THE PRINCIPAL CHEMICAL RESEARCHES ON COD LIVER OIL

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HISTORY AND PREPARATION

The literature comprising the chemical studies on cod liver oil is very extensive, and it contains a mass of contradictory assertions. The confusion follows from the fact that samples of this oil may be widely dissimilar, and that the oil of commerce is prepared by divers methods, occasionally from materials other than the liver of *Gadus morrhua*. Moreover, the mystery surrounding its therapeutic action has led investigators into strange fields of speculation. At present the chemical reactions and composition of cod liver oil are just beginning to be understood; it is well, therefore, to distinguish the substantial researches of the past from those which appear inconsequential.

In the ancient method of preparing fish liver oils the livers are submitted to natural disintegration, with or without the aid of solar or artificial heat, and the oil which separates above the tissue mass is decanted. The early stages of this "rotting process" are, I believe, autolytic rather than putrefactive, for the oils possess considerable bactericidal power, and the enzymes of fish liver obviously must be active at low temperatures.

Dubail (1854) described the methods of rendering once in vogue at the principal fishing grounds of the world. The rotting process was in general use, but he stated that the London apothecaries in 1849 employed a steam-jacketed apparatus, and that shortly thereafter one Dr. Fleury of Newfoundland devised a tinned copper cooker for rendering the oil without decomposition. He added that Fleury had studied the storage of oil in the cod-fish liver—the remarkable fattening which follows the early

summer feeding migration. Dubail failed to mention that Fox (1848), possibly as agent of the London pharmacists, established at St. John's the first factory for the manufacture of "steam process" oil. Möller (1853) introduced this process into Norway. He used "a water bath of large dimensions." Berthé (1855) observed, apparently to his surprise, that the steamed oil gave the sulfuric acid color reaction of Gobley (1844) just as did rotted oil.

In the modern manufacture of medicinal oil direct steam has all but universally replaced the steam jacket. Fresh livers are steamed for 20 to 30 minutes, and, as the heating is rapid and uniform, enzymic decomposition is practically nil. The oil which separates is filtered from suspended coagulated protein, and chilled to remove "stearine." Nearly all phases of the cod liver oil industry not covered in Möller's book (1895) have been described in the important papers of Drummond and Zilva (1922), Zilva and Drummond (1923), André (1923), and Drummond (1924). The therapeutic history was reviewed by Bennett (1841), Dubail (1854), and Guy (1923).

The early studies on rotted oil were amply considered by Möller (1895), who discussed more than fifty compounds alleged to occur in this material. I shall make little attempt to interpret these investigations, for the reason that the rotted product is really not cod liver oil, but a more or less generally adulterated decomposition product thereof. It is now seldom used in medicine, being confined to technical arts under the name of "cod oil."

Cod liver oil of the best grade ranges in color from golden to pale yellow. It is *devoid of taste-qualities*, but it exhibits a faint, not unpleasant, fishy smell, especially noticeable when the oil is held in the mouth. (The sense of smell is popularly confused with taste.) Even the best oil is subject to considerable variation in composition, the result of seasonal, sexual, nutritional, or other differences in the fish or mode of rendering. The numerous determinations of the physico-chemical constants of cod liver oil, published in every treatise on fats, serve but to

emphasize its indefinite nature. The behavior of cod liver oil with 250 organic solvents has been studied by Bills (1926).

THE FATTY ACIDS

According to Tolman (1909) most marine oils are chemically similar,[1] and very different from vegetable oils and the oils of land animals. Cod liver oil consists of about 99 per cent of simple and mixed fatty acid glycerides and 1 per cent of unsaponifiable matter. It is usually "frozen" ("refined") at a few degrees of frost to remove the high-melting fats which are technically, but erroneously termed "stearine." The "stearine," according to Heyerdahl (1895) and Lewkowitsch (1922) contains much unsaturated material, although it is less unsaturated than the original oil. The existence in the oil of certain fatty acids is well established; that of others is doubtful; no chemist in recent times has attempted a quantitative separation.

Palmitic acid, $C_{16}H_{32}O_2$. Passing over the questionable "gadic acid" of Luck (1856), one finds that Heyerdahl (1895) demonstrated palmitic acid among the fatty acids liberated upon the acidification of soap prepared from refined cod liver oil. The palmitic acid separated at ordinary temperature from the total fluid acids, and was purified by recrystallization from alcohol. The analytical data and the melting point, 62°, clearly identified the product.

Jecoleic acid, $C_{19}H_{36}O_2$; *Fahrion's acid*, $C_{17}H_{32}O_2$. Jecoleic acid, the existence of which has been questioned by Bull (see below), was investigated by Heyerdahl in the following manner. The fluid acids remaining after the separation of crude palmitic acid were oxidized by concentrated alkaline permanganate at 0°. The insoluble oxidation product was washed with cold water, and recrystallized from chloroform to a melting point of 114° to 116°. Ultimate analysis and the acid value indicated the formula $C_{19}H_{38}O_4$ for the oxidized acid, and acetylation indicated two hydroxyl groups, thus: $C_{19}H_{36}O_2(OH)_2$. This

[1] A few exceptions were noted, mainly in the unsaponifiable matter.

compound, "dihydroxyjecoleic acid," may be presumed to have arisen by the oxidation of the jecoleic acid, $C_{19}H_{36}O_2$, originally present in the oil. Dihydroxyjecoleic acid may be compared with the "asellic acid," $C_{17}H_{34}O_4$, m.p. 116°, previously obtained by Fahrion (1893) by somewhat similar methods. Asellic acid presupposes the existence in cod liver oil of an unnamed acid, $C_{17}H_{32}O_2$.

Therapic acid, $C_{17}H_{26}O_2$. The fluid fatty acids were brominated in cold acetic acid. The purified, alcohol-insoluble bromine compound was found to be an addition product, $C_{17}H_{26}O_2Br_8$, of an acid, $C_{17}H_{26}O_2$, originally present in the oil. Heyerdahl called this "therapic acid" because of its supposed therapeutic virtues. Heiduschka and Rheinberger (1911) prepared from the mixed fatty acids a tetrachlorotetraiodide (instead of the octobromide), and dehalogenated this with zinc and acetic acid. Apparently the reduction did not stop at therapic acid, but continued to a dihydrotherapic acid, $C_{17}H_{28}O_2$. Lewkowitsch (1921), who is always skeptical of odd-carbon fatty acids, pointed out that Heiduschka and Rheinberger's analytical data agree better with C_{18} than C_{17}. He believed that therapic acid is nothing but impure clupanodonic acid (see below).

Myristic acid, $C_{14}H_{28}O_2$. For the identification of several acids we must refer to the important work of Bull (1906). With a special apparatus he distilled the methyl esters prepared by the sodium methylate method from pure Lofoten oil. Eighty per cent of the esters could be distilled up to 240° at 10 mm. Hg, and it was found that these esters fractionated themselves fairly definitely at several narrow temperature ranges. The fraction, 161.5° to 165°, upon recrystallization from alcohol, yielded the pure methyl ester of myristic acid. M.p. = 19°. Saponification number = 231.8 (theory = 231.4). The next fraction, 185° to 186°, contained much methyl palmitate, which was also crystallized from alcohol. M.p. = 29.5°. Saponification number = 207.4, exactly theoretical. By saponification palmitic acid was obtained, m.p. = 62°, thus confirming Heyerdahl.

Palmitoleic acid, $C_{16}H_{30}O_2$. The alcoholic solution from the 185° to 186° fraction was saponified, converted into the barium

salt, the latter crystallized from ether, and purified as the zinc salt. The free acid melted at −1° and corresponded to the formula $C_{16}H_{30}O_2$. Bull did not name this acid; the designation, "palmitoleic," was conferred by Lewkowitsch (1906). André (1923) asserts that Bull has recently proposed to call this substance "zoomarinic acid," because of its somewhat general occurrence in marine animal oils.

Stearic acid, $C_{18}H_{36}O_2$. The fraction, 205° to 206° was saponified, and the potassium salt converted into the lead salt. On long standing an ether solution of the lead salt precipitated a small quantity of lead stearate.

Oleic acid, $C_{18}H_{34}O_2$. The ether-soluble portion of the lead salt yielded an acid solidifying at 4°. The acid number, the iodine number, and the solubility ratio of the sodium and potassium salts in alcohol indicated that this was oleic acid.

Gadoleic acid, $C_{20}H_{38}O_2$. The fraction, 223° to 225° was saponified, and the free acid prepared. This was exactly half neutralized with KOH, and twice recrystallized from alcohol. The liberated free acid melted constantly at 24.5°. The analysis, acid number, and iodine number agreed with the formula $C_{20}H_{38}O_2$. This acid, named "gadoleic," occurs in large amount. Bull's excellent distillation curve gave no evidence of Heyerdahl's jecoleic acid. Gadoleic acid oxidized with alkaline permanganate at 0° yields an acid which Bull termed "dihydroxygadic acid" (not related to Luck's gadic acid, however). Lewkowitsch, 1921, calls this more appropriately "dihydroxygadoleic acid." One may suspect that Heyerdahl's dihydroxyjecoleic acid, m.p. 114° to 116°, is a eutectic mixture of dihydroxygadic acid, m.p. 127.5° to 128°, and dihydroxystearic acid, 141° to 143° (the oxidation product of oleic acid), in which case "jecoleic acid" becomes a mixture of gadoleic and oleic acids. It is indeed true that odd-carbon higher fatty acids are extremely rare,[2] although inseparable eutectic mixtures of fatty acids are not uncommon.

[2] According to Lewkowitsch (1921) the identity of possibly only daturic acid, $C_{17}H_{34}O_2$, has been established beyond reasonable doubt.

Erucic acid, $C_{22}H_{42}O_2$. The liberated acid of the final fraction, 239° to 240°, was recrystallized from alcohol. It melted sharply at 34°, and gave an acid number of 165.4 (theory = 165.9). The solubility of the salts further indicated erucic acid.

Eicosapentenoic acid, $C_{20}H_{30}O_2$; *Eicosahexenoic acid,* $C_{20}H_{28}O_2$. More recently Bull (1917) prepared a mixture of the bromides of unnamed fatty acids which, he believes, represents $C_{20}H_{30}O_2$ and $C_{20}H_{28}O_2$. The former may be identical with the acid later designated "eicosapentenoic" by Brown and Beal. The latter I have not found elsewhere described, but in keeping with modern nomenclature it might be termed "eicosahexenoic acid."

Hexadecatrienoic acid, $C_{16}H_{26}O_2$; *Clupanodonic (octodecatetrenoic) acid,* $C_{18}H_{28}O_2$; *Arachidonic acid,* $C_{20}H_{32}O_2$; *Docosapentenoic acid,* $C_{22}H_{34}O_2$; *Docosahexenoic acid,* $C_{22}H_{32}O_2$. Brown and Beal (1923), in their study of the unsaturated fatty acids of menhaden oil, had occasion to examine also salmon, herring, and sardine body oils, and cod liver oil.[3] The saponification numbers, iodine numbers, and refractive indices of all five oils were very similar. Each oil was esterified by the methyl alcohol-hydrogen chloride method, and the esters were fractionally distilled at 15 mm. Hg over 10° ranges. From the iodine number, polybromide number, and percentage of bromine in the bromides of the fractions it was stated that these oils were "decidedly similar in character." The menhaden ester fractions were worked up more thoroughly than the cod liver esters, but it was assumed, on the basis of the indicated similarity, that the cod liver oil possessed essentially the same composition as the menhaden.

Further analytical procedures applied to the *menhaden* oil indicated the presence of several series of acids: C_{14}, C_{16}, C_{18}, C_{20}, and C_{22}, thus confirming Twitchell (1917). Roughly, the degree of unsaturation increases with the molecular weight. Bull's finding of myristic acid, and Heyerdahl's and Bull's recovery of palmitic acid were supported. Methyl clupano-

[3] A dark amber, American cod oil—personal communication.

donate was obtained in a state of purity believed to be hitherto unequaled. Unfortunately, the literature on clupanodonic acid is confounding. Tsujimoto (1906) gave this name to an acid, $C_{18}H_{28}O_2$, found in sardine oil. Later (1920, 1922) he decided that the formula should be $C_{22}H_{34}O_2$. In the meantime, however, other investigators perpetuated the designation, clupano-

TABLE 1

ACID	FORMULA	MOST PROBABLE MELTING POINT	ATOMS OF HALOGEN ABSORBED
Myristic	$C_{14}H_{28}O_2$	53.8°	0
Palmitic	$C_{16}H_{32}O_2$	62.6°	0
Palmitoleic	$C_{16}H_{30}O_2$	−1°	2
Stearic	$C_{18}H_{36}O_2$	69.3°	0
Oleic	$C_{18}H_{34}O_2$	12°–17°*	2
Clupanodonic (octodecatetrenoic)	$C_{18}H_{28}O_2$		8
Gadoleic	$C_{20}H_{38}O_2$	25.4°	2
Erucic	$C_{22}H_{42}O_2$	33°–34°	2
Docosapentenoic	$C_{22}H_{34}O_2$		10

* Dimorphous.

TABLE 2

ACID	FORMULA	ATOMS OF HALOGEN ABSORBED
Hexadecatrienoic	$C_{16}H_{26}O_2$	6
Fahrion's	$C_{17}H_{32}O_2$	2
Therapic	$C_{17}H_{26}O_2$	8
Jecoleic	$C_{19}H_{36}O_2$	2
Arachidonic	$C_{20}H_{32}O_2$	8
Eicosapentenoic	$C_{20}H_{30}O_2$	10
Eicosahexenoic	$C_{20}H_{28}O_2$	12
Docosahexenoic	$C_{22}H_{32}O_2$	12

donic, for the original $C_{18}H_{28}O_2$, which nomenclature is quite properly retained by Brown and Beal. Hexadecatrienoic, arachidonic, eicosapentenoic, and docosahexenoic acids were probably present. The presence of arachidonic acid in cod liver oil may be indicated in a very early report by Bull (1899). Docosapentenoic acid, $C_{22}H_{34}O_2$, was also found, and its existence, in cod liver oil, was almost contemporaneously confirmed

by Tsujimoto and Kimura (1923). The Japanese authors, however, called this acid *clupanodonic* (*sic*); they prepared it by methods similar to those of Brown and Beal.

In passing it may be noted that all the known fatty acids of cod liver oil fall into the broad series, $C_nH_{2n-2x}O_2$, where n = 14 to 22, and x, the number of double bonds, = 0 to 6. That there are many unfilled places in this series is not remarkable, for the iodine number of cod liver oil may itself vary well over 70 units, and the unsaturated acids are rather unstable. Those acids the existence of which is reasonably well established are listed in table 1.

The presence of the acids listed in table 2 is less well established.

"MORRHUIC ACID" AND CHEMOTHERAPY

Recent chemotherapeutic investigations on unsaturated fatty acids have revived the term, "morrhuic acid," in a new meaning. Originally this name was given by Gautier and Mourgues (1888) to a nitrogenous putrefaction product, hydroxydihydropyridine butyric acid, $C_9H_{13}NO_3$, found in rotted oil. Lately, in its new sense, "morrhuic acid" or "sodium morrhuate" is merely an elegant synonym for cod liver oil soap prepared for intravenous injection by the method of Rogers, Muir, Knowles, Cochrane, Davies, and Brierley (1919), Ghosh (1920), or Cutting (1926). While a therapeutic dose of the soaps thus prepared would, I believe, contain an insignificant amount of vitamin D, possibly also vitamin A, the basis for the alleged value of the soap in the treatment of tuberculosis and leprosy must be sought elsewhere.

It is well known that the fat of animals is modified by the fat of their diet. Bell (1851) wrote that pigs which ate cod livers became very fat, and their fat acquired the color and odor of cod liver oil. Channon, Drummond, and Golding (1924) observed that the butter fat of cows receiving cod liver oil tended to acquire the constants of the oil. Williams (1912a, 1912b), perhaps influenced by Heyerdahl's theories, attributed the beneficial effect of cod liver oil to the direct action of the unsaturated acids absorbed in the tuberculous tissues. Lansberg (1919) observed that tubercle bacilli disintegrate in cod liver oil, but

not in vegetable oils. Lindemburg and Pestana (1920, 1921) observed that cod liver oil, like chaulmoogra oil, inhibits the growth of cultures of the leprosy and tubercle bacilli. Ghosh (1920) found that cod liver oil "morrhuate" exhibited the same action in leprosy as the sodium hydnocarpate prepared from chaulmoogra oil, and that it furthermore appeared to exert some action in tuberculosis. Ghosh attributed the action of both cod liver oil and chaulmoogra oil to the highly unsaturated acids. (However, no cyclic acid of the chaulmoogric-hydnocarpic series has yet been recognized in cod liver oil.) The following additional medical references are of interest in this connection: Rogers (1919), Biesenthal (1920), Tambe (1920), Campbell and Kieffer (1922), Tewksbury (1922), Fine (1922), Hume (1924), Grigaut and Tardieu (1924), Pernet, Minvielle, and Pomaret (1925), Caussade, Tardieu, and Grigaut (1925), Jessel (1925). It must be stated, however, that the chemotherapeutic action of cod liver oil has not been thoroughly substantiated. Perhaps, after all, the value of the oil lies mainly in its remarkable content of vitamins.

OXIDATION AND HYDROGENATION

It is evident, from the number of unsaturated acids in cod liver oil, that many hydroxy acids can exist. However, Heyerdahl (1895) has shown that perfectly fresh oil is "hydroxyl-free." On exposure to air it becomes rancid, the rancidity being due to the gradual formation of hydroxy-fats, not to the liberation of fatty acid. The free fatty acids, indeed, oxidize with great ease, but medicinal oil contains only a fractional percentage of acid not combined as glycerides.

Lund (1925) confirmed Heyerdahl's observation that rancidity is caused by oxidation, and proceeded to investigate the mechanism of the oxidative process. By means of the guaiacum reaction he detected organic peroxides during the initial stages of oxidation. These peroxides *per se* imparted no rancidity to the oil; moreover, a rancid oil long after contact with oxygen showed no peroxide. But a non-rancid oil, containing its own peroxide or an added varnish, became rancid on standing, even

in the absence of oxygen. On the other hand, in the presence of an easily oxidized substance, such as liver débris or pyrogallol, the rancidity was delayed. It is evident, therefore, that the oxidation of cod liver oil is an autocatalytic process, accelerated by organic peroxides, and retarded by foreign substrates.

In keeping with that of Lund is the earlier work of Drummond, Zilva, and Coward (1924) on the spontaneous deterioration of cod liver oil emulsions. They found that oil emulsified with Irish moss or gum acacia, which contain oxidases, gradually lost its vitamin A potency. On the other hand, gum tragacanth containing no oxidase caused no appreciable destruction. Other evidence of oxidation induced by emulsifying agents appears in the work of Krauss (1914).

The general subject of the autoxidation of unsaturated fatty acids has been reviewed by Fahrion (1920, 1921). He showed that various degrees of oxidation may take place, that oxidation may be accompanied by polymerization or dehydration, and that in certain cases only volatile products may ensue. Kugelmass and McQuarrie (1925) observed that cod liver oil during oxidation gave off an emanation, probably a vaporous substance, which fogged photographic plates. Servais (1903) detected a malodorous, volatile aldehyde among the products of oxidation by air.

Oxidation becomes a practical problem in the manufacture of cod liver oil. The formation of oxidation products, like those of enzymic hydrolysis, may be satisfactorily avoided only by conducting the rendering operations expeditiously. Other procedures, such as the "gassing" processes that have been exploited during the past 70 years, are, as Lewkowitsch (1922) pointed out, superfluous. Gassing originated in the British patent of Murray (1853) which specified that the "unpleasant odours or fetid flavours" of (rotted) oil are improved by treating the oil with carbon dioxide. Gas exerts no protective influence on cod liver oil which has been properly rendered and bottled.

By hydrogenating cod liver oil edible hard fats are prepared in Norway. The commercial hydrogenation processes unfortunately destroy the vitamins. Schuck (1918), Marcelet (1921),

and Grosser (1925) observed that the fishy smell of cod liver oil disappears during hydrogenation. Schuck found that the highly unsaturated fats, to which the smell is largely due, are reduced by hydrogen even in the absence of a catalyst. His patented process nevertheless destroys the vitamins.

INORGANIC CONSTITUENTS

Years ago considerable importance was attached to the inorganic composition of cod liver oil. For example, in 1853 the *Académie Impériale de Médecine* entertained a special report on whether the oil contains a trace of phosphorus. The nineteenth century literature yields many other discussions of a similar character, some of which were reviewed by Möller (1895). The original references are mainly of historical interest. Since Möller's time the literature has increased disproportionately to the importance of the subject. After examining some forty references I believe that the available information may be accurately digested in a few sentences.

Direct ignition of small quantities of pure cod liver oil yields no weighable ash. Some of the inorganic substances may be determined by carbonizing the oil or its soap, and extracting the char; others may be identified in the unsaponifiable fraction, or in Kjeldahl digests. Iodine is undoubtedly present to the extent of 0.0001 to 0.0005 per cent. Traces of Cl, Br, P, N, Zn, organic S, and alkalies may sometimes be found. Rotted oil may contain any of these elements, and especially N and P, in amounts considerably larger than traces. Certain pharmacologically active nitrogenous bases are characteristic of rotted oil.

UNSAPONIFIABLE FRACTION—VITAMINS

The unsaponifiable fraction of cod liver oil consists of approximately half cholesterol and half pigment, vitamins, hydrocarbons, complex alcohols, and unidentified matter.

Cholesterol. Allen and Thomson (1881) saponified the oil with alcoholic sodium hydroxide, evaporated the soap to dryness with sodium bicarbonate and sand, and extracted the dry mass with petroleum ether. Cholesterol was isolated from the extract.

Cholesterol is a secondary olefinic terpene alcohol, $C_{27}H_{46}O$, melting in the neighborhood of 148°, and giving a great number of derivatives in keeping with its structure.

Pigment. The early assumption, based on the sulfuric acid color reaction, that the pigments of cod liver oil are biliary substances, was doubted by Buchheim (1875). He demonstrated that aqueous extracts of the oil contain no gall-substance, that an emulsion of the oil and bile completely separates on standing, and that the principal bile pigment indeed does not give exactly this color reaction. Salkowski (1887) studied the pigment by means of the improved color reaction of Hager (1885). This test, now called the "Hager-Salkowski reaction," consists in adding to a chloroform solution of the substance to be examined a few drops of concentrated sulfuric acid, whereupon the color evoked passes into the chloroform stratum. Cod liver oil itself gives a light violet color, gradually changing to reddish brown; cholesterol, a blood red, slowly darkening; the purified fatty acids, a dark brownish red; and the concentrated pigment from the oil, an intense indigo blue, rapidly changing to purple violet. Thus the color reactions of cod liver oil may correspond, not to one substance, but to several conjointly—a fact of significance to investigators in search of specific vitamin reactions. Salkowski concluded that the pigment of cod liver oil belongs to the group of "lipochromes." Rosenheim and Drummond (1920) claimed that the substance which gives this lipochrome reaction may be distinct from carrotene (carotin) and xanthophyll and any other known lipochrome. Palmer (1922) regarded this lipochrome as not a carotinoid. In private correspondence he writes that the pigment does not give the ferric chloride test for carotinoids. Moreover, it does not disappear when cod liver oil is heated with oil-bleaching charcoal. In fact, the oil thus becomes green, just as *bilirubin* becomes green upon oxidation to biliverdin. De Kadt (1920) reported that when the lipochrome is removed from cod liver oil by the siliceous adsorbent, "tonsil," the treated oil no longer gives the sulfuric acid test, while a carbon disulfide extract of the adsorbent exhibits the lipochrome in concentrated

form. Whether the tonsil removes cholesterol and the vitamins is not known.

Vitamin A and vitamin D. It is not within the scope of this review to discuss the very extensive literature of the fat-soluble vitamins, this field having recently been covered by McCollum and Simmonds (1925), Drummond, Channon, and Coward (1925), and others. Briefly, it may be stated with regard to cod liver oil that biological assays indicate in all samples the presence of vitamins A and D to a greater or less extent. The xerophthalmia-preventing factor (or factors?) known as vitamin A was recognized in this oil by Osborne and Mendel (1914); and the calcification-promoting, rickets-preventing vitamin D by McCollum, Simmonds, Becker, and Shipley (1922). Both vitamins influence growth.

Neither vitamin has yet been isolated, but potent concentrates of one or the other have been prepared by several investigators, notably Zucker (1923), Dubin (1925), Takahashi, Nakamiya, Kawakami, and Kitasato (1925), and Drummond, Channon, and Coward (1925). The general procedure for concentrating the vitamins may be summarized: Cod liver oil, or an extract thereof obtained by means of an immiscible solvent such as alcohol or acetic acid, is saponified with alcoholic alkali. The unsaponifiable matter is extracted by a suitable organic solvent, either from the semi-aqueous solution of the alkaline soap, or from an insoluble precipitate of calcium or magnesium soaps. The crude extract is then further concentrated by fractional crystallization, high-vacuum distillation, treatment with digitonin, and other special operations.

Various researches would indicate that vitamin A originates in green plants, reaching the liver of the cod through the intricate stages of marine nutrition. It is often associated, but certainly not identical, with the lipochromes. It is resistant to saponification, *gentle* hydrogenation, and acetylation, is easily oxidized, is destroyed by short light waves, is volatile with steam or high vacuum, is dialyzable through rubber, is not precipitated by digitonin, may be of an aldehydic nature, and may be responsible for certain color reactions of cod liver oil.

The origin of vitamin D is unknown. It may be exogenous, like A, or endogenous, as by enzymic synthesis within the fish. Vitamin D is resistant to saponification and *gentle* hydrogenation, is only less readily oxidized than A, is destroyed by short light waves, may be dialyzable through rubber, is not precipitated by digitonin, is destroyed by nitrites, and may be related to the sterols. Substances exhibiting antiricketic properties are produced by the action of ultraviolet rays on cholesterol, sitosterol, and ergosterol; and by the action of Florida earth on cholesterol.

Hydrocarbon. The presence in pure cod liver oil of a small amount of hydrocarbon was demonstrated by Drummond, Channon, and Coward (1925), who obtained an unsaturated material, probably squalene, $C_{30}H_{50}$, by the high-vacuum distillation of the unsaponifiable fraction. Recent investigations have shown that squalene is undoubtedly identical with spinacene.

Alcohols. Drummond, Channon, and Coward further report that the unsaponifiable fraction appears to contain a considerable amount of one or more unsaturated fluid alcohols, and a small amount of a saturated solid alcohol melting at 60° (impure), which is possibly batyl alcohol, $C_{20}H_{42}O_3$. Weidemann (1926) believes that the unsaturated alcohols are mainly dihydric, and more unsaturated than selachyl alcohol, $C_{20}H_{40}O_3$, which they otherwise resemble.

IDENTIFICATION AND ASSAY

A great many tests, based on physical and chemical properties, have been proposed for the identification of medicinal cod liver oil and the detection of adulteration, but almost every one of these is, by itself, liable to misinterpretation. The following tests are, in my opinion, the most practical.

1. The oil should be golden to pale yellow in color, and possess an inoffensive, fishy smell.

Fish body-oils, and rotted cod oil, unless bleached and deodorized, usually have a dark color and strong smell. Vegetable oils lack the fishy odor. Blubber oils, foreign liver oils, and bleached oils may escape this test.

2. If to a few cubic centimeters of genuine cod liver oil in a test tube, a similar quantity of the test-sample at the same temperature be added, and partially mixed, no well-defined diffusion striæ should appear.

In this test, originated by Merz (1876), for recognizing the purity of any oil, diffusion striæ must be distinguished from color striæ. Rotted cod oil cannot always be distinguished from steamed, but extensive adulteration by almost any foreign oil is fairly well revealed. Diverse samples of pure cod liver oil produce only feeble striæ on mixing.

3. The Hager-Salkowski color reaction, performed in accordance with the U. S. P. X should be positive, as described.

Consideration of the work of Liverseege (1904), Thaysen (1914), Bohrisch (1918), De Kadt (1920), Poulsson and Weidemann (1923), and Holmes (1924) leads to the conclusion that this long-known reaction may be given by several fish liver oils, and that occasionally cod liver oil fails to give it. However, there is good evidence that such abnormal cod liver oil must have been exposed to conditions of oxidation, insolation, bleaching, etc., which are likely to be detrimental to the vitamins. This test, therefore, is of value principally as a means of determining that the oil has not been improperly treated.

These tests afford no information on the vitamin potency of a cod liver oil. To gain such information, methods of biological assay have been devised which consist in determining the minimum of oil sufficient to cure or prevent certain avitaminoses. The U. S. P. X describes a procedure, based on the growth-resumption method of Zilva and Miura (1921), for the assay of vitamin A. The official standard is low. For the cure of xerophthalmia a larger dose of oil appears to be required than for the resumption of growth. For the assay of cod liver oil for vitamin D the most satisfactory procedure appears to consist in determining the percentage of oil which must be incorporated in a rickets-producing diet to render it curative of rickets. When a large number of assays are performed the vitamin D potency of medicinal oils may be found to vary six or eight times, while the variation in vitamin A may be considerably greater.

Several attempts have been made to estimate these vitamins, particularly vitamin A, by color reactions. The work of Drummond and Watson (1922), Poulsson and Weidemann (1923), Sjörslev (1924), Rosenheim and Drummond (1925), Fearon (1925), Carr and Price (1926), Willimott and Moore (1926), and Rosenheim and Webster (1926) indicates that cod liver oil contains a chromogenic substance which is associated, but not necessarily identical, with vitamin A. Cocking and Price (1926) compare the several reactions, and conclude that the color developed by antimony trichloride affords the most promising results. However, it is true that the value of all the color reactions proposed for the estimation of vitamins is at present highly problematical. Color reactions for vitamin D have not as yet been developed even to the point of extensive parallelism between the tests themselves and the biological findings.

REFERENCES

ALLEN, A. H., AND THOMSON, W.: Chem. News **43**, 267. (1881).
ANDRÉ, E.: Bull. sci. pharmacol. **30**, 267–280, 352–364. (1923).
BELL, J.: Pharm. J. Trans. **11**, 320–321. (Editorial.) (1851).
BENNETT: Treatise on Oleum Jecoris Aselli, London. (1841).
BERTHÉ: J. pharm. chim. (III) **28**, 121–123. (1855).
BIESENTHAL, M.: Am. Rev. Tuber. **4**, 781–783. (1920).
BILLS, C. E.: J. Biol. Chem. **67**, 279–285. (1926).
BOHRISCH, P.: Apoth. Z. **33**, 519–520, 523–524, 531–532. (1918).
BROWN, J. B., AND BEAL, G. D.: J. Am. Chem. Soc. **45**, 1289–1303. (1923).
BUCHHEIM, R.: Arch. exp. Path. **3**, 118–124. (1875).
BULL, H.: Chem. Z. **23**, 996. (1899).
BULL, H.: Ber. **39**, 3570–3576. (1906).
BULL, H.: Tids. kemi farm. terapi **14**, 1–2. (1917).
CAMPBELL, H. B., AND KIEFFER, J.: Am. Rev. Tuber. **6**, 938–949. (1922).
CARR, F. H., AND PRICE, E. A.: Biochem. J. **20**, 497–501. (1926).
CAUSSADE, G., TARDIEU, A., AND GRIGAUT, A.: Presse méd. **33**, 55. (1925).
CHANNON, H. J., DRUMMOND, J. C., AND GOLDING, J.: Analyst **49**, 311–327. (1924).
COCKING, T. T., AND PRICE, E. A.: Pharm. J. **117**, 175–178. (1926).
CUTTING, R. A.: J. Lab. Clin. Med. **11**, 842–845. (1926).
DE KADT, P. J.: Pharm. Weekblad **57**, 756–758. (1920).
DRUMMOND, J. C.: Chem. and Ind. Rev. **2**, 928–932. (1924).
DRUMMOND, J. C., CHANNON, H. J., AND COWARD, K. H.: Biochem. J. **19**, 1047–1067. (1925).
DRUMMOND, J. C., AND WATSON, A. F.: Analyst **47**, 341–349. (1922).
DRUMMOND, J. C., AND ZILVA, S. S.: J. Soc. Chem. Ind. **41**, 280T–284T. (1922).

DRUMMOND, J. C., ZILVA, S. S., AND COWARD, K. H.: J. Soc. Chem. Ind. **43**, 236T–238T. (1924).
DUBAIL: J. pharm. chim. (III) **25**, 36–47. (1854).
DUBIN, H. E.: J. Am. Pharm. Assoc. **14**, 215–219. (1925).
FAHRION, W.: Chem. Z. **17**, 684–686. (1893).
FAHRION, W.: Chem. Umschau **27**, 158–160, 201–204. (1920); **28**, 5–7, 20–21. (1921).
FEARON, W. R.: Biochem. J. **19**, 888–895. (1925).
FINE, M. J.: Am. Rev. Tuber. **6**, 934–937. (1922).
FOX, C.: Cited by Munn, W. A.: Montreal Pharm. J. **25**, 192–197. (1914).
GAUTIER, A., AND MOURGUES, L.: C. r. **107**, 740–743. (1888).
GHOSH, S.: Indian J. Med. Research **8**, 211–215. (1920).
GOBLEY: J. pharm. chim. (III) **5**, 306–310. (1844).
GRIGAUT, A., AND TARDIEU, A: Presse méd. **32**, 1022. (1924).
GROSSER, J.: Z. deut. Oel- Fett- Ind. **45**, 622–624, 639–640. (1925).
GUY, R. A.: Am. J. Diseases Children **26**, 112–116. (1923).
HAGER: Pharm. Zentralhalle **26**, 13–15. (1885).
HEIDUSCHKA, A., AND RHEINBERGER, E.: Pharm. Zentralhalle **52**, 837–838. (1911).
HEYERDAHL, P. M.: In F. P. Möller's Cod Liver Oil and Chemistry, London. (1895).
HOLMES, A. D.: J. Am. Pharm. Assoc. **13**, 532–535. (1924).
HUME, J.: Lancet (II) 162. (1924).
JESSEL, G.: Tubercle **6**, 223–224. (1925).
KRAUSS, L.: Süddeut. Apoth.-Z. **54**, 364. (1914).
KUGELMASS, I. N., AND MCQUARRIE, I.: Science **62**, 87–88. (1925).
LANSBERG, L. M.: Pharm. Weekblad **56**, 1726–1727. (1919).
LEWKOWITSCH, J.: Jahrbuch Chem. **16**, 394–415. (1906).
LEWKOWITSCH, J.: Chemical Technology and Analysis of Oils, Fats and Waxes 6th ed., Vol. 1, London. (1921).
LEWKOWITSCH, J.: Chemical Technology and Analysis of Oils, Fats and Waxes 6th ed., Vol. 2, London. (1922).
LINDEMBURG, A., AND PESTANA, B. R.: Brazil-Medico **34**, 603–609. (1920).
LINDEMBURG, A., AND PESTANA, B. R.: Z. Immunitäts. **32**, 66–86. (1921).
LIVERSEEGE, J. F.: Analyst **29**, 210–215. (1904).
LUCK: Neues Jahrb. Pharm. **6**, 249–251. (1856).
LUND, A. Tids. kemi bergvæsen **5**, 102–114. (1925).
MCCOLLUM, E. V., AND SIMMONDS, N.: The Newer Knowledge of Nutrition 3rd ed., New York. (1925).
MCCOLLUM, E. V., SIMMONDS, N., BECKER, J. E., AND SHIPLEY, P. G.: J. Biol. Chem. **53**, 293–312. (1922).
MARCELET, H.: C. r. **173**, 104–107. (1921).
MERZ, G.: Chem. Zentr. 8. (1876).
MÖLLER, P.: Cited by F. P. Möller, 1895. (1853).
MÖLLER, F. P.: Cod liver Oil and Chemistry, London. (1895).
MURRAY, J.: British Patent 365, Feb. 11. (1853).
OSBORNE, T. B., AND MENDEL, L. B.: J. Biol. Chem. **17**, 401–408. (1914).
PALMER, L. S.: Minnesota Agr. Expt. Sta., Ann. Rept. 43–44. (1922).
PERNET, J., MINVIELLE, M., AND POMARET, M.: Presse méd. **33**, 55. (1925).
POULSSON, E., AND WEIDEMANN, G.: Tids. kemi bergvæsen **3**, 169–174. (1923).

ROGERS, L.: Brit. Med. J. (I) 147–148. (1919).
ROGERS, L., MUIR, E., KNOWLES, R., COCHRANE, A. W. R., DAVIES, C., AND BRIERLEY, C. S.: Indian J. Med. Research, special number 236–256. (1919).
ROSENHEIM, O., AND DRUMMOND, J. C.: Lancet (I) 862–864. (1920).
ROSENHEIM, O., AND DRUMMOND, J. C.: Biochem. J. **19**, 753–756. (1925).
ROSENHEIM, O., AND WEBSTER, T. A.: Lancet (II) 806–807. (1926).
SALKOWSKI, E.: Z. anal. Chem. **26**, 557–582. (1887).
SCHUCK, W. P.: United States Patent 1,260,072, Mar. 19. (1918).
SERVAIS, L.: Chem. Rev. Fett- Harz- Ind. **10**, 231. (1903).
SJÖRSLEV, N.: J. Biol. Chem. **62**, 487–493. (1924).
TAKAHASHI, NAKAMIYA, KAWAKAMI, AND KITASATO: Cited by Drummond, Channon, and Coward, 1925.
TAMBE, G. R.: Tubercle **2**, 58. (1920).
TEWKSBURY, W. D.: Am. Rev. Tuber. **6**, 929–933. (1922).
THAYSEN, H.: Ber. pharm. Ges. **24**, 136–140. (1914).
TOLMAN, L. M.: J. Ind. Eng. Chem. **1**, 340–345. (1909).
TSUJIMOTO, M.: 1906, 1920, 1922. Cited by Brown and Beal, 1923.
TSUJIMOTO, M., AND KIMURA, K.: J. Chem. Ind. (Japan) **26**, 1162. (1923).
TWITCHELL, E.: J. Ind. Eng. Chem. **9**, 581–584. (1917).
WEIDEMANN, G.: Biochem. J. **20**, 685–691. (1926).
WILLIAMS, O. T.: Brit. Med. J. (II) 700–702. (1912-a).
WILLIAMS, O. T.: Pharm. J. **89**, 806–809. (1912-b).
WILLIMOTT, S. G., AND MOORE, T.: Biochem. J. **20**, 869–872. (1926).
ZILVA, S. S., AND DRUMMOND, J. C.: J. Soc. Chem. Ind. **42**, 185T–188T. (1923).
ZILVA, S. S., AND MIURA, M.: Biochem. J. **15**, 654–659. (1921).
ZUCKER, T. F.: British Patent 208,145, Dec. 3. (1923).