition of folic acid did promote chick growth on the ration containing the same meal which had been heated for 150 minutes at 300°F. This last-named meal had lost about one-third of its folic acid due to the prolonged heating, while that heated for 75 minutes did not have its natural folic acid content impaired.

Since fish meal, condensed fish solubles, and similar protein products are normally fed at levels of 2.5 to 6.0% in chick or turkey poult starter rations, experiments designed to determine whether such products possess special growth-promoting properties are being carried out. Four experiments have been successfully concluded and have been the subject of a short article in Progress Reports No. 92 (publication No. 4 in List of Publications). It has been shown that better growth of chicks is obtained with a mixed protein supplement containing soyabean meal and herring meal together than is obtained when either is used alone as the main protein supplement. Furthermore, with a corn-soyabean meal ration containing all known B vitamins, a slight but not significant improvement in chick growth resulted when 2.5 or 5.0% fish meal or penicillin was added, but when both were added together a significant increase in growth rate was observed. With turkey poults raised on a corn-soyabean meal ration supplemented with all known B vitamins the addition of 5% of herring meal improved growth as did aureomycin, but the inclusion of both these supplements in the ration caused better growth than did either used alone. These experiments indicate that herring meal contains a factor (or factors) which is apparently not one of the known B vitamins and which has definite growth promoting properties for chicks and turkey poults. Certain other laboratories have reported similar findings. Further work will be designed to attempt to concentrate the active principle(s).

In connection with the research concerning uncharacterized growth factor(s) in fish products a number of materials were prepared from herring (See Summary No. 37). These included whole blended herring, blended herring autolyzed at pH 3, a lactic acid bacterial fermentation product prepared with an industrial (Danish) culture ("L.K.C." product) and a somewhat similar material prepared from identical material using known lactic acid bacteria. These were fed, in addition to typical local commercial samples of condensed fish solubles and herring meal. Two experiments were carried out using a typical all vegetable protein basic ration to which 2.5 and 5.0% of the different products were added. In the second experiment a similar all-vegetable basal ration was used as the control. It was supplemented with a complete mixture of vitamins of the B complex in one case and the vitamin mixture plus aureomycin in another.

The results of these experiments are given in Tables I and II. The first experiment showed that all the herring products tested at the two levels caused a small increment in growth. In the second experiment it was found that the

addition of 5% of herring meal to the ration caused an increase in growth rate with all three types of ration. On the other hand none of the remaining products enhanced chick growth with any of the three rations.

These results may seem rather inconsistent, but tests in which the growth increment due to some uncharacterized factor(s) is never large are rarely consistent. Synthesis of growth factors by the chick intestinal flora, thiaminase or other "anti-vitamins" in the unheated fish products, may play a part in causing rather irregular results. However, these experiments indicate that no special object appears to be gained by preparing autolysates or fermentation products rather than fish meal as far as chick feeding is concerned.

TABLE I. Growth response of chicks to various herring preparations as supplements to an all-vegetable starting ration.

Supplement	Weight of chicks (grams) using	
	2.5% of supplement	5% of supplement
None (wt. of controls, 313 gm.)	-	-
Herring meal	342	354
Condensed herring solubles	358	342
Ground whole herring	347	362
Autolyzed " "	334	334
"L.K.C." product	319	341
Laboratory fermentation product	344	344

TABLE II. Growth response of chicks to various herring preparations as supplements to all-vegetable starting rations.

Supplement (5%)	Weight of chicks (grams) with		
	No addition	Vitamin mixture	Vitamin mixture plus aureomycin added
None	408	411	477
Herring meal	480	476	522
Ground whole herring	400	405	463
Autolyzed whole herring	400	394	442
Laboratory fermentation product	381	382	434

During the early stages of this investigation concerning the nutritive value of B.C. herring products it was suggested that herring meals be stored for at least a year to ascertain whether any differences in their nutritive value became apparent after that time. Accordingly, samples were stored under normal warehouse conditions and were used as the chief protein supplements in similar rations to those used in the original tests. The results of this experiment are given in Table III. The average weights of the chicks fed the

November 1950 low temperature meal were arbitrarily given values of 100 in both the original test and in the test conducted on the stored meals. Before storage, the meals, apart from the November 1950 low temperature dried (ideal) meal, were very similar in nutritive value. After storage, however, there were evident differences in the quality of the meals. The two oldest commercial meals did not promote so rapid a rate of growth relative to the other meals as they did formerly. The least drop in nutritive value occurred with the low temperature meal which had been most recently processed. It is apparent that changes in the nutritive value of herring meals occur during storage and that these changes are influenced to some extent by the processing methods used in the manufacture of the meals. It remains to be ascertained whether toxicity of oxidized oil or some other factor is responsible for these differences.

TABLE III. The relative nutritive value of different herring meals before and after one year storage as measured by growth response of chicks.

Description of herring meal	Relative nutritive value	
	Before storage	After storage
Low temperature processed Nov. 1950	100	100
Commercial processed Feb. 1950	78	64
Commercial processed Nov. 1950	81	68
Commercial processed Feb. 1951	81	79
Low temperature processed Feb. 1951	80	86

SUMMARY NO. 27

THE EFFECT OF OVERHEATING OF THE AVAILABILITY OF ESSENTIAL AMINO ACIDS IN HERRING MEAL DESTINED FOR ANIMAL FEEDING — Miss H.M. Bissett

This investigation is the continuation of work reported in the past two Station Annual Reports. The main objectives of the work were to determine the effect of heat on the nutritive value of herring meal and also to detect any seasonal variation in amino acid content of herring meals. For this purpose three lots of meal were prepared. The first was a November meal prepared under ideal conditions from industrial herring presscake by air-flow drying at 100° to 110°F. Portions of this meal were heated for ½, 1, 2 and 3-hour periods at 300°F. in a rotating heated steel drum. The second lot of meal was a February meal and was treated in the same manner as the first meal. Since these methods of preparation did not duplicate conditions in commercial flame driers, it was thought that one set of meals should be prepared under commercial conditions to give a truer picture of the available essential amino acid content of herring meal. For this purpose the B.C. Packers Ltd. and Canadian Fishing Co. Ltd. jointly prepared air-flow-dried meal, meal dried under normal commercial conditions and meal dried under abnormal conditions as regards heating.

The November and February meals were assayed for the 11 essential amino acids. The commercially prepared November meal was assayed only for the three most important amino acids. The methods used were the same as those reported in the 1950 Station Annual Report. Both chemical hydrolysis (for total amino acids) and enzyme hydrolysis (to indicate available amino acids) were employed. Sc. fecalis was used to assay for methionine. All results are expressed in

grams of amino acid per 16 grams of nitrogen, which is roughly equivalent to the percent amino acid in fish protein. The complete tables of resulting data are too extensive to reproduce here; the accompanying table represents the type of data secured.

It was found that the essential amino acids lysine, arginine and methionine present in the whole herring and the presscake remained almost the same in the ideal meal. It was also found that moderate overheating did not seriously affect the availability of the amino acids. The available amino acid content was approximately two-thirds of the total amino acid content, as judged by the in vitro enzyme method used. At the same time, in meals which were heated for 3 hours there was only about one-fifth of the total amino acids still available. These results indicate that prolonged and severe heating is necessary to seriously affect the availability of amino acids in fish meal. This is supported by the fact that the overheated flame-dried meal which was exposed to a high temperature for a comparatively short time gave just as high results as did the ideal flame-dried meal. This finding is important since it parallels very well the biological assays carried out using chicks. It is also interesting to note the great contrast which is evident when the above results are compared with those which have been published for essential amino acids in soyabean meal in other laboratories.

Effect of different methods of preparation on certain essential amino acids in November 1951 herring meals prepared from the same load of herring (gm. of amino acid per 16 gm. of nitrogen).

Meals	Lysine		Arginine		Methionine	
	Acid	Enzyme	Acid	Enzyme	Acid	Enzyme
Ideal meal: Dried B.C. Packers Smokehouse meal (69.52% protein)	10.8	9.0	7.4	6.3	2.7	2.7
Normal commercial meal: (73.9% protein)	10.9	9.2	7.2	6.8	2.5	2.9
Overheated flame dried meal: (76.62% protein)	10.4	9.3	7.3	6.7	2.5	2.8
Overheated flame dried meal (sacks caught fire): (75.65% protein)	10.6	9.3	7.8	6.7	2.8	2.8
Ideal flame dried meal hexane extracted: (76.05% protein)	11.3	9.1	7.3	6.0	2.7	2.8

In soy meal, which is rich in carbohydrates, a Maillard reaction occurs, and the important essential amino acid lysine is rapidly inactivated and becomes biologically unavailable, though it can still be found to a large extent after chemical hydrolysis. In fish meal prolonged overheating causes a progressive decrease in availability of all the essential amino acids.

One unexplained discrepancy has occurred in the amino acid assays of fish meals which have been carried out. Thus the values obtained for a given amino acid while very similar for one series of meals analyzed have often differed considerably for another series of meals. Since the microbiological assay methods used have usually been identical, it is thought that irregularities in the hydrolysis procedure may have been responsible. Experiments designed to test this point will be carried out, fish meals being hydrolyzed for various lengths of time under the same conditions, the liberated essential amino acids being determined at intervals.

SUMMARY NO. 28

THE RIBOFLAVIN CONTENT OF HERRING MEALS — Miss B.A. Southcott

Included in the study of the nutritive value of herring meals was a series of riboflavin assays on meals prepared in various ways; the purpose of this investigation was to determine the effect of the method of preparation of a meal upon its riboflavin content.

Samples were taken from lots which had been processed, as listed in the accompanying table, especially for use in the various tests included in the herring meal programme. Each sample was acid hydrolyzed, filtered, suitably diluted, stored at 0°C. in the dark and later assayed by the microbiological method of Snell and Strong, using Lbc. casei ATCC 7469 as the test organism. Values were obtained by titration with 0.1 N NaOH and comparison with standard riboflavin samples.

Sample treatment	Riboflavin, µgm./gram
Whole herring	1.3
Herring presscake	0.9
Ideal meal	2.3
Ideal meal, heated 300°F. 60 min.	2.3
" " " " 120 "	2.6
" " " " 180 "	2.2
" " hexane extracted	3.0
" " " " heated 300°F. 60 min.	2.2
" " " " " " 120 "	3.2
" " " " " " 180 "	2.6
Commercial meal	2.2

These results indicate no definite trend in the change of riboflavin values when herring meal is heated, hexane extracted or both.

SUMMARY NO. 29

COBALAMIN DISTRIBUTION IN HERRING MEALS

H.L.A. Tarr
Miss B.A. Southcott

An extensive study of the cobalamin content of herring meals was made. It was found that normal commercial flame-dried herring meals had about the same total cobalamin content as specially prepared meals dried at 38 to 43.5°C. Chromatographic separation, elution and assay of the cobalamins in herring meal samples indicated that substantially all of the activity found by direct assays of the meals was due to the vitamin itself. Hexane extraction of the meals had little effect on the cobalamin content, but heating the meals at 300°F. for extended periods reduced it. A paper describing the results has been accepted for publication in the Board's Journal.

Further work on the cobalamin assay method is not contemplated at the time of writing.

SUMMARY NO. 30

THE DETERMINATION OF FAT IN FISH MEAL

P.J. Schmidt

It is difficult to extract the oil which has become oxidized in old fish meal or in fish meal that has been excessively heated. The oil contents found for such meals can be much lower than the true value. A method was tried whereby the fish meal is heated in boiling acetone for one hour using a reflux condenser. Hydrochloric acid is added to hydrolyze the tissue. The mixture is then centrifuged. The one-hour extractions are repeated until no further oil can be removed.The acetone is evaporated off from the combined acetone extracts and the residue is extracted with ether.

The following samples of fish meal were prepared for this experiment:

Sample A - This was fish meal prepared by Dr. Tarr for another experiment and was an "ideal" meal, having been heated to a temperature of only 38-43°C. for 24 hours.
Sample B - Some of Sample A was heated in a flat pan in an air oven for 60 hours at a temperature of 60°C.
Sample C - This was Sample A heated at 60°C. for 180 hours in the air oven.

The fat in these samples was determined in two ways; the acetone—ether extraction method using the soxhlet apparatus, and the reflux method described above. The results were as follows:

Sample	% Ether extract on a dry weight basis	
	Soxhlet method	Reflux method
A	11.8	14.6
B	9.92	14.1
C	9.30	13.4

These data show that more oil is extracted by the reflux method and although the amount that can be extracted from the heated meals does become less, the difference is not as great as in the case of the soxhlet method. On this basis the reflux method is certainly preferable.

SUMMARY NO. 31

MICROBIOLOGICAL FORMATION OF COBALAMINS — H.L.A. Tarr

In last year's Station Annual Report the status of the work on this subject was reviewed (p. 33-34). With the purchase of a proper rotary shaker for aerobic fermentation studies it has been possible to carry out a number of further experiments. Since these results are not yet published they will be described in some detail.

In one experiment sterile suspensions containing either 2% (dry wt.) of "ideal" (air-flow-dried) fish meal or 2% (dry wt.) of herring stickwater and 2 p.p.m. of cobalt were inoculated with growth from seven different Streptomyces cultures and shaken at 250 r.p.m. and 28°C. on a rotary shaker (pH 6.8). After 5 days the pH was readjusted (the solutions were markedly alkaline in reaction) to 6.8. The suspensions were then heated for 15 min. at 120°C. and the filtrates assayed microbiologically. A cup-plate assay using a vitamin B_{12}-requiring E. coli mutant proved unsatisfactory, because the zones of exhibition were rarely clear cut, but usually hazy and indistinct. Assays were therefore carried out using L. leichmannii with the usual aseptic addition technique. In a few instances the cobalamin (which was entirely in the hydroxo or similar chromatographic form) was separated using KH_2PO_4-treated filter paper and water saturated n-butanol solvent, eluted and assayed. In a further similar experiment a tryptic peptone from starry flounder, lemon sole flesh and chum salmon kidney were all studied using 2% of each (dry wt. basis). The results are given in the accompanying table. They show that there was no very great general difference in the quantity of cobalamin formed by the different Streptomyces species, though in most cases S. aureofaciens was the best producer. The yield from herring meal appeared to be a little greater than that from stickwater. In kidney which is itself an exceptionally rich source of the vitamin, the actual increase was only about the same as that found with other substrates. No evidence for the existence of a substance which was specifically utilized in the syntheses of cobalamins was obtained in these experiments. It will be observed that in these shake cultures assays carried out after chromatographic separation indicated that not all the microbiologically active substances found by direct assay were actually cobalamins.

Further experiments carried out along very similar lines with the three Streptomyces cultures used in the latter part of the above experiment showed that regular readjustment of the pH to about 6.8 during fermentation, or addition of 1% glucose and 0.5% calcium carbonate as buffer, had no important effect on the cobalamin yield. When the ideal herring meal and stickwater were used at 2, 4 and 5% level in a 5-day shake culture experiment, the cobalamin yield was not altered to any important extent, though there was a tendency in some cases for a lower yield with 4% solids.

Further studies in an attempt to determine possible relation between substrate and cobalamin formation were made as follows. A synthetic basic solution (Czapek) containing inorganic salts but not nitrate or ammonia nitrogen was prepared. To this was added 0.2% $NaNO_3$ plus 1% of formic acid, glucose or arabinose. Another series contained 0.2 and 1% of asparagine or 1% of glycine but no nitrate. All these solutions were prepared in triplicate, and one of each was inoculated with S. griseus (ATCC), S. aureofaciens or S. olivaceus. The results, after 7 days shaking at 28°C., showed that there was very considerable variation in the amount of cobalamin formed.

Substrate (2% dry wt.)	Culture used	Cobalamin content (mμg./ml.) Direct assay	After chromatographic separation
Ideal herring meal	uninoculated control	6.5	-
" " "	S. olivaceus	184	106
" " "	S. aureofaciens	425	290, 313
" " "	S. griseus[1]	202	-
" " "	S. griseus[2]	284	-
" " "	S. griseus[3]	337	-
" " "	S. griseus[4]	294	-
" " "	S. griseus[5]	260	-
Stickwater	uninoculated control	23	-
"	S. olivaceus	69 (poor growth)	-
"	S. aureofaciens	200	155
"	S. griseus[1]	106	-
"	S. griseus[2]	99	-
"	S. griseus[3]	101	-
"	S. griseus[4]	136	-
"	S. griseus[5]	136	-
Starry flounder peptone	uninoculated control	2.1	-
" " "	S. olivaceus	201	121
" " "	S. aureofaciens	168	-
" " "	S. griseus[5]	75	-
Lemon sole muscle	uninoculated control	0.63	-
" " "	S. olivaceus	243	148
" " "	S. aureofaciens	246	-
" " "	S. griseus[5]	199	-
Chum salmon kidney	uninoculated control	182	-
" " "	S. olivaceus	467	-
" " "	S. aureofaciens	452	-
" " "	S. griseus[5]	361	203

1 = Waksman's strain No. 3478
2 = Strain from Dr. P. Trussell
3 = Strain from Merck & Co.
4 = Strain from U. of Texas
5 = A.T.C.C. No. 10137

In the formate solution growth was absent. In the nitrate plus glucose or nitrate plus arabinose solutions S. griseus grew hardly at all, while both S. olivaceus and S. aureofaciens grew and formed 10-34 mμg./ml. of apparent cobalamin. Both glycine and asparagine supported growth and cobalamin formation, but to a variable extent (2 to 23 mμg./ml.).

In a further experiment the above inorganic substrate plus 0.5% asparagine and 0.1 mμg./ml. of arabinose was used as substrate in order to determine whether two known "fragments" of the cobalamin molecule increased cobalamin formation. Both 5,6-dimethylbenziminazole and 1,2-diamino-4,5-dimethylbenzene

were tested in 1 and 10 µg./ml. amounts. The results, employing the three Streptomyces species used previously, indicated that there was no marked increase or suppresion of cobalamin formation with these substrates. A further test was made with S. aureofaciens in shake cultures using stickwater (2% solids) with 10, 20 and 50 µg./ml. of the above two compounds. Growth was good in all cases, but accurate microbiological assays for cobalamin could not be carried out because of the high level of antibiotic produced in the solutions. S. griseus was used instead of S. aureofaciens in a similar test with the same levels of the two chemicals. There was some depression in cobalamin formation with 50 µg./ml. of 5,6-dimethylbenziminazole. However, production of cobalamin was not very different since the amounts formed only varied between 163 and 237 mµg./ml.

The results obtained in these experiments in which attempts were made to relate the yield of cobalamin to the substrate used were not encouraging, and the investigation of the microbiological formation of cobalamin will be carried out from a different angle.

SUMMARY NO. 32

PREPARATION OF 1,2-DIAMINO-4,5-DIMETHYLBENZENE AND 5,6-DIMETHYLBENZIMINAZOLE

H.L.A. Tarr

These compounds were desired for the work described in Summary No. 31, and, since they were not available at the time, they were prepared as follows.

The first attempt to make the diamine was by the following route:

o-xylol (1) —nitration→ 4-nitro-o-xylol (2) —reduction→ 4-amino-o-xylol (3) —nitration→

4-amino-5-nitro-o-xylol (4) —reduction→ 4,5-diamino-o-xylol, (5)

which is 1,2-dimethyl-4,5-diaminobenzene, a synonym for the compound desired. Compounds (2) and (3) were prepared readily, but attempts to nitrate compound (3) at -15°C. with nitrosylsulphuric acid, as described in the literature, were unsuccessful. The following method was adopted, and proved satisfactory.

o-xylol (1) —nitration→ 4-nitro-o-xylol (2) —nitration→ 4,5-dinitro-o-xylol (6)

—reduction with PtO_2 in methanol→ 4,5-diamino-o-xylol (5) (= 1,2-diamino-4,5-dimethylbenzene) (M.P. 114-115°C.)

The yield of (5) from (3) was quantitative, but the crystals of (5) were somewhat discoloured. Recrystallization from hot water with a little charcoal yielded white crystals which were rendered anhydrous by drying in vacuo over P_2O_5.

5,6-dimethylbenziminazole (M.P. 204-205°) was prepared from the above compound (5) by the method of Folkers et al. by condensation of the diamine in presence of formic acid.

SUMMARY NO. 33

CHROMATOGRAPHIC SEPARATION AND MICROBIOLOGICAL ASSAY OF INDIGENOUS AND ADDED COBALAMINS (FORMS OF TRUE VITAMIN B_{12}) IN CRUDE ANIMAL PROTEIN MATERIALS

H.L.A. Tarr

On page 31 of last year's Station Annual Report the difficulties experienced in obtaining reproducible assays for vitamin B_{12} were described. Failure to obtain reproducible results under different conditions of extraction and assay prompted a thorough investigation of the assay procedure. This was also necessitated because of the extremely confusing and conflicting reports of various investigators.

Since the work which was carried out has been published in detail during the year (No. 14 in List of Publications), the results will be reviewed briefly. Extensive chromatographic separation experiments conducted during the past two or three years have shown that all, or nearly all, the cobalamins in crude fish materials occur as the hydroxo form, or at least in a form or forms which exhibit closely similar Rf values on paper partition chromatograms. These forms may be chloro, nitro or other cobalamins. Since vitamin B_{12} itself (cyano-cobalamin) will cause very different microbiological response under different conditions of assay using, for example, Lactobacillus leichmannii, and different cobalamins cause different response under identical assay conditions, it was considered essential that a technique be found with which identical response of the assay organism to different cobalamins would result. After considerable exploratory work it was decided to adopt the technique of aseptic addition of the cobalamin-containing solution to the previously sterilized assay medium. Since only cyano (vitamin B_{12})- and hydroxo (vitamin B_{12a} and B_{12b})-cobalamins were available, all work was carried out using these, and all assays were controlled with both vitamins. It was found that aseptic addition of sterile aqueous solutions of these forms of the vitamin to Skegg's L. leichmannii assay medium caused identical response of the assay organism, providing the assay conditions (particularly inoculum and incubation) were kept strictly constant. It was found essential, using this method, that growth of the assay organism be rapid, for prolonged incubation of the vitamins in the uninoculated assay medium containing reducing agents caused much more rapid inactivation of cyano- than of hydroxo-cobalamin. The reverse was the case when the vitamins were sterilized in the assay medium in presence of reducing agents.

Using this technique an extensive study of the cobalamin content of certain fish organs and protein by-products has been made. In many cases recovery experiments were made using added cyano- or hydroxo-cobalamin, and assays were carried out on crude extracts, and on the same materials after the cobalamins had been separated by paper partition chromatography and eluted into sterile water. The method of chromatography was that which has been employed for some time at this Station in work on fermentation production of cobalamins, for it separates not only cyano- and hydroxo-cobalamins from one another, but also from certain interfering substances such as deoxyribosides.

From 91 to 107% of the total cobalamins in herring meal as indicated by direct assay was recovered after chromatographic separation on KH_2PO_4-treated filter paper, elution and assay. When 500 mμg. of either cyano- or hydroxo-cobalamin was added per gram of herring meal and the aqueous suspensions were autoclaved at 120°C. for 5 minutes, direct assays yielded 96 to 111% of the expected amounts, and of this 81 to 99% was recovered after chromatographic

separation. When 10% aqueous herring meal suspensions were autoclaved at pH 7.0 with 0, 1, 10 and 100 µg. of KCN per ml. the following respective ratios of hydroxo- to cyano-cobalamin were found: 95:5; 90:10; 64:36 and 50:50. No important difference in the total cobalamin content of herring meal was found as a result of cyanide treatment, but autoclaving with 0.5 mg. per ml. of mercaptosuccinic acid caused a fictitiously high recovery. Chromatographic separation of the cobalamins of raw and autoclaved fish kidney and beef liver indicated that hydroxo-cobalamin, or at least forms with closely similar chromatographic properties, account for at least 95% of their total (chromatographically separable) cobalamin content. The following results were obtained by direct assay: sockeye salmon kidney 3664, chum salmon kidney 2110, and beef liver 1580 mµg. of total apparent cobalamin activity per gram of wet weight, of which 71, 91 and 55% respectively was recovered following chromatographic separation, elution and assay.

Ample supplies of the following have been very kindly donated for certain problems outlined in this Summary and in other Summaries by the writer and his associates:

Vitamin B_{12f}, Professor Conrad Elvehjem and Dr. Lewis, Dept. of Biochemistry, Madison, Wisconsin.

Pseudocobalamins B_{12a} and B_{12b}, Dr. J.J. Pfiffner, Parke Davis and Co., Ltd., Detroit, Mich.

Crystalline vitamin B_{12} (Cyanocobalamin) (assay standard). Dr. H.B. Woodruff, Merck and Co., Rahway, New Jersey.

Vitamin B_{12}, U.S.P. crystals, Miss A.M. Wilson, Merck and Co., Montreal.

Vitamin B_{12a}, Dr. H.B. Woodruff, Merck and Co., Rahway, New Jersey.

Vitamin B_{12b}, Chas. Pfizer and Co., Brooklyn, N.Y.

Vitamin B_{12}, U.S.P. crystals, Chas. Pfizer and Co., Brooklyn, N.Y.

Terracin (Terramycin hydrochloride concentrate), Thiolution (an anti-fungal anti-yeast antibiotic) and Terramycin hydrochloride, Chas. Pfizer and Co., Brooklyn, N.Y.

Standard Aureomycin hydrochloride, a culture of an E. coli mutant used in vitamin B_{12} assays, a culture of Bacillus cereus used in the Lederle pad-plate assay for aureomycin, vitamin B_{12b} (Lederle's original preparation) and a preparation of pseudocobalamin, Lederle Laboratories, Pearl River, N.Y.

Samples of the following antioxidants from Tennessee Eastman Co. Ltd.:
Tenox BHA (Butylated hydroxyanisole); Tenox II (70% propylene glycol, 20% butylated hydroxyanisole, 4% citric acid, 6% propylgallate); Texox PG (propylgallate) and Tenox x 3 ("solubilized" butylated hydroxyanisole).

SUMMARY NO. 34

SEPARATION OF DIFFERENT FORMS OF VITAMIN B_{12} — Miss B.A. Southcott

In order to determine the extent to which different forms of vitamin B_{12} occur in various fishery materials, it became necessary to employ a method which would separate vitamin B_{12} from vitamins B_{12a} and B_{12b} and also from the pseudo B_{12} vitamins. Tests were carried out with solutions of the pure vitamins in the hope of finding a method applicable to fish product extracts.

Originally, a bioautographic technique was used in which water-saturated n-butanol was the solvent. Filter-paper sheets, saturated with 0.66M KH_2PO_4 and spotted with various vitamin B_{12} forms, were developed with this solvent and plated using agar seeded with the vitamin B_{12}-requiring mutant, E. coli 113. This procedure was found efficient for separating vitamin B_{12} from B_{12a} and B_{12b}, but not for distinguishing between B_{12a} and B_{12b}, and the pseudo form, B_{12f}. Vitamin B_{12f} divided into two zones, one slow-moving and one fast-moving to the same degree as vitamin B_{12}. In a cyanide atmosphere, B_{12f} gave the same results as it did without cyanide, so that even in this way it could not be separated from vitamins B_{12}, B_{12a} and B_{12b}. It appears that the B_{12f} which was supplied was impure and in the cyano form.

Recently, samples of pseudo vitamin B_{12} and pseudo vitamin B_{12b} have been received, along with the information that B_{12f} had been discovered to be a mixture rather than a pure substance.

Tests are now being carried out to determine the best method of separating these new pseudo forms from vitamins B_{12}, B_{12a} and B_{12b}. Points which are being investigated include the relative efficiency of various times and temperatures of development, the effects of the use of phosphate-treated and untreated filter paper sheets, the results of the use of a cyanide atmosphere, and the degree of separation of various forms obtained with different solvents. At present it appears that pure vitamin samples might be conveniently developed on phosphate-treated papers at 37°C. in a cyanide atmosphere, using as the solvent n-butanol: acetic acid: water in the proportion of 4:1:5. Further work is to be done concerning the method of extraction of fishery materials, and the combination of temperature, time and solvent required for satisfactory separation of the various forms of the vitamin B_{12} in such materials.

SUMMARY NO. 35

ASSAY METHODS FOR VITAMIN B_{12} — Miss B.A. Southcott

In an attempt to find a rapid vitamin B_{12} assay which would give reproducible results with crude fisheries products, several methods using a vitamin B_{12}-requiring mutant of Escherichia coli were investigated. It was hoped that an E. coli plate assay, in addition to being more rapid and simple than the Lactobacillus leichmannii tube-turbidimetric assay, might provide a method which would give results with all forms of vitamin B_{12} and prove adaptable for use with fisheries products.

The cup-plate method was first used. Samples were pipetted into cylinders placed on top of a simple agar seeded with E. coli 113, and the diameter of the growth zone around each cylinder was measured after overnight incubation at 37°C.

Solutions containing from 5 to 500 mμg. vitamin B_{12} per ml. could be conveniently assayed. This cup-plate method was applied to a series of stickwaters which had given values of approximately 100 mμg. B_{12} per ml. when assayed by the tube-turbidimetric method. Filtration of the stickwaters while hot was found necessary in order to avoid the occurrence of precipitates inside the cylinders in even the diluted samples; however, following this treatment certain stickwaters still produced growth zones which were diffuse or irregular.

In a further series of assays with the same stickwaters, bisulphite treatment was employed. Twenty-millilitre samples were autoclaved for one-half hour at 15 lb. pressure with the addition of 0.1% $Na_2S_2O_5$, and were filtered while warm. Sixteen stickwaters were used and values of from 50 to 150 mμg. per ml. were obtained; zone types varied from diffuse to clear cut. When the same samples were steamed one-half hour with 0.05% KCN, fourteen of the sixteen gave zones of excellent type, but the values were low — from 5 to 50 mμg. per ml. The effect of bisulphite and cyanide treatment on pure vitamins B_{12} and B_{12b} was next investigated, since the effects on stickwaters had been irregular with respect to zone diameter and zone type. Solutions of the vitamins containing 250 mμg. per ml. were prepared and treated.

Treatment	pH when treated	B_{12} cm. zone	B_{12b} diameter
Untreated	4.5	2.6	2.2
"	6.0	2.7	2.1
Autoclaved 1 hr.	4.5	2.6	1.6
" "	6.0	2.5	1.6
" " with 0.001% $Na_2S_2O_5$	4.5	1.8	1.5
" " " 0.01% "	4.5	1.2	1.0
Steamed 1/2 " " 0.001% KCN	6.0	2.6	1.9
" " " " 0.002% KCN	6.0	2.6	1.9

The zones obtained were of excellent type, but measurements showed that solutions of vitamins B_{12} and B_{12b} of equal concentration did not give zones of exactly the same size. Cyanide treatment of the pure vitamins did not result in a lowering of results as had the same treatment of stickwaters.

A series of tests was designed to compare values obtained when identical samples were assayed by the cup-plate method as used above and the pad-plate method, in which samples are spotted on filter paper discs ¼" in diameter. The discs, when dry, were placed on the same *E. coli* seeded agar employed for the cup assay. When pure vitamin solutions were used, no significant differences were noted between cylinder zones and pad zones; in each case the values for vitamin B_{12b} were slightly below those for vitamin B_{12}. The use of filter paper pads was found especially convenient because samples could be spotted, dried and then stored until assayed; in addition, the use of pads makes it possible to cover a wide range of values in the standard solutions and to prepare quickly a large number of aliquots of a sample.

It is hoped that further work will show that the pad-plate technique could be adapted for use in routine vitamin B_{12} assay of crude fisheries products, and that the use of pads might overcome some of the difficulties encountered in both the tube-turbidimetric and cup-plate assays of such products.

SUMMARY NO. 36

A STUDY OF THE EFFECT OF FISHERY PRODUCTS ON YEAST GROWTH

R.A. MacLeod

There are persistent rumours from various sources to the effect that fishery products, in particular whale solubles, are of considerable benefit in ruminant feeding. Since whale solubles have been shown to be a mediocre source of known nutritional essentials, the opinion has been expressed that the effects purportedly observed are due to stimulation of the rumen flora by unknown factors in the whale solubles. We have been informed recently by a representative of a private American laboratory concerned with fishery products that evidence has been obtained in their laboratory which they believe supports the above hypothesis. These investigators claim to have observed that fishery products when added to a medium suitable for the growth of yeasts greatly stimulate the yeasts to utilize inorganic nitrogen salts. They infer from this that if yeasts can be stimulated to utilize inorganic nitrogen, so also can the rumen population, thereby permitting a higher percentage of less costly inorganic nitrogen to be included in the diet of ruminants. Their results with yeasts, we understand, are being used to support a patent application to cover the inclusion of fishery products in prepared cattle feeds.

In view of the report referred to above, it was of interest to us to determine whether we too could demonstrate the presence of a factor or factors peculiar to fishery products which could effect the growth of yeasts. In addition, if yeast growth promoting activity were observed and could be correlated with the chick growth promoting action of fishery products, a convenient assay for the chick factor would be at hand.

The two strains of yeast chosen for the following study were Saccharomyces cerevisiae, a typical baker's yeast, and Saccharomyces carlsbergensis 4228, a brewery yeast. These yeasts were carried as stock cultures on malt agar slants held at 4°C. Inoculum cultures were prepared from these by transferring cells to fresh slants and incubating the latter for 24 hours at 30°C. At the end of this time cells from a slant were added to 10 ml. of sterile water in a test tube until the turbidity as measured in an Evelyn colorimeter corresponded to a concentration of 1 mg. of cells per ml. (as determined by reference to a previously calibrated curve). The 10-ml. suspension was then added to 90 ml. of sterile water. One ml. of this final dilution of yeast cells was added to each assay tube or flask.

The components of the basal medium used in this investigation were the same as are normally included in standard yeast assay media. The composition of the medium is presented in Table I. The nitrogen content and the vitamin content of the medium was increased over that normally employed in assay media to minimize the possibility that any stimulation from supplements could be due to their ability to supply known nutritional essentials.

Supplements for the above medium were prepared to contain approximately 50 mg. of solids per ml. (One-ml. volumes of each of the supplements were added to the appropriate tubes or flasks.) These supplements included two samples of whale solubles, one from a local fishing company, the other from a supply on hand from the South Polar region; a sample of herring solubles; one of stickwater, and one of enzymatically hydrolyzed halibut muscle. Where necessary, samples were clarified by filtration or centrifugation.

TABLE I. Composition of basal medium for the growth of yeasts.

Component	Amounts present in 1 litre of final medium
Glucose	50 gm.
$(NH_4)_2SO_4$	3.5 gm.
KH_2PO_4	0.55 gm.
KCl	0.425 gm.
$MgSO_4.7\ H_2O$	0.125 gm.
$CaCl_2.2\ H_2O$	0.125 gm.
$FeCl_3.6\ H_2O$	0.0025 gm.
$MnSO_4.4\ H_2O$	0.0025 gm.
Potassium citrate	5 gm.
Citric acid	1 gm.
HCl-hydrolyzed casein (charcoal treated) (equivalent to 8.5 gm. original casein)	100 ml.
Asparagine	200 mg.
Tryptophan	200 mg.
Cystine	200 mg.
Thiamin	1 mg.
Inositol	50 mg.
Biotin	50 ug.
Calcium pantothenate	5 mg.
Pyridoxine	1 mg.
Nicotinic acid	1 mg.

Assays were set up in the following manner. Five ml. of double strength medium were added to each tube or flask followed by the supplement and sufficient water to give a volume of 9 ml. The tubes or flasks were then sterilized by steaming for 15 minutes, cooled and inoculated with the 1 ml. of yeast suspension described above. For aerobic experiments the 10-ml. volumes of inoculated medium were incubated at 30°C. on a rotary shaker in 125-ml. flasks. For anaerobic experiments, the medium was placed in 18 x 150-mm. test tubes and incubated in a desiccator made oxygen deficient by the reaction of chromium metal with H_2SO_4 in the presence of Na_2CO_3.

Although fishery products are claimed only to increase the utilization of inorganic nitrogen by yeasts, such an effect would ordinarily have to result in an increased cell yield if products suitable for ruminant nutrition were to be formed. For this reason cell yield rather than inorganic nitrogen utilization was determined in these experiments.

Since the medium shown in Table I is capable of supporting the growth of yeasts, the effect of an unknown growth factor could only be expected either to

increase the speed of attainment of maximum growth or to effect the extent of growth of the organisms. To check the first possibility, 1-ml. volumes of the growth suspension were removed aseptically after 24 hours from flasks incubated aerobically. A 1:10 dilution of this aliquot was made with water and the turbidity of the resulting suspension determined. By reference to a curve relating mg. moist weight of yeast per ml. to turbidity, the actual weight of yeast cells in the original medium was obtained.

Since maximum growth of the organism under aerobic conditions is usually achieved before 40 hours of incubation, the effect of fishery products on the extent of growth of the organism was determined by measuring the weight of cells produced in 45 to 48 hours.

Results typical of those obtained using Saccharomyces carlsbergensis as the test organism are shown in Table II. In reporting these results growth in the unsupplemented medium has been assigned the value of 100. Yeast extract and enzymatic casein hydrolysate (charcoal treated) were included to compare the effect of non-fishery materials on the growth of the yeasts. The effect of adding further vitamins and acid-hydrolyzed casein (charcoal treated) was also determined to ensure that any effects which might be obtained from the supplements were not due to inadequate supplies of these in the basal medium.

TABLE II. Effect of various supplements on the aerobic growth of Saccharomyces carlsbergensis 4228.

	Additions to basal medium*	Growth response** (as percent of unsupplemented medium), after incubation time 24 hrs.	45 hrs.
1.	No additions	100	100
2.	Whale solubles (local)	105	110
3.	" " (South Polar)	115	112
4.	Herring solubles	56	110
5.	Stickwater	113	112
6.	Halibut hydrolysate	109	117
7.	Yeast extract	111	115
8.	Enzymatic casein hydrolysate	103	116
9.	Vitamin supplement	95	106
10.	Acid hydrolyzed casein (charcoal treated)	101	114

* Except for items 9 and 10 supplements were added in amounts calculated to supply approximately 50 mg. of solids per flask. Items 9 and 10 were added in amounts equal to those present in the original medium.

** The calculated limits of accuracy after 24 hrs. incubation are ± 5%; after 48 hrs. incubation ± 10%.

Although no attempt has been made to analyze the results statistically, it can be calculated from known errors inherent in the method and from the dilution factors involved that the results in Table II cannot be more accurate than ± 5% after 24 hours and ± 10% after 48 hours in this particular experiment.

With this fact in mind, a study of the table will reveal that the addition of fishery materials to the medium neither stimulates early growth of the yeast nor affects the extent of growth of the organism to any really significant degree. In the case of one supplement, herring solubles, growth inhibition is obtained after 24 though not after 45 hours, a result which has been observed several times.

The corresponding results with Saccharomyces cerevisiae are shown in Table III. The calculated limits of accuracy in this experiment are ± 11% at 24 hours and ± 7% at 48 hours. With one possible exception, that of the effect of enzymatic halibut muscle hydrolysate on the extent of growth of the yeast, no significant effect of the supplements on growth was observed.

TABLE III. Effect of various supplements on the aerobic growth of Saccharomyces cerevisiae.

	Additions to basal medium *	Growth response ** (as percent of unsupplemented medium), after incubation time 24 hrs.	48 hrs.
1.	No additions	100	100
2.	Whale soluble (local)	96	98
3.	" " (South Polar)	98	98
4.	Herring solubles	95	110
5.	Stickwater	101	103
6.	Halibut hydrolysate	108	116
7.	Yeast extract	99	96
8.	Enzymatic casein hydrolysate	105	106
9.	Vitamin supplement	98	101
10.	Acid hydrolyzed casein (charcoal treated)	102	106

* Except for items 9 and 10 supplements were added in amounts calculated to supply approximately 50 mg. of solids to each flask. Items 9 and 10 were added in amounts equal to those present in the original medium.

** The calculated limits of accuracy after 24 hrs. incubation are ± 11%; after 48 hrs. incubation ± 7%.

A number of assays were arranged to determine the effect of fishery materials on anaerobic growth of the yeasts, since the rumen is an anaerobic system and results under these conditions might better reflect the action to be expected there. Unfortunately, yeasts grow rather erratically under anaerobic conditions. Sufficient experiments were run, however, to conclude that fishery materials had no more effect on the growth of the yeasts under anaerobic than under aerobic conditions.

The results reported above do not support the conclusion that a growth factor peculiar to fishery products affects the growth of yeasts. There is thus a discrepancy between these results and those of the American workers.

This variance may be due of course to a difference in the strains of yeast used. The effect of this discrepancy, however, on the validity of the hypothesis that since fishery products will enhance the growth of a strain of yeasts they will enhance the growth of the rumen flora, is obvious. If the phenomenon reported cannot be reproduced in organisms so closely related as other strains of yeast, then it is difficult to justify the conclusion, without direct experimental evidence, that fishery products will stimulate such a widely different group of organisms as are found inhibiting the rumen. Further doubt is cast on this possibility when it is realized that yeasts are not among the micro-organisms usually found to compose the native flora of the rumen.

SUMMARY NO. 37

PREPARATION OF AUTOLYSATES AND BACTERIAL DIGESTS OF HERRING FOR CHICK FEEDING TESTS

H.L.A. Tarr

In Southen California enzymic autolysates of fish waste[**] have been made for some years for livestock feed or fertilizer purposes. In certain Scandinavian countries bacterial digests (e.g. "L.K.C. Product"), or acid preserved (formic and/or other acid) autolysates (see also Summary No. 38) have been made for use in animal feeds. In order that this type of product could be tested in the current chick biological assay programme the following products were prepared. In all instances, for the sake of uniformity in testing, frozen Nov. 1951 B.C. herring from which the corresponding "Ideal" (air-flow-dried) meal had been prepared was used. The following were made:

(a) Whole herring blended in a "silent cutter".

(b) Blended herring (as in a) were mixed thoroughly with 35% HCl (55 ml. for 2 kg.) to bring the pH to 2.9 to 3.0. The product was allowed to autolyze at 37°C. for 18 hours, when it had become a free flowing liquid through "fish pepsin" action. The samples were stored another day at 37°C. (pH 4.1), and then transferred to a 0°C. room until required. This product appeared to be very stable and resisted bacterial spoilage at cool temperatures. However, it was somewhat susceptible to mould development.

(c) 1805-gm. portions of blended herring + 100 gm. of sucrose + 80 gm. of soybean meal + 15 ml. of concentrated HCl were mixed thoroughly. The pH was 5.0. Sufficient was prepared to make 16 kg., and this was inoculated with 16 ml. (0.4% wt.) of an 18-hr. 30°C. bran culture of a Danish "L.K.C." lactic acid bacterial mixture. (Probably Lactobacillus plantarum and possibly other organisms.) The mixture was incubated in four separate glass containers at 25°C. with frequent stirring. After 18 hours the mixture was somewhat gassy, and the pH was 5.5. The following pH values were recorded during incubation: 1 day, 5.3; 2 days, 4.9; 4 days, 4.5; 6 days, 4.5. The mixture was then stored at 0°C. until required.

(d) A product very similar to (c) above was made, but the inoculum was 8 ml. per kg. of a mixture of bran infusion cultures of Lactobacillus plantarum, Propionobacterium casei and P. shermanii. During incubation at 30°C. the pH values observed were: 2 days, 4.7; 3 days, 4.5; 5 days, 4.5. This mixed culture was used because it has been claimed that Lactobacilli and Propionobacteria when cultivated together form large amounts of cobalamin. As yet this product has not been assayed for the vitamin.

The above products are being tested at about 5% level in chick rations in order to ascertain whether they contain growth factors which are not present in herring meal.

SUMMARY NO. 38

FISH ENSILAGE

P.J. Schmidt
A.W. Lantz

Experiments were started this year to show how waste fish material could be utilized in the form of a "fish ensilage". Material of this kind has been prepared in some European countries for animal feeding. A distinct disadvantage in utilizing such a feed is the high cost of transportation of the large quantity of water present in the feed. However, the process could be used to advantage in areas where there are no reduction plants and where the producer is near the consumer.

To produce ensilage, the lean fish or fish wastes must be ground in a meat grinder. To 100 lb. of fish material in a wooden barrel is added 2¾ lb. of 85% formic acid and 20 lb. of water. The pH of the mixture should be in the vicinity of 4.5. The material is stirred once a day for 2 weeks and then can be put into wooden barrels with covers.

Experiments were made, using sole fillet waste and whitefish fillet waste. The heads and viscera were present in these wastes. The process softened the bones to the extent where they could be crushed by the fingers. The material rapidly became much less viscous. Some of the ensilage produced is now 4 months old and still has no objectionable odour.

More work in this direction is contemplated (See also Summary No. 37). Experiments using cheaper acids like acetic acid should be conducted. The production of soil fertilizers by this process is also a possibility.

SUMMARY NO. 39

COBALAMIN CONTENT OF STICKWATERS AND CONDENSED FISH SOLUBLES PREPARED FROM THEM

H.L.A. Tarr

It had been suggested frequently to a local company that there was an apparent discrepancy between the cobalamin content of their herring stickwaters and the condensed fish solubles prepared from them. The company finally decided to collect samples representative of a season's production.

Total cobalamin content (mμg./gm. dry wt.)

Herring stickwaters (Range: 6.0 to 10.0% dry wt.)						Condensed herring solubles (Range: 45.4 to 49.8% dry wt.)		
							Duplicates	
1	1520	15	1540	29	1230			
2	1450	16	1490	30	1530	1	980	940
3	1390	17	1490	31	1280	2	835	975
4	1500	18	1270	32	955	3	830	795
5	1310	20	1130	33	1300	4	880	975
6	1300	21	1110	34	1210	5	800	790
7	1130	22	1200	35	1330	6	1200	1250
8	925	23	1430	36	1210	7	890	1000
9	1200	24	1270	37	1700	8	715	550
10	1070	25	1330	38	1420	9	1190	950
11	1090	26	1120	39	1380	10	770	770
12	1140	27	975	40	1350	11	705	725
13	1260	28	975	41	1680	12	550	560
14	1320					13	560	650

The total cobalamin content of all these samples was determined using the L. leichmannii assay referred to in Summary No. 33. Duplicate analyses were carried out on several samples. The results tabulated herewith indicate that, on a dry weight basis, the stickwaters contain apparently considerably more cobalamin than the condensed solubles prepared from them. The reason for this is not known, but it is intended to check the figures by separation of the cobalamin chromatographically, followed by elution and assay. It may be that some of the cobalamin is destroyed during acidification of the stickwater during preparation of the solubles.

SUMMARY NO. 40

THE DETERMINATION OF MOISTURE IN FISH

P.J. Schmidt

Since there are several methods for the determination of moisture, an attempt was made to compare the results obtainable from four of these. Briefly, the methods compared were as follows:

Method 1 - The sample of 5 to 10 grams of ground fish is mixed with asbestos and 20 ml. of water and heated in an air oven for 4½ hours at 100°C.

Method 2 - Twenty grams of prepared sand is mixed with the sample and then dried in an air oven at 115°C. for 3½ hours.

Method 3 - This is the same as method 2, except that the sample is dried in a vacuum oven at 80°C. for 5 hours.

Method 4 - A 5 to 10-gram sample is heated in an air oven for 1 hour at 70°C. and then for another 4 hours at 130°C.

The results of some analyses using these methods are given below:

Method	Percent moisture	
	Halibut fillets	Salmon viscera
1	76.7	73.8
2	76.8	74.5
3	76.6	73.2
4	76.9	73.6

The data shows that the best drying was obtained when the sample was mixed with sand and then dried in an air oven at 115°C. for 3½ hours (Method 2).

SUMMARY NO. 41

MOISTURE CONTENT CHANGES PRODUCED BY GRINDING — P.J. Schmidt

It was suspected that the Station's laboratory grinder ("Mikro-pulverizer"), a type of hammer mill, caused some drying of the material being ground. A great deal of air appeared to be blown through the grinder when the machine was operating. It was found that fish meal with 37.5% moisture (water added several days prior to grinding) contained only 30.1% moisture after grinding. A different type of cutter was then obtained for the grinder, which has proven to be quite satisfactory.

SUMMARY NO. 42

MOLECULAR DISTILLATION OF FATTY ACID ESTERS - HERRING OIL — L.A. Swain

In the summer of 1950, a period of over two weeks was spent at the Atlantic Fisheries Experimental Station to use and study the molecular still located there. A Pacific whale liver oil was fractionated with the still, with the expected increase in its vitamin A content (due to conversion of kitol). Following the favourable reaction of the Board at its meeting last January to the suggestion that determination be made of the composition of the oils from some of the fishes of the Canadian Pacific coast, a molecular still was ordered to assist in the fractionation of the fatty acids. This study was considered by the writer to be a continuation of the work on the composition of pilchard oil, reported by Brocklesby and Harding in 1938.

Two one-gallon samples of herring oil from the extreme ends of the B.C. coast, and two samples of salmon oil were obtained through the courtesy of B.C. Packers Ltd., and placed in cold storage. Two methods of converting oil to its methyl esters, and three methods of fractionating the resulting esters prior to distillation, were tried before selecting the methods to be used.

The still received suffered two defects — a gas-leaking metal connection, and an improperly connected temperature-recording instrument. Cognizant of the delicate and complex construction of this molecular still, the writer spent nearly three months of extremely careful manipulations and consultations with authorities searching for and correcting these difficulties. The apparatus now works satisfactorily.

The sample of herring oil from northern B.C. had been prepared from fish caught in January, 1952. It had a saponification value of 184.3 and iodine value 117.7, and yielded 1.06% unsap. Methyl esters prepared from it were separated into three fractions - (1) the crystals separating from acetone solution at -25°C., (2) the crystals separating from the diluted mother liquor at -75°C., and (3) the mother liquor. The crystal fraction obtained at -75°C. was divided into three portions by molecular distillation. The five fractions so prepared were then each subjected to further fractionation with the molecular still, yielding a total of 51 sub-fractions. The saponification equivalent and iodine value were determined on each sub-fraction, and from these data was calculated the fatty acid composition of this sample of herring oil with the results shown in the accompanying table. It is planned to continue

this analysis at intervals to follow seasonal variations in the composition of this and other important Pacific coast fish oils.

Fatty acid composition of Pacific herring oil (Prince Rupert; January, 1952) (% by wt.).

Methyl esters, crystallized from acetone at	-25°C.	-75°C. Distillate fraction			Mother liquor, -75°C.
		1	2	3	
Iodine value	9.4	54.0	95.0	99.8	213.2
No. of distillate sub-fractions prepared	6	10	9	13	13
Saturated					
Myristic		1.9			
Palmitic	4.1	5.1	0.2		
Stearic	1.9	0.7	0.4		
Arachidic		trace	trace		
Mono-unsaturated					
C_{14}		0.5			
C_{16}	0.4	3.8	0.6		
C_{18}	0.3	3.5	4.3		
C_{20}		1.3	0.1		
Unsaturated (includes some mono-unsaturated)					
C_{16}					5.4
C_{18}			5.9	0.4	15.6
C_{20}			5,7	8.9	15.4
C_{22}				9.8	1.7
C_{24}				2.0	

Note: The high iodine value of the mother-liquor fraction indicates an average of 2.5 double bonds per molecule of the 38% of the fatty acids in this fraction.

SUMMARY NO. 43

VITAMIN A IN WHALE LIVER — P.J. Schmidt

Certain assays of vitamin A in B.C. whale liver oils in 1950 (Progress Reports No. 83, pp. 28-29, July 1950) showed that these oils possessed maximum ultra-violet light absorption at the wavelengths 322-325 mμ. This indicated that very little kitol (absorption maximum at 296 mμ.) was present. Commercial samples of whale liver oil were assayed and their maximum absorption was at 310-312 mμ. This year some fresh whale liver samples were obtained and the assays were made in the same way. A cold extraction of the oil with ethyl ether was made and the vitamin A potency determined as directed in the USP method. By this method a correction for irrelevant absorption is made.

The results of the assays were as follows:

Species	Sex	Length ft.	Date of butchering	Ether extract % of liver	Vitamin A USP units/g. of oil (corrected)	Maximum absorbancy mμ.
Finback	male	60	July 8/52	2.43	145,000	312
Blue	"	60	July 9/52	3.24	194,000	324
Sperm	"	55	July 9/52	4.30	130,000	312

The vitamin A potencies found were exceptionally high. This may be due to the fact that the liver samples were very fresh. The writer personally removed the samples from the animals, and they were immediately frozen. When the samples were thawed for analysis, they did not have the objectionable odour that whale liver usually has.

In the case of the finback and blue livers, the maximum absorption was found to be at 312 mμ. and not in the vicinity of 325 as in the previous samples. Norwegian workers have recently reported absorbancy maxima which vary from 295 to 325 mμ. The reason for this variation is not known.

SUMMARY NO. 44

METHODS FOR THE DETERMINATION OF PEROXIDE VALUE IN FISH OILS

Ulf Fagerlund

Measuring of the peroxide content is in the literature regarded as one of the most accurate methods for determination of rancidity in oils and fats. However, from time to time different techniques have been suggested, which techniques often give different values for the same oil. Further, the results may be expressed in different manners.

Following a request from the industry for a simple and accurate method for determination of the peroxide value, that could be generally acceptable and adopted by the industry, an investigation of different methods was started.

Skellon and Wills (The Analyst, 73, 78, 1943) describe a simple method making use of sodium bicarbonate for removal of air oxygen. They obtained good results when determining peroxidic oxygen in benzoyl peroxide and thermally oxidized fats, which contain a large quantity of peroxides. This method, somewhat changed, was used with good result on properly purified benzoyl peroxide in the present investigation.

When used in determination of the peroxide value of a freshwater fish oil which had a peroxide value of 35 milliequivalents (meq.) of active peroxidic oxygen per kilogram of oil, a value less than that of "thermally oxidized fats" (250-1500 meq./kg.), the procedure was changed as follows. (a) Solvent: Chloroform had to be added as the glacial acetic acid did not keep the oil in solution when water was developed in the course of neutralization of the carbonate and added in the form of potassium iodide solution. (b) Order of addition of the reagents: It was shown by experiments that glacial acetic

acid acts as a very efficient oxygen carrier when introduced to KI solution. To prevent contact of air with either oil or KI in acetic acid solution it is suggested that the carbonate be added to the acid before the oil is added. (c) Potassium iodide solution: Solid powdered KI gave as good results as did a saturated water solution. (d) Sodium bicarbonate: Sodium carbonate was found to give a more violent CO_2-evolution than bicarbonate and accordingly a more staisfactory removal of air oxygen, which was demonstrated by the lower blank values obtained. (e) Concentration of thiosulphate solution: 0.005 N sodium thiosulphate solution gave the highest degree of accuracy. (f) Reaction time: This particular oil gave higher peroxide values when the reaction time was extended from 15 to 60 minutes. This condition has to be further investigated.

When these changes were applied the greatest variation obtained between any two of three determinations was within 6.3%. That does not give enough accuracy and therefore a method employing de-aeration was considered.

The improved Lea "cold" method (J. Soc. Chem. Ind. 1946, 268), though giving satisfactory results, makes use of a too complicated apparatus for routine determinations. Skellon and Thurston (The Analyst, 73, 97, 1948) describe a modified apparatus, which was used in this investigation and gave promising results.

The investigation will be continued.

SUMMARY NO. 45

COLD CLEARING DURING CENTRIFUGATION OF HERRING OIL — Ulf Fagerlund

In 1937 Dr. Carter experimented with centrifugation during cooling of pilchard and herring oils. In the case of pilchard oil, which was then more important than herring oil, a reasonable increase in the iodine value of the centrifugated oil was thus obtained. Herring oil, however, became semi-solid throughout the sample, because of the rather rapid cooling.

This year it was suggested more thorough investigations be made of how much the iodine value of cold-cleared herring oil could be raised by slow cooling. Centrifugation, as an easy way of separation, was applied during cooling to prevent the oil from becoming semi-solid.

Four centrifugations were performed, of which three were done in the Station's cold room. Considerable trouble was experienced in arranging a gradual lowering of the temperature from 20°C. to about -10°C. One experiment was made in a new refrigeration centrifuge borrowed by the Station.

The length of the experiments varied from 6 hours to 7 days, and 27 samples were taken on which the iodine value and the percentage of stearine were determined. The oil was poured off the centrifuged stearine, which remained as a cake in the bottom of the cups.

In one run herring oil with an iodine value of 130 was cooled slowly. After 74 hours, when the temperature had fallen to -4.4°C., 29% of cold-cleared oil was obtained, with an iodine value of 138. After 96 hours, when the temperature had fallen to -6°C., only 4% of cold-cleared oil was obtained,

with an iodine value of 145, the highest value obtained in this investigation.

The experiments showed that slow cooling is necessary to prevent occlusion of unsaturated glycerides with the more saturated ones. The difference in unsaturation of glycerides in herring oil does not seem to be great. Accordingly only a small rise in iodine value of the cold-cleared oil could be obtained by this method.

SUMMARY NO. 46

VACUUM DISTILLATION OF ESTERS PREPARED FROM BUTE INLET WAX

L.A. Swain

Further work was done on the wax from Bute Inlet, reported upon last year (Station Annual Report for 1951, p. 28), which served as exploratory procedures for the planned studies on fish oils. The fatty acids obtained from a sample of the wax were separated by the lead salt method. Both resulting fractions were converted to their methyl esters and distilled under vacuum in the Todd "Precise Fractionation" still, possible because these fatty acid mixtures were quite saturated and therefore unaffected by the lengthy exposure to heat. Some of the fractions so obtained were further separated by "amplified distillation" (Weitkamp, J. Am. Oil Chem. Soc. 24, 236, 1947) but the method led to erratic results. (Of five commercial mixtures of hydrocarbons tested, only "Mentor 29" had the right boiling point range for this purpose. A gallon of this product was kindly donated by Imperial Oil Ltd.) Calculation of the composition of the methyl ester distillates by the procedures described by Rapson et al. (J. Am. Oil Chem. Soc. 24, 84, 1947) led to the results shown in the accompanying table.

Fatty acids in Bute Inlet wax (% by weight)

Carbon content	Saturated	Mono-unsaturated
14	31.2	1.5
16	34.0	3.9
18	3.2	4.7
20	2.4	7.3
22	1.5	8.2
24	1.6	0.4

Methyl esters prepared from the fatty acids of a hydrogenated sample of the wax were separated into 8 fractions in the Todd still. The "amplified distillation" technique for further separation was unsatisfactory.

The unsap from the same sample of hydrogenated wax was acetylated and distilled in vacuum in the Todd still; 8 fractions were collected. The first fraction was hydrocarbon as indicated by hydroxyl value. The remaining fractions after saponification corresponded approximately, by hydroxyl value, to the C_{20} alcohol. Depression of the melting point of benzene caused by these acetates calculated fairly uniformly to a C_{20} alcohol ester.

Acetates of the unsap of a sample of the original wax were similarly fractionated, yielding 8 portions. Saponification equivalents of the fractions suggested, on the other hand, a mixture of C_{20} and C_{22} alcohols (or a C_{21} alcohol). From previous work on fractionation of the unsap by crystallization, it was clear that these alcohols are similar in unsaturation, with one double bond per molecule of the size indicated by the saponification equivalents. The iodine values of these distillate fractions correspond either to a mixture of C_{20} and C_{22} alcohols, or to a C_{21} alcohol. The first fraction was a saturated hydrocarbon, with molecular weight corresponding to a C_{20} molecule.

The origin of this wax remains a mystery. The suggestion that lodgepole pine pollen is the source, mentioned in last year's Report, was followed up by storage experiments with pollen from the Jack pine (same species as lodgepole) growing on Lulu Island.

This pollen was extracted with acetone and the extract was extracted with light petroleum. This procedure was repeated at three time intervals with pollen stored in water at outdoor temperature. The resulting material was always a solid; Bute Inlet wax is a liquid at room temperature. Results follow:

Storage time (days)	Extract (% of dried pollen)	Unsap		Fatty acids	
		%	Iodine value	%	Iodine value
0	6.9	47.5*		52.5	70.8
13	18.0	17.8		73.7	
57	20.6	14.1	60.8	71.7	59.7
153	12.3	20.7		72.9	
Bute Inlet wax		54.3	78.4		37.8

* 70% alcohol by chromatographic analysis.

Although this pollen was not exposed to the activities of the microflora characteristic of Bute Inlet, it does not seem reasonable to postulate a relation between the material extracted from it and the large quantities of liquid wax that have been periodically observed in Bute Inlet.

At the request of Radio Station CBU, a 4-minute interview was presented during an early morning "Fishermen's Broadcast", in which some of the more interesting features of this Bute Inlet wax were described.

SUMMARY NO. 47

ALCOHOL IN TUBE WORMS

Ulf Fagerlund
L.A. Swain

During an investigation last year on the distribution of vitamin B_{12} in marine fauna (last year's Station Annual Report, p. 34), Mr. Maxwell noted an ethereal odour (suggestive of capryl alcohol) during steam distillation of the tube worm Eudistylia vancouveri. This year the source of the odour was further investigated. An oily liquid, possessing the characteristic odour, could be

obtained from ground up worms in 0.05% yield by steam distillation, or by boiling with added water. This oily liquid had a small acid value (2.4), a very low saponification value (35), and a high content of unsaponifiable matter (30.5%). The odour was in the unsaponifiable fraction, and chromatographic analysis of the latter suggested it to be mostly alcoholic. The odour was associated with this portion.

No definite boiling point could be observed when a small sample of the chromatographed liquid was distilled from a 1-cc. distillation flask, the main part distilling over between 80 and 160°C., which fact would indicate the presence of a mixture of alcohols.

The esters resulting from esterification with 3,5-dinitrobenzoic acid and with p-nitrobenzoic acid melted at 59.5°C. and 13°C., respectively. The ester of the first-mentioned acid with known capryl alcohol melted at 30.0°C.

With the vast number of isomers of the possible low-molecular-weight alcohols (of which only a few are recorded in the chemical literature), it is not possible to identify the alcohol or alcohols with the methods employed.

This work was described in Pacific Progress Reports No. 92, during the year.

SUMMARY NO. 48

OILS - MISCELLANEOUS

L.A. Swain

A sample of oil was analyzed at the request of a resident up the coast. From the characteristics determined it was apparently a herring oil of fair quality.

A sample of fatty fibrous material was submitted from Prince Rupert, with a request for determination of its origin. The sample contained 74.8% of fatty material, 3.9% of a fibrous material; the rest was water. The fatty material contained 4.5% unsap and had an iodine value of 34. The fatty acids appeared to be mainly stearic, palmitic and oleic acids. Presumably it was from a land animal, but further identification was not attempted.

At the request of Dr. R.H. Wright, B.C. Research Council, a sample of benzyl bornyl ether, prepared in his laboratory, was purified in the molecular still. This equipment was very suitable for the purpose because of the negligible opportunity for thermal decomposition during the distillation.

SUMMARY NO. 49

REPORT ON USE OF EQUIPMENT ON LOAN TO UNIVERSITY OF BRITISH COLUMBIA BY VANCOUVER STATION

Dr. L.W. Shemilt
Div. of Chem. Eng.,
University of B.C.

(A) Pilot Plant Spray Drier

This has continued in use for Chemical Engineering Research Work at the B.A.Sc. level, and for demonstration use in studying the unit operation of drying.

From the operational standpoint the following may be noted:

(a) Continued difficulties with control of the spray wheel have been found. Operation is now carried out by using a Strobotac for speed measurement.

(b) The Taylor Wet-Bulb Recording thermometer used in the inlet air stream has never operated correctly and needs removal for repair.

(c) The inlet air blower was calibrated up to its maximum value of 0.08 lb. of air per second at 200°F., 1 atm.

(d) The drier efficiency, i.e., the variation in heat to be added to the inlet air to evaporate one pound of water, was determined for various air inlet rates.

(e) 10% milk solution was dried to a milk powder of 7% moisture and microphotographs taken of the product. Good uniformity of product was obtained.

(f) 10% tannin solution was dried to a powder of 7% moisture content. Microphotographs indicated good spraying but non-uniform drying. Further checking of operating characteristics is needed.

(B) Pilot Plant Heat Pump Concentrator

This equipment continued in use for B.A.Sc. research programs and general class demonstration use.

Considerable difficulties in attempted operation of this unit have continued to be found. Operation has indicated that leaks between the vacuum and pressure sections still exist and endeavours are being made to correct this situation. An indicating manometer has been installed on the feed line. Efforts will continue on improving the apparatus so that its operational characteristics may be determined.

SUMMARY NO. 50

CLEANING HERRING SPAWN — L.A. Swain

In studying the extent of bird predation on herring spawn, scientists at the Biological Station in Nanaimo were required to remove spawn from vegetation. The eggs, which adhered firmly to the vegetation, could be removed by scraping, but such a procedure was very time-consuming, and suggestions from the Vancouver Station were invited.

In an attempt to facilitate this separation, egg clusters on rockweed and on eelgrass were exposed to two concentrations of solutions of several alkaline chemicals at two temperatures. None was successful. A sample of the enzyme hyaluronidase was later sent to Nanaimo with suggested procedure for its use. The results obtained were moderately successful, and further work is planned with this enzyme.

SUMMARY NO. 51

ANALYSES OF WATER SAMPLES FROM THE KITSAULT RIVER — P.J. Schmidt

The death of a larger than usual number of salmon in the Kitsault River is a problem that came up again this year. It was felt that this could be caused by a toxic effluent from a smelter on the river. Last year analyses for cyanide were made on samples of salmon but nothing could be detected. This year samples of water from the river were sent to us by Mr. Stokes, the Department's Regional Biologist:

Sample	Date collected	Location	Water temperature	Air temperature
A	Oct. 18, 1951	above mill	32.0°F.	28.0°F.
B	Oct. 18, 1951	below mill	32.0°F.	28.0°F.
C	Oct. 18, 1951	8½ mi. from river mouth	34.0°F.	30.0°F.
D	Oct. 19, 1951	river mouth	35.0°F.	32.0°F.

At time of sampling, the river was low and no fish of any species could be observed.

Each of the samples contained small amounts of suspended organic material. Results of examinations of the samples by the writer are:

Sample	Turbidity	pH by pH meter	Alkalin.* by methyl orange titr. HCO_3 ion (mg./100 ml.)	Alkalin.* by methyl orange titr. Calculated as ppm. of $CaCO_3$	Calcium (ppm.)
A	very clear	7.16	6.70	88.9	13.6
B	turbid	7.82	8.54	113	19.1
C	less turbid than B	8.00	8.85	117	20.4
D	less turbid than C	7.70	7.63	101	25.9

* There was no phenolphthalein alkalinity but there was methyl-orange alkalinity, indicating that only bicarbonates are present, and no carbonate or hydroxide, for they do not exist in appreciable quantities in solutions of pH less than 8.3.

No cyanides or sulphides could be detected.

No indication of pullution could be found. However, these tests do not positively prove that a toxic effluent is not present in the river. The detrimental action of a given waste may result from a compound which was not tested for or for which no test may be known. Bioassays may be required. A survey of the polluted area may be advisable. The Department of Fisheries has of course been informed that further tests would gladly be made if their biologists thought that was necessary.

SUMMARY NO. 52

ANALYSIS OF PICKLING TANK EFFLUENT — P.J. Schmidt

The Department of Fisheries submitted for analysis samples of the effluent from a metal-pickling process at the Burrard Dry Dock Co., North Vancouver. The analysis was requested to ensure that no effluent harmful to fish life was being ejected. A sample was taken at the discharge ejector of the pickling tank. It was found to have a pH of 0.70 and a hydrochloric acid content of 2.49% by weight. Another sample was taken at the outer end of the drain leading from the sump where dilution took place. Its pH was 7.65, and was considered to be harmless to fish life.

SUMMARY NO. 53

FISH FLESH FOR CANNED BABY FOOD — R.A. MacLeod

Information concerning the nutritive value of salmon and tuna was requested by a Mr. T.H. Summerell of Winnipeg who is interested in the possibility of using fishery products in canned baby foods. He was given fairly complete information on the comparative analyses of the flesh of these two fishes, including values for protein, fat, vitamins A and D, some members of the vitamin B complex, and for six of the important inorganic constituents.

SUMMARY NO. 54

ANALYSIS FOR FORMALDEHYDE — Ulf Fagerlund

An analysis for formaldehyde in a sample of shrimp for bait was requested by the firm of Hart & Howes Ltd. Drop tests with malachite green and fuchsin indicated the presence of small amounts of aldehyde.

SUMMARY NO. 55

AMBERGRIS — L.A. Swain, N.M. Carter

A sample of "Is this ambergris?" was brought to the Station and was immediately recognized as a jellyfish. This was not the first time jellyfishes have been so submitted. The usual quota of other recognizable (and some unrecognizable) materials were submitted to the Station during the year.

The continuing public interest in materials suspected of being ambergris led the Station to request permission to secure photographs of samples of various grades of the true material. These photographs (See Summary No. 56), in both colour and black and white, turned out successfully, and will prove to be of great use in lectures and in showing the frequent enquirers what ambergris really looks like. Several kinds of interesting inclusions in the samples were also examined.

SUMMARY NO. 56

PHOTOGRAPHY

E.G. Baker

The Station photographic equipment was used a great deal in the past year by all members of the staff for photographing new equipment and recording results of experimental work. A number of lantern slides were also made for use by the staff in giving lectures.

Most of the new equipment purchased is for use in making movies. This included a direct focuser for the 16-mm. movie camera and a viewing and splicing apparatus. Several new photographic flood lights were also purchased, and a tripod which can be used with all cameras.

The writer started the making of a movie showing the work being done by this Station in the different departments but this had to be discontinued for the present due to the urgency of other work. It is hoped it can be completed next year.

Samples of several types of ambergris were secured for the purpose of making a set of 35-mm. colour slides and black-and-white prints. (See Summary No. 55.)

INDEX OF AQUATIC ANIMALS USED IN INVESTIGATIONS, EXPERIMENTS, ETC.
(Numerals refer to Investigators' Summaries except when preceded by "p." = page)

INDEX OF OBJECTS OF INVESTIGATIONS, EXPERIMENTS, ETC.
(Numerals refer to Investigators' Summaries except when preceded by "p." = page)