SOME OBSERVATIONS ON THE MANUFACTURE AND VITAMIN CONTENT OF COD LIVER OIL AND ALLIED OILS.

HUGH BRANION, M. A.

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By Hugh Branion, M. A.

Foreword.

"Fish and marine animal oils have been articles of commerce for several hundred years, being used variously, according to their nature and origin, as lubricants, illuminants, in the manufacture of cheaper grades of soft soap, in leather dressing and jute batching and for medicinal purposes."

"For centuries cod liver oil has been prepared from cod livers as a by-product of the cod fisheries off the coasts of Japan and Norway and more recently the coasts of Alaska, Newfoundland and Nova Scotia, the value of oil for medicinal purposes having been recognized long before anything was known of vitamins or the role these play in diseases of malnutrition. To-day the oil is regarded as of more importance than merely as an offshoot of the fishing industry", particularly in the last two years since the recognition of the value of cod liver oil as a nutritional factor in the food of poultry, cattle, sheep and pigs. "Originally produced from livers which were stored in barrels until the livers rotted whilst exposed to the atmosphere, when presently the oil floated to the top and was removed, the steaming process came into use about 1850 in both Norway" - where it was introduced by Peter Möller - "and Newfoundland, the heating taking place in steam-jacketed pans. Latterly the process of heating by direct steam, in which live steam is blown into the mass of livers, has come into use". (Jour. Soc. Chem. Ind. 47, 1335, 1928).

Along the coast of Nova Scotia the old rotting process still is in use although the larger commercial firms almost solely use direct steam. Drummond and Zilva (J. Soc. Chem. Ind. 41, 280, 1922) describe the various processes of manufacture and the resultant oils,-

Rotting Process:- "Occasionally in the very small fishing hamlets where the supply of livers is not worth the introduction of modern methods this process may be seen in operation. The livers are thrown into large wooden vats as they are collected from time to time and allowed to rot for long periods, the oil rising to the surface as the liver tissue slowly disintegrates. The oil yielded by this process is usually a clear golden brown coloured product, the depth of colour being to a large extent dependent on the length of time the contents of the vat have been standing. It also tends to possess a rather objectionable odour owing to the presence of traces of products of putrefaction." This 'black' oil was the sole cod liver oil produced when it attained its therapeutic reputation. It is now practically never used for medicinal purposes, its principal use being in the tanning industry.

"Steam-jacketed Pan Process:- The sound livers after stripping the gall bladders are placed in barrels. All discoloured or spotted livers are discarded. The barrels of livers are taken to the factory and tipped into the jacketed vats, which are heated by low pressure steam. When in the opinion of the operator, the oil has been separated satisfactorily the protein matter is removed by passing the liquid through a sieve ladle whilst the oil is kept warm and allowed to stand and separate. The product which is a clear white oil constitutes the first fraction and on cooling it tends to deposit crystals of "stearine". The residue is again heated and yields a second fraction of high grade oil of slightly inferior taste, but this fraction is mixed with the first. The hot residue is then usually allowed to drain through a number of cloth filtering bags hung up over a tinned gutter which collects the oil as it drops through. This oil is usually of a clear yellow colour and possesses a more pronounced taste. This oil is sometimes mixed with the other two fractions but it is more generally sold for industrial use." "The residue from the filtering bags is treated by one of three processes. In some cases it is put back into the steam kettles and afterwards passed through a hand press which yields a dark yellow oil mainly used for technical purposes." The residue is suitable for drying into a commercial liver meal for which there is now an increasing demand for stock food. "The second method consists of heating over an open fire in pans with stirring, until all the water is driven off, with the formation of a dark brown oil."

"More frequently, however, the residue is thrown into vats outside the factory and allowed to rot for 2 - 3 months with the formation of a deep golden brown oil which is skimmed from the top when enough has accumulated."

The Direct Steam Process:- "The selected livers are thrown into a conical vat. Steam is blown into the livers until they are, in the opinion of the operator, "cooked". The steam is turned off and the contents are allowed to stand, after which the deep layer of oil at the top is ladled off and poured through an unbleached linen filter to remove protein matter. The protein remaining on the filter is thrown back into the vat. The crude oil is allowed to settle to allow the small amount of moisture and traces of protein to settle out, after which it is run into storage tanks. The liver pulp remaining behind in the vat is generally run off into rotting vats outside the factory and there allowed to yield after some months the usual type of golden brown oil. Sometimes in the better factories the pulp is subjected to pressure to yield the second grade of oil and the residue is dried for liver meal." The 'chum' or residue from this process is also used for meal.

Other Methods:- "In addition to the very many factories where these widely-employed methods are used there exist some in which the processes are highly elaborated and scientifically controlled and in which oils are carefully prepared under conditions (use of vacuum, atmosphere of carbon dioxide etc.) which protect against oxidation."

U. S. Bulletin 1475 outlines an electrolytic process which consists in passing minced fresh livers, suspended in brine heated to about 155° F., through a series of cells composed of pipes containing carbon rods. The electric current breaks the oil cells and frees the oil. Centrifuging is used to free the oil from the brine and solids. The time required for the whole process is about 3 minutes.

Oil has also been obtained by various workers by subjecting the livers to low temperature, the freezing rupturing the oil cells. The yield of oil by this method is small but the oil is very pale and palatable.

Refining:- Bleaching processes for reducing pigmentation based on oxidative changes, ozone, chlorine, sunlight and aeration are occasionally used. Medicinal cod liver oil is usually refined by freezing to remove "stearine" or by mixing with some absorbent to remove pigment and passing through a filter press. The crystallization of "stearine" is carried out at low temperatures (0^{o} to $-10^{o}C$) and the oil is subsequently passed through filter presses. The residue is sometimes expressed under pressure to yield a second grade medicinal oil. The stearine is used for technical purposes.

Along Canadian coasts two types or grades of oil are prepared. The first is a medicinal cod liver oil and the second is termed "coast cod oil" or "shore oil" and is used for technical purposes or for animal food. This latter grade is the bulk of oil manufactured.

The present trend of modern manufacture is to protect the oil from oxidation as it is believed that contact with air tends to impart an undesirable flavour to the oil and to be responsible for the unpalatable qualities of the oil. Some commercial firms have put flavoured (use of wintergreen, peppermint, etc.) oils on the market. The old idea that pale oils were of less therapeutic value than dark oils has been generally discarded.

The concensus of opinion is that chilling of medicinal oil is unnecessary and may even reduce the vitamin potency of the oil. It also seems apparent that the more the oil is refined the greater the danger of destroying the vitamins. Oxidation and bleaching processes are certainly harmful, but are little applied to medicinal oil.

Therapeutic Value

The remarkable action of cod liver oil in the treatment of rickets, incipient tuberculosis, xerophthalmia and in diseases of malnutrition generally has called forth considerable research. Active principles were discovered, shone overnight and were discarded. Minute traces of various compounds in the oil such as iodine, the alkaloids of Gautier and Mourgues and so on were at one time or another held responsible. Möller, about 1880 says "the active principle of cod liver oil is the oil itself".

There the matter stood until McCollum and Davis and Osborne and Mendel simultaneously discovered vitamins in 1913. We now know that the therapeutic value of cod liver oil lies in its vitamin potency. Two fat soluble, growth promoting vitamins, A and D, antixerophthalmic and antirachitic respectively are found in it.

The need for vitamin A in the dietary of the adult is not so marked as in that of the young growing animal. The lack of vitamin A is manifested in the young by failure of growth and the development of an eye disease called xerophthalmia. Adult animals kept on a diet deficient in vitamin A show some impairment of nutrition in the form of an increased liability to contract bacterial infections and a diminished power of reproduction. The definite disturbances in calcium and phosphorus metabolism resulting in defective ossification (rickets), formerly ascribed to vitamin A, are now known to be due to lack of another vitamin, antirachitic or vitamin D, which is usually associated with A. Vitamin D exerts its full growth promoting influence only in the presence of vitamin A and vice versa.

It was shown by Steenboch, Windaus, Hess, Bills and others that light rays of wavelength 2800 to 3130 Angstrom units acted in place of vitamin D and it is well established that there is an intimate relation between vitamin D and ultraviolet radiations.

Further research showed that the unsaponifiable matter of cod liver oil which contains the vitamins, also contained cholesterol. It was thought that cholesterol was the provitamin, which on activation, became vitamin D. Later it was shown that the provitamin was ergosterol which was present in minute quantities. Ergosterol can be activated by ultraviolet light to become, as it were, vitamin D. Preparations of activated ergosterol are now on the market - notably Acterol made by Mead Johnson & Co., with about 100 times the potency of cod liver oil (biologically tested).

In the past the only vitamin estimation made on cod liver oil was for A. Clare and Soames (Lancet #5447,214, 150) report "the content of vitamin A in cod liver oil bears no relationship to that of vitamin D". This may be explained in part by the fact that vitamin A is less stable than vitamin D and hence more likely to be affected by methods of preparation and storage. In view of this it would seem essential that an estimation of vitamin D should be made.

The U. S. Pharmacopoeia only requires 50 units of vitamin A per gram of the oil. This is extremely low and should be raised to at least 250 units. Ayerst cod liver oil is rated as containing 500 units or better (their standard) if .002 grams of the oil fed daily causes resumption of growth and the cure of the incipient xerophthalmia.

As the writer has pointed out, vitamin A is not so essential for the adult as for the young. It would seem that, on first glance, these preparations of activated ergosterol would not suffice as a substitute for cod liver oil in the dietary of babies. However vitamin A is very readily obtained in other foodstuffs and it is quite probable that medicinal cod liver oil will be replaced in the near future by preparations of activated ergosterol. It would seem that its chief use in the future will be in the direction of supplementing the rations of poultry, cattle, swine etc. and for technical purposes.

<u>Assays for Vitamin A.</u>

1. <u>Biological</u>:- Until recently the sole method of assay was to place young albino rats 20 - 30 days old, weighing 35 - 55 grams, on a diet adequate in all respects (including vitamin D) except vitamin A, until growth ceased for seven days and the typical eye disease, xerophthalmia, associated with lack of this vitamin developed. A known quantity of the oil under test was then given daily to the rats for 28 - 35 days. The minimum amount of oil given daily required to cure the induced symptoms of vitamin A starvation and causing a gain in weight of 10 - 20 grams in 35 days is termed the unit and the oil is labelled as containing so many units per gram. Sherman and Munsell (Jour. Amer. Chem. Soc. 47, 1658, 1925), Steenboch (J. Biol. C. 72, 765, 1927) and the Accessory Food Factor Committee (Lancet, 214, #5447, 148, 1928) give the details of this assay. This biological assay is necessarily long and tedious and the degree of accuracy is small unless a prohibitive number of animals is used.

2. <u>Colorimetric</u>:- A great many papers have appeared in the past few years dealing with colorimetric assays for vitamin A. Fearson (Biochem. Jour. 19, 888, 1925) found that cod liver oil reacted with phosphorus pentoxide to give a blue colour which varied in a general way with vitamin A potency. The test is carried out as follows:-

Phosphorus Pentoxide 1:- "About a gram of phosphorus pentoxide is added to 5 c. c. of the oil to be tested, either undiluted or dissolved in dry light petroleum, employing a well dried tube. On gentle shaking a deep violet colour develops. The colour slowly changes to a muddy brown depending on the amount of moisture present in the oil."

2. "A drop of the oil to be examined is let fall on to the centre of a small heap of phosphorus pentoxide on a dry filter paper. If the test is positive a purple colour develops in a minute, and persists until the pentoxide has begun to deliquesce". Negative oils give only a yellowish colouration.

<u>Pyrogallol and Resorcinol</u>:- Fearon (Biochem. Jour. 19, 893, 1925) also put forward another colour reaction, the pyrogallol test for vitamin A. It is carried out as follows:-

"One or two drops of the oil, or dry ethereal solution of the substance to be tested, are placed in a dry 10 or 25 c. c. flask. A few dry crystals of pyrogallol are added, and then 5. c. c. of the condensing agent (12% trichloracetic acid in dry light petroleum). The contents are well shaken. A bluish pink colour slowly develops if the test be positive, changing to deep rose, and reaching its maximum intensity in 15 to 20 minutes."

He also states that phloroglucinol, naphthol and substituted phenols (vanillin and amidol) all give definite colour reactions with cod liver oil in the presence of trichloracetic acid.

Willimott and Moore (Biochem. Jour. 20, 869, 1926) attracted by the permanency of the reaction advanced a modification of Fearon's pyrogallol test for quantitative application. They used resorcinol with a suitable control in concentration. "Since the rapidity of colour formation varies in an inverse manner with the resorcinol concentration, it was necessary to employ such a concentration of the latter as to cause a full carmine colouration in a reasonable time without the formation of purple." Their quantitative technique is carried out "with resorcinol present to the extent of 0.5% of the oil taken. The oil is first dropped in varying quantities into dry test tubes. The appropriate quantity of ethereal resorcinol is added, and then, with thorough vertical shaking, 5 c. c. of 10% trichloracetic acid in toluene and 0.5 c. c. of benzoyl peroxide in the same solvent. After two hours the colours are compared against a solution of magenta toned with methylene blue."

However Rosenheim and Webster (Biochem. Jour. 20, 1342, 1926) claimed that Fearson's test was non specific for vitamin A since it was not given by the unsaponifiable fraction of the oil. They made the suggestion that the chromogen of the reaction is associated with aldehydic oxidation products of the unsaturated fatty acids.

Later, Willimott, Moore and Wokes (Biochem. Jour. 20, 1292, 1926) also pointed out that the pyrogallol test was non specific. The phosphorus pentoxide and sulphuric acid test were also said to be less sensitive than the arsenic or antimony trichloride tests.

Sulphuric Acid:- The colour reaction with sulphuric acid has been known for some time. The United States Pharmacopoeia gives the following tests:-

1. "If a drop of the oil be dissolved in 20 drops of chloroform and the solution shaken with 1 drop of sulphuric acid the solution will acquire a violet red tint, rapidly changing to rose red and finally brownish yellow."

2. "If a glass rod moistened with sulphuric acid be drawn through a few drops of the oil on a porcelain plate a violet colour will be produced."

The British Pharmacopoeia gives the following colour test:-

"A drop of sulphuric acid added to a few drops of the oil on a porcelain slab develops a violet colouration."

Drummond (Analyst, 47, 314, 1922) feels certain that the blue colour produced is caused by vitamin A. Rosenheim and Drummond advanced the following colour reaction for vitamin A:-

"Dissolve one or two drops of cod liver oil in about 5 c.c. of an anhydrous fat solvent. Add one drop of concentrated sulphuric acid and shake gently. A deep violet colour is a positive test. The colour soon fades or is replaced by a red or brown shade. The violet colour is the essential part of the test."

Arsenic and Antimony Chloride:- Rosenheim and Drummond (Biochem. Jour. 19, 753, 1925) found also that arsenic trichloride produced a blue colour with cod liver oil. The reaction is carried out,-

"By adding 1 c. c. of pure arsenic chloride to 1 drop of cod liver oil and shaking the test tube at once. The oil dissolves immediately to form a clear blue solution which in the course of a few seconds assumes a purple tint and gradually fades. The reaction is characterised by a well defined adsorption band extending from λ 550 - 559." This test is 20 times more sensitive than the sulphuric acid test.

Similar blue reactions were given by dimethyl sulphate, trichloracetic acid, acetyl chloride and benzoyl chloride (the last two only in the presence of zinc chloride).

Carr and Price (Biochem. Jour. 20, 497, 1926) suggested a reaction based on the use of antimony trichloride. They also found that the colour producing agent in the case of trichloracetic acid was present in impure fractions. Their list of reagents includes,-

1. Antimony trichloride in chloroform giving a brilliant blue, not undergoing perceptible change for 3 minutes.

2. Stannic chloride in chloroform giving a deep blue colour rapidly changing to purple.

3. Anhydrous ferric chloride, added to a solution of the oil in chloroform, producing a fluorescent reddish violet colour.

4. Anhydrous aluminium chloride, added in the form of a fine powder to the oil, producing a red-violet colour fading to brown.

5. Silicon tetrachloride giving a rose-pink colour.

6. Phosphorus oxychloride producing a transient blue rapidly fading to red.

The Accessory Food Factor's Committee (Lancet, 214, 5447, 148, 1928) made a report on a comparison between the colorimetric and biological method of determining vitamin A in cod liver oil. The values obtained by the $AsCl_3$ method and the $SbCl_3$ modification agreed within the limits of error of each method ($\pm$ 0.50 units). The colorimetric method afforded information consistent with that derived from the biological tests.

The technique is as follows:-

1. <u>Arsenic Chloride Method</u> -- "The oil is measured from a Wright's capillary pipette graduated by means of mercury to hold 25 c. mm. and delivering 20 mg. oil, into a test tube of clear white glass of 10 mm. internal diameter. One c. c. of the reagent (pure $AsCl_3$) is delivered from a standard 10 c. c. burette into the test tube and a reading taken immediately (the time limit one minute) in a Lovibond's colorimeter."

2. <u>Antimony Chloride Method</u> -- "By means of a standard pipette 2 c. c. of the oil are measured into a measuring flask of 10 c. c. capacity. The pipette is rinsed out three times with chloroform, and the solution made up to volume with the same solvent; 0.2 c. c. (= 40 c. mm. oil) of the solution are measured into a test tube (10 mm. diameter) and mixed with 2 c. c. of a 30 per cent antimony chloride solution in chloroform delivered from a standard burette. Readings are taken as above described."

Rosenheim and Schuster (Biochem. Jour. 21, 1329, 1927) brought forward a new colorimeter based on the Lovibond colour system applicable to the testing of cod liver oil by means of the arsenic or antimony trichloride reaction. Lovibond's tintometer was not suitable for the rapid matching necessary, owing to the transient nature of the blue colour developed. Standard glasses are arranged in frames containing ten each of the red, yellow and blue units, inside a wooden box, in such a way that, by means of knobs, they can be rapidly moved horizontally behind a window and below the cell or test tube containing the coloured solution to be matched. The colours are arranged in units and tenths of units.

A suitable colour standard for comparison is provided by mixing 100 c. c. of crystal violet solution (1:10,000) with 50 c. c. of methylene blue of the same concentration both dyestuffs being dissolved in alcohol.

Leim and Chipman used thymol blue in a pH range of 8.6 to 10.0 with suitable buffers.

<u>Absorption Band</u>:- Morton and Heilbron (Biochem. Jour. 22, 987, 1928) report a correlation between vitamin A and an absorption band in the region of 3280 Ångstrom units. An examination of various oils shows that the intensity of the 328 $\mu\mu$ band varies directly with the depth of blue colour obtained with the antimony trichloride test.

The writer, through the cooperation of the Department of Physics, University of Toronto, is investigating this property of vitamin A with oils which have been biologically and colorimetrically assayed. The results should be available in the near future.

Assay of Vitamin D.

Biological:- There are two general methods for estimating the vitamin D content of cod liver oil. In both, experimental rats, at about the same age and weight as for vitamin A, are placed on a diet high in calcium and low in phosphorus with no vitamin D. Rickets result in about 18 days. Either preventitive or curative types of experiments are carried out. In the former the oil is fed with the rachitic diet and the protection afforded is estimated by chemical analysis of the bones, radiographic and histological evidence. Chick, Korenchevsky and Roscoe (Biochem. J. 20, 622, 1926) outline a suitable method for bone analysis. In the curative type, the rats are allowed to develop rickets and the minimum amount of oil necessary to effect a cure is determined. The amount of healing is estimated either by the "line test" of McCollum and Simmonds, (J. Biol. C. 51, 41, 1922) and Bills, Honeywell and MacNair (J. Biol. C. 76, 251, 1928); pH of the faeces; or radiography. Either the line test or radiography has proved equally satisfactory.

Mead Johnson & Co. use as a standard "that 1 part of oil in 400 parts of diet shall induce a distinct healing of rickets in five days".

Colorimetric:- Shear (Proc. Soc. Expt. Biol. Med. 23, 546, 1926) gives the following colour reaction for vitamin D,-

"Add 1 part of concentrated HCl to approximately 15 parts of aniline. Introduce 3 c. c. of cod liver oil or the solution under investigation into a wide test tube and add an equal volume of the aniline reagent. Mix the contents of the tube thoroughly, heat to boiling with constant shaking and boil for about half a minute. The yellow emulsion should turn green and in a few seconds change to red. Within a minute or two the emulsion should separate into two layers, the lower one being coloured an intense red. On standing, the colour deepens and persists."

Certain oils which give no red colour with this test have been shown to give a positive reaction after irradiation with ultraviolet light.

Another colour reaction for vitamin D using phosphomolybdotungstic acid has been described by Bezssonov (Comptes Rendus 179, 572, 1924).

Rosenheim and Webster (Biochem. J. 20, 544, 1926) report that these reactions are non-specific.

It is of interest that Rosenheim (Biochem. J. 23, 47, 1929) has reported that ergosterol (provitamin D) gives a blue colour reaction with chloral hydrate and with trichloracetic acid, by means of which it may be detected in the presence of other naturally occurring sterols. The reactions are as follows:-

1. "A few crystals, 1 mg. or less, of ergosterol are added to about 0.5 g. of chloral hydrate, liquified by warming in a water bath. They dissolve and immediately give rise to a carmine red solution, showing a broad absorption band at 500 $\mu\mu$. The red colour changes within a minute into a green and finally into a deep blue which persists for a considerable time."

2. "An aqueous solution, prepared by dissolving nine parts of pure crystallized trichloracetic acid in one part of water is added to ergosterol dissolved in a few drops of chloroform. An immediate red solution (band at 500 $\mu\mu$) is produced which changes gradually into a clear blue (bands at 570 - 580 and at 650 - 680 $\mu\mu$) without showing the intermediate green phase of the chloral hydrate reaction."

Haussler and Brauchli (Helvetica Chimica Acta 12, 187, 1929) have also reported the reaction of Tortelli and Jaffe (Ch. Z. 39, 14, 1915) as a specific colour reaction for ergosterol. The reaction is carried out by dissolving 5. c. c. of the oil or fat with 10 c. c. of chloroform, and 1 c. c. of glacial acetic acid and adding 2.5 c. c. of a solution of 10% bromine in chloroform. On shaking a green colour is developed. This reaction is progressively destroyed as the ergosterol is irradiated.

In the Writer's limited experience Rosenheim's test is also negative with irradiated ergosterol.

Spectographic:- Three absorption bands at 2935, 2815 and 2700 Ångstrom units were observed by Morton and Heilbron (Nature 10, 122, 1928) and shown to be criteria of provitamin D.

The spectogram of cod liver oil shows two absorption bands in the region of 3280 Å and 2790 Å. The latter is associated with vitamin D.

Source of Vitamins.

The animal world is dependent upon the plant world for its supply of vitamins. Drummond, Zilva and Coward (Biochem. J. 16, 518, 1922) followed the stages in the transfer of the vitamins from their vegetable source to their final place of storage in the cod's liver. The cod is carnivorous and feeds on smaller fish such as herrings, caplins, crustaceans and squids which in turn feed on the small organisms floating on the surface of the sea, classed as plankton. The plankton comprises minute crustaceans, worms, decapods, copepods, and numerous larval forms which feed on the minute diatoms. Diatoms have been grown in the laboratory by Drummond, Jameson and Coward (Biochem. J. 16, 482, 1922) and in the presence of diffuse sunlight they were capable of forming vitamins from the purely inorganic constituents of the medium in which they grew.

It would appear, in view of the recent work on the activation of ergosterol that these diatoms may synthesize the sterols but that untraviolet light from the sun activates them forming the vitamins, Modern trend of research is leaning toward the view that vitamin A is also a sterol.

Comparison of Indirect and Direct Steam Extraction.

Chipman and Mahen reported in 1927 from their work at the Fisheries Experimental Station that the water bath method (indirect steam) produced a good light oil but that the yield was not comparable with the direct steam method unless the temperature was raised to 100°C. At this temperature the oil became darker in colour.

This work was repeated by the writer using essentially the same methods. In the direct steam method the livers were removed in fresh condition from the fish, the gall bladders removed, and they were then washed in cold water. All diseased or spotted livers were discarded.

The livers were then placed in a two-litre beaker to about two-thirds of its depth and steam was led in by a tube which extended to the bottom. The livers were not minced as it had been shown previously by the above workers that such procedure had little or no effect on the percentage yield of the oil.

Some of the steam condensed and the oil floated on the water and rose to the top as the hepatic cells were ruptured by the steam. The mass of livers was subjected to steam for thirty minutes. After cooling, the mass was placed in a flannelette or canvas bag and allowed to drain for several hours. The oil was separated from the water by means of a separatory funnel. After draining, the chum in the bags was pressed, being subjected to increasing pressure until no more oil could be expressed. All supplies of oil were combined and filtered through wood charcoal to remove small particles of stearine and liver. Later work in the Department of Biochemistry showed that Chardin folded filter paper was more satisfactory. The oil was not chilled.

From several determinations it was shown that on an average the charcoal absorbed 9% of the oil. Approximately the same amount of charcoal was used each time. The residue from the bags after pressing was analysed for fat content and from 24 - 25% remained. Möller reported (Cod Liver Oil and Chemistry) that about 25% of the oil remained in the livers after extraction.

In the water bath or indirect steam method the livers were placed in a tin which was immersed in a water bath completely surrounded by water except for a small opening through a funnel in the top. The temperature of the water bath and the inside temperature was closely watched. The duration of cooking was 30 minutes and the oil after cooking was given the same treatment as in the case of direct steam extraction. In later work the livers were placed in a closed can and the container was slung inside a steam retort under low pressure.

The colour of the oil was determined by the Rosenheim-Schuster tintometer.

In all experiments the conditions were kept as constant as was possible. It will hardly be necessary to state that in this work -- as in all experiments with biological material -- the variation of results is considerable. The yield of oil depends on many factors which may all be summed up under the heading "the physiological condition of the fish" -- its food, the time of spawning, its physical condition, age, etc. In this connection it may be pointed out that Hess, Bills, Weinstock, Honeywell and Riokin (Proc. Soc. Expt. Biol. Med. 25, 653, 1928) report that the antirachitic potency of the liver of the cod is in inverse ratio to its content of oil. Oil from livers very small and poor in fat showed them to be exceptionally potent -- "far exceeding in potency any oil which has been heretofore reported."

It was considered that the only safe way of overcoming these individual variations was to make a large number of determinations and average them. In cases where comparable results were needed the livers were divided into the necessary number of parts.

In this connection it must also be remembered that although these laboratory preparations are, in themselves, comparable, in commercial extraction, using much greater quantities, the percentage yields would be very much increased.

The results of a number of experiments on the yield of oil from the two methods of extraction are given in the following table:-

Table 1

Percentage Yield of Oil

	Direct Steam Method		Indirect Steam Method	
Cod Livers	14.0		4.3	
	24.2		18.3	
	25.5		17.7	
		Average 21.2%		13.2%
Ling Livers	38.4		15.0	
	32.8		8.9	
		Average 35.6%		11.9%

Table 2

Colour of Oil

	Direct Steam Extraction			Indirect Steam Extraction	
	Yellow	Red		Yellow	Red
	2.6 units	0.3 units	80°C	40 units	0.7 units
	2.6 "	0.3 "	100°C	40 "	1.1 "
	2.7 "	0.4 "			
	2.3 "	0.6 "			
Average	2.6 units	0.4 units		40 units	.9 units

These results corroborate in general the work of the previous workers. The yield from the indirect steam method of extraction is much smaller than from the direct steam method. Chipman and Mahen reported that "the water bath method produced a good light oil but the yield is not comparable with the direct steam method unless the temperature is raised to 100°C. At this point the oil appears to become darker in colour". The temperature of the water bath varied from 80 - 100°C but the oil was always darker in colour and at 100°C the yield was still much smaller.

It was considered that the darker colour might be due to burning of the livers, so the livers were covered with water before heating the bath. The colour of the resulting oil was not so dark but it was still darker than that from direct steam extraction.

Experiments on the Rotting Process:- Experiments were also carried out using the rotting process and direct steam process. In the former the livers were simply placed in a tin and allowed to rot for three weeks in the sun. These livers using the direct steam method at once yielded 31.2% oil; the rotting process yielded 34.0%. The colour of the oil obtained by this method was yellow 28.0 units, red 9.9 units. The vitamin A potency of the 'rotted' oil using the $SbCl_3$ test was 7.6 units and for 'fresh' oil from the same livers was 9.0 units.

The liver oil obtained by the rotting process is high in free fatty acids and the decomposition products give it objectionable odour, colour and flavour. However the free fatty acids can be removed and the objectionable colour and odour decreased by various processes. This method has certain commercial advantages in that it requires no attention and no equipment except a barrel.

From these results it appears that oil produced by the rotting process is inferior in vitamin potency, to oil produced from fresh livers. The percentage yield is about the same.

Holmes (Ind. Eng. Chem. 16, 964, 1924) reports concerning the rotting process that "vitamin potency decreased as decomposition progressed". Since it is well established that both sunlight and oxidation destroy vitamin A the inferior potency can be easily accounted for.

Effect of Time of Heating on Extraction.

As was previously pointed out the livers, in commercial manufacture, are heated "until in the opinion of the operator" they have been sufficiently cooked. Investigation by Chipman and Mahen reported a drop in percentage yield when the steaming was prolonged. This may be due to emulsification, hydrolysis or the loss of some volatile fatty acid.

The direct steam method of extraction was used; steaming cod livers for 15, 30, 45, 60, 90 and 120 minutes. The results (averages) are tabulated in Table 3. Two types of yields were obtained and these are both shown.

Table 3

Time of Steaming	Percentage Yield		Colour of Oil	
15 minutes	27.2%	24.9%	Yellow 3.9 units	Red .8 units
30 "	37.4	28.9	3.8	.7
45 "	35.0	33.6	4.3	.8
60 "	38.0	30.6	4.2	.9
90 "	38.4	19.6	5.1	1.1
120 "	38.8	18.4	5.1	1.4

The effect of prolonged steaming on vitamin A potency is shown in Table 4. The vitamin potency is expressed in blue units as measured on the Rosenheim-Schuster tintometer using the $SbCl_3$ method.

Table 4		
Time of Steaming	Vitamin A Potency	
15 minutes	7.6	7.8
30 "	7.4	7.5
45 "	6.6	7.5
60 "	6.4	7.3
90 "	5.8	7.5
120 "	5.8	6.8

These results show that if the yield is not decreased by prolonged heating, it certainly is not appreciably increased. Furthermore, prolonged steaming will cause more destruction of the vitamin and gives the oil longer contact with air, affording an opportunity for oxidation of the fatty acids and the subsequent unpleasant taste. There was also a progressive darkening of the oil as the time of steaming was increased.

In view of these facts, it would seem that from 30 minutes to 1 hour is the most satisfactory time for steaming. Less than 30 minutes' steaming would result in loss of oil.

In the case of the indirect steam method (water bath) another factor comes into force. The temperature of the centre of the mass of livers was 68° on one occasion when the temperature of the bath was 80°C. On re-extracting the chum from this experiment by the direct steam method 14% of oil was extracted. It would seem that longer heating was necessary to obtain better yields using this method. Results (averages) of a series of experiments in this connection are given in Table 5.

Table 5			
Time of Extraction	Percentage Yield	Colour of Oil	
30 minutes	17.0%	Yellow 4.0	Red .9
60 "	20.7	4.6	1.2
120. "	24.1	6.0	1.0

Prolonged heating increases the yield, using the indirect steam method of extraction, but the oil becomes progressively darker. This method would seem unsatisfactory on all counts. Prolonged heating in this method seemed to have little or no effect on the vitamin potency due probably to the fact that there is less opportunity for oxidation. The potency was 7.4, 7.6, 7.6 respectively.

Pressing of 'Chum'.

It has been questioned by some workers as to whether pressing the chum was worth while. Two lots of oils were extracted by the direct steam method and the oil was allowed to drip from the chum in the flannelette bags for 12 hours. This oil was kept and the chum was pressed and the oil obtained was kept separate. The results are shown in Table 6.

Table 6

Percentage Yield		Colour of Oil Yellow	Colour of Oil Red
30 minutes steaming			
Drip	10.1%	3.0 units	1.2 units
Press	10.1	5.6	1.5
1 hour steaming			
Drip	17.6%	4.1 units	1.1 units
Press	6.0	5.0	1.5

The oil from the press is undoubtedly darker in colour and for medicinal purposes should be kept separate from the first fraction. The percentage yield seems large enough to warrant the pressing of the chum.

Reheating the chum after direct steam extraction yielded little oil and does not warrant the expense

Storage of Livers.

Investigations were also carried out with a view towards developing a method for keeping the livers in storage for some time before extracting the oil. If a satisfactory method was found, fishermen with small catches would be able to store the livers until such time as a supply was obtained large enough to be economically extracted. It would also be of use in the case of trawlers which are out on the banks for several days.

Since it is evident that exposure to the air and sunlight causes a progressive decrease in vitamin potency and darkening in colour of the oil, methods using low temperatures and exclusion of air were tried. In the first method the livers were kept in ice and water (0°C) in sealed jars for varying times and the colour and vitamin potency of the oil determined. A typical result is given:-

Table 7

Percentage Yield		Vitamin A Potency	Colour of Oil	
Direct Steam Extraction			Yellow	Red
At once	30.6%	6.8 blue units	2.7 units	0.8 units
0°C 1 day	37.4	6.8	3.5	1.0
2 "	38.2	6.6	5.0	1.4
3 "	38.4	6.4	4.7	1.3
4 "	40.6	5.7	4.9	1.6
5 "	34.4	5.8	7.8	1.8
6 "	35.0	5.7	11.0	2.0
7 "	38.8	5.5	8.0	2.0

A few experiments were made leaving the jars unsealed but even at this low temperature the oil was very rancid and dark in colour.

This method although of some use since in unsealed jars the colour of the oil at the end of 7 days was yellow 20.0 units, red 3.7 units with a vitamin potency about the same as in the sealed jars, is not satisfactory. It would be of some use in case of necessity. The National Fish Company has equipped their trawlers with barrels that can be sealed for holding livers.

A second method was tried keeping the livers in sealed cans in cold storage around - 12°C for two weeks. The livers were divided in half and comparable tests made extracting at once and after storage. The results are shown in Table 8

Table 8

Direct Steam Extraction Fresh Livers	Percentage Yield After Cold Storage
27.4%	45.3%
31.4	40.2
33.1	44.8
36.3	43.4
25.6	30.3

Another method using atmospheres of air, CO_2 and nitrogen at the same temperature was tried. The yields, using direct steam extraction, were, from fresh livers 25.6%; after cold storage in air 30.3%, in CO_2 41.3%, in N 38.9%. The resultant oil was of good colour and there was no difficulty in extraction from the frozen livers. A typical result with regard to colour being,-

Oil from fresh livers	Yellow	3.4	units,	Red	0.9 units
" " storage livers in air	"	3.7	"	"	0.9 "
" " " " " CO_2	"	3.6	"	"	1.0 "
" " " " " N	"	3.7	"	"	1.0 "

The percentage yield of oil was always increased. The reason for this increase was thought to be due to increased rupture of oil cells during the freezing but one lot of livers were rapidly frozen in a brine tank which should cause minimum rupture and the yield was again increased from 35.5% to 40.1%.

The vitamin content of the oil determined by the $SbCl_3$ method was not appreciably changed. Oil from fresh livers gave 6.8 blue units and from cold storage livers gave 6.6 blue units.

Holmes (Ind. Eng. Chem. 16, 295, 1924) reports that "oils from livers which had been stored at a low temperature out of contact with air had as high a vitamin potency as oils rendered from fresh livers".

This method seems worthy of further investigation and it would be feasible for companies with cold storage facilities to adopt this method not only for holding livers but also as a means of increasing the yield of oil.

Refining.

The refining of medicinal cod liver oil by chilling and the removal of the stearine which crystallizes out around $0^{\circ}C$ by means of a filter press was investigated. It resulted in a loss of over 40% of the original oil. The colour of the remaining oil was changed from yellow 2.5, red 0.4 units to yellow 1.9, red 0.1 units; the vitamin content of the unrefined oil was 10.5 blue units and the refined 10.0 units using the $SbCl_3$ test. The colour of the stearine removed by the chilling on being brought back to its liquid state was yellow 2.0, red 0.1 units with a vitamin potency of 9.9.

This would appear to be a wasteful process which does little or no good. Holmes (Ind. Eng. Chem. 18, 188, 1926) reports "little difference between the chemical and physical characteristics of crude and pressed cod liver oils" and that there is "no significant difference in vitamin potency". Drummond and Zilva (J. Soc. Chem. Ind. 41, 280, 1922) say "it would appear unnecessary to remove the stearine" and further "it seems a pity that so valuable a source of the vitamin should be used for technical and non-dietetic use".

Mixing of Livers.

Since it is almost impossible to eliminate the tendency of fishermen to mix pollock, cusk, hake or haddock livers with cod livers the question arises as to the effect of this "adulteration" on the vitamin potency and yield of cod liver oil. Investigations were made on the pollock, haddock and hake, cusk not being available at the time. The livers were obtained in fresh condition and the oil was extracted by the direct steam method as outlined previously. The results (averages) are given in Table 9.

Table 9

Percentage Yield		Vitamin A Potency	Colour of Oil	
Cod	28.2%	9.0	Yellow 2.5	Red 0.4
Haddock	25.8	6.4	4.0	1.0
Pollock	45.5	12.2	2.9	0.9
Hake	47.9	11.1	2.8	0.7

In the case of pollock oil on extraction it is colourless but on standing exposed to air it rapidly darkens and in a few hours becomes quite deeply pigmented.

Zilva and Drummond (Lancet 11, 753, 1921) say "coal-fish (pollock) is even a little more potent than cod and haddock oil although not as active, showed a high potency".

Holmes (J. Metabol. Res. 2, 360. 1922) reports that "the potency of pollock livers is slightly in excess of that of cod liver oil obtained from cod fish of similar physical condition".

Holmes (J. Metabol. Res. 5, 259, 1924) reports that haddock liver oil contained less vitamin A than hake or cod liver oils.

Chipman however reported in 1927 that haddock liver oil contained no vitamin A. This would appear to be incorrect. He reports hake liver oil as a potent source of the vitamin.

It is evident that as regards colour, yield and vitamin potency that haddock livers are inferior to the rest but are still potent sources of the vitamin; the livers of the other fish examined are superior and there appears to be no adequate reason why cod livers should not be mixed with the livers of these other fish.

In view of the fact that it is generally believed that sexual condition and age of the fish, as well as food, influence the potency of the liver oil, it would seem necessary that further research on liver oils from these fish at different seasons should be carried out. However, Zilva, Drummond and Graham (Biochem. J. 18, 178, 1924) claim that age and sexual condition, i.e. in relation to time of spawning, have no influence.

Liver Oils from Other Fish.

Dogfish. The average yield of oil was 33.6% with a vitamin A potency of 4.0 units using the $SbCl_3$ test. This is a low potency and is in contradiction to the reports of Brocklesby (Can. Chem. Metall, Sept. 1927); Holmes (Ind. Eng. Chem. 17, 310, 1925) and Chipman on oil obtained in 1927. Oil obtained at the same time by Cook, chemist of National Fish Co. was also inferior. This may be due to a seasonal variation.

Skate. The percentage yield was 11.6 with a vitamin A potency of 3.8.

Catfish. No oil was obtained from several specimens.

Burbot (fresh water cod or ling). Work on the liver oil from this freshwater fish was begun at the Station and continued at the Department of Biochemistry, University of Toronto. The livers were shown to give a yield of oil varying from 31.2 to 46.0% with an average of 38.1%. The vitamin A potency is as good as, if not better than, medicinal cod liver oil in both colorimetric and biological assays. The biological assay ranks the oil as containing about 500 units per gram and the colorimetric assay gives 13.0 blue units using the $SbCl_3$ test. Complete results will be published elsewhere in the near future.

Summary

1. The direct steam method of extraction, from 30 to 60 minutes' duration, is shown to give better cod liver oil as regards yield, colour and vitamin A content.

2. Removal of stearine by chilling is not advocated.

3. Storage of livers at -12°C either in air or atmospheres of CO_2 or nitrogen is shown to appreciably increase the percentage yield of oil without materially affecting the colour or vitamin content of the oil.

4. The mixing of livers of haddock, hake and pollock with cod livers in the manufacture of cod liver oil is not injurious to colour, yield or vitamin content, and with the exception of haddock increases yield and vitamin potency.

5. Liver oils from the dogfish, skate and burbot (ling) were investigated.

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