CONCLUSION.

Using a period of 60 seconds immersion, and using a sufficient number of frogs, it is possible to detect variations of plus or minus ten per cent in the concentration of local anesthetics, analgesics, hypnotics and sedatives.

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MEDICINAL COD LIVER OIL—OBSERVATIONS ON COLOR AND VISCOSITY.*

BY GEORGE E. ÉWE.

COLOR.

Medicinal cod liver oil appears in the market showing various shades of yellowish or brownish yellow color. This color is to a large extent due to its content of biliary constituents of the liver from which the oil is obtained, although, as will be shown further on the source of the oil, the iron content, extent of oxidation, manufacturing manipulations, degree of exposure to sunlight, age, etc., materially affect the color of the oil. The presence of biliary matters can be demonstrated by applying Pettenkofer's test to a water-extract of cod liver oil and also by applying Gmelin's test to the residue obtained by evaporating a fresh alcohol-extract of the oil.

When the oil is obtained by the "steaming" process a pale colored oil is procurable whereas when the "rotting" process is employed a much darker product results. While there is no data available on the relative content of biliary con-

* Scientific Section, A. PH. A., Madison meeting, 1933.

stituents in oils made by the 2 processes the possibility is presented that the "rotting" process imparts a larger proportion of these constituents to the oil for the following reasons: the "steaming" process, since it employs heat, coagulates the proteids of the liver thus locking-up the biliary constituents and rapidly segregating them from the oil whereas in the "rotting" process the liberation of the oil is dependent upon the slow disintegration (autolysis) of the liver and consequently the oil is brought into intimate and prolonged contact with the liberated biliary constituents. This should permit the oil to dissolve much more of these coloring substances than is possible with the rapid "steaming" process.

A possible source of color which has not been thoroughly recognized as yet is the iron content of the livers. Iron is a regular constituent of cod liver oil. Four samples examined by Briod, Van Winkle, Jurist and Christiansen (1) were found to contain 0.47, 0.13, 0.39 and 0.34 parts of iron per million, respectively. These proportions seem negligible, all being less than 1 part per million. However, minute proportions of iron can materially affect the color of cod liver oil as shown by the following experiments: the color of pale yellow cod liver oil to which $2^1/_2$ parts per million of iron in the form of ferric oleate was added was distinctly darkened in color by this addition, while the addition of 5 parts per million made the oil several shades darker in color.

It does not seem inconceivable that traces of iron are carried into the oil from the iron-rich liver during the separation of the oil from the liver, especially in view of the slight acidity shown by even the best quality of cod liver oil.

While it is possible that the iron content of the livers is a source of the iron content of cod liver oil, the influence of the metallic equipment used in producing, refining, storing and shipping the oil must not be overlooked. Briod and Christiansen (2) found that darkening will be occasioned if the oil is allowed to remain in contact with iron, especially if a trace of moisture is present. Under these conditions the traces of free fatty acids common to even a good grade of oil may eventually attack the iron or iron rust of the container to produce ferrous fatty acid salts which are soluble in the oil and which later oxidize to the much darker ferric condition and so discolor the oil. Thus, the state of oxidation of the iron content is also a factor influencing the color of cod liver oil.

To ascertain something of the effects of light and oxidation and also of the possible effect of iron upon the color of cod liver oil under the influences of light and oxidation samples of plain oil, the same oil with the addition of $2^1/_2$ parts per million of iron in the form of ferric oleate and the same oil with 5 parts per million of added iron were filed away in flint glass, corked bottles in diffused sunlight; a corresponding series being filed away in the dark. After one month all samples kept in the light were lighter than those kept in the dark whether corked or uncorked. Furthermore, there was no detectable difference in color between the corresponding corked and uncorked samples so that in the stated period of time light had exerted a notable bleaching effect but the bleaching action of oxidation was not yet evident. After 4 months all samples kept in the light were still lighter than the corresponding ones kept in the dark and, in addition, the unstoppered samples were all lighter than the corresponding stoppered ones, thus illustrating the bleaching action of oxidation. The samples exposed to both light and air were bleached to a greater extent than the corresponding ones exposed to either air

alone or light alone, so that the bleaching effects of light and air are additive. Whether the light treatment or the oxidation treatment was the more potent bleaching factor could not be satisfactorily determined in this series of experiments for although the colors of corresponding samples exposed to light alone or to air alone were approximately the same in depth, they were different in quality, the colors of the samples exposed to light alone being of a brownish cast whereas those exposed to air alone were of a yellowish cast. However, the light treatment was more rapid, since it showed its effects after 1 month, whereas the effect of oxidation was not evident at this time. The samples exposed to both light and air all showed a whitish haze after 4 months, which suggests the formation of water or other insoluble substance by the combined effect of light and air. In all cases, the colors of the samples containing $2^1/_2$ parts of added iron per million were still distinctly (but only slightly) darker, after 4 months, than the corresponding samples of plain oil, whereas the samples containing 5 parts of added iron per million were several times darker than the samples with $2^1/_2$ parts of added iron per million which presents the possibility that the color of cod liver oil may be darkened by iron to a greater degree than would be predictable by direct mathematical proportion.

The possibility that the color of cod liver oil may be contributed to by yellow carotinoid pigments derived from the plankton upon which cod fish feed abundantly, has evidently not been thoroughly investigated. It is known, however, that plankton feed upon diatoms and other forms of life which have been shown to contain carotinoid pigments.

The depth of color of cod liver oil will be found to vary when the oil is successively obtained from different sources, due primarily to the various manufacturing and storage methods employed by different producers. Variations in color of successive batches of the oil from a single source of production will also be occasioned if variations in the production method used in the particular plant are permitted. When uniform methods of production are used successive batches of medicinal oil of high potency and very pale color scarcely varying in depth of color can be consistently produced.

When cod liver oil is of initially dirty yellow or brown color this can be usually ascribed to unsatisfactory preparation and such oils are considered inelegant, if not actually inefficient, preparations.

When cod liver oil is exposed to air and sunlight (direct or diffused) it undergoes an initial bleaching, but if excessively exposed, ultimately becomes much darker than if protected against oxidation and sunlight. This bleaching process greatly impairs the taste and odor, and possibly the vitamin content, of the oil and consequently is not permissible in the production of the best grade of medicinal oil.

The U. S. Dispensatory (21st Edition) states: "In the best equipped establishments (for making high quality medicinal cod liver oil)...the oil...is... bleached by treatment with fuller's earth or by exposure to sunlight." While this may be the practice in some establishments it is certain that the use of fuller's earth or exposure to sunlight is entirely unnecessary for the production of pale colored medicinal cod liver oil of extreme palatability, unoffensive odor and high vitamin A and D potency. Oils produced without these treatments are to be pre-

ferred since the effect of these treatments upon the taste, odor and possibly the vitamin content, is not favorable.

High quality, pale colored medicinal oil has been observed to darken slightly when kept in tightly stoppered, incompletely filled, flint-glass bottles for some months in diffused sunlight, and even when kept in the dark. In the latter case, even amber-glass bottles did not entirely prevent a change in color. However, in all these cases, the color, while unmistakably darker, was no darker than that of many oils ordinarily marketed as medicinal oils and the oils were still entirely fit for use.

J. C. Drummond (3) states that the nearly colorless cod liver oils derived from spawning fish are considerably lower in vitamin content than the pale yellow oils obtained from feeding fish. Drummond goes on to state that it is not at all undesirable that medicinal cod liver oil should possess a pale lemon color since such oils are generally superior to so-called "white" oils, and that only dirty yellow or brown oils, are undesirable, since these colors usually indicate unsatisfactory preparation.

There is no direct relation between the depth of color of a medicinal cod liver oil and its vitamin content and biological assay, rather than color, must be depended upon as the criterion of vitamin potency. Hare ("Practical Therapeutics," 1930) states "The oil is pale or dark according to its freedom from foreign materials. Although the paler oils are generally prescribed, there can be little doubt that the darker ones are more medicinally active." However, since pale oil has been repeatedly observed to darken with age while the vitamin content has certainly not coincidently increased, Hare's statement cannot be concurred in. It is also well known that oil prepared by the "rotting" process while highly colored is not higher on the average in vitamin content than the light colored oils obtained by the "steaming" process.

Pale yellow color as a result of careful preparation, storage and preservation is a desirable feature of medicinal oil since the pale yellow oil is generally considered the more elegant product. However, pleasant taste and odor and high vitamin potency must also be possessed by such a product and products made pale by chemical, adsorbent or light treatments at the expense of taste, odor or potency must be guarded against. An oil possessing some color but of satisfactory taste, odor and vitamin potency is more to be desired than a pale oil of poor taste and odor and low vitamin potency, or a "white" oil of low potency.

VISCOSITY.

The viscosity (or consistency) of cod liver oil calls for important consideration because a more viscous oil would be less readily swallowed.

At least one producer of medicinal grades of cod liver oil assures prospective users that his product is uniform in consistency. However, in this investigation no material difference was found in the viscosities of various market brands of medicinal cod liver oil when compared at 25° C.

To the eye, different brands of cod liver oil of the same grade will often appear to vary in viscosity when compared, but a more reliable measure of viscosity must be used to obtain a true comparison. The U. S. P. IX method for determining

the viscosity of Liquid Petrolatum affords a simple and sufficiently accurate way of comparing the viscosities of various cod liver oils of the same grade.

Various brands of medicinal cod liver oils on the market showed the following viscosities by the U. S. P. IX method for Liquid Petrolatum: 5.74, 5.77, 5.39, 5.45, 5.45, 5.61, 5.80, 5.64 and 5.80, respectively. Some of these oils were flavored but it is not likely that the viscosities of the original unflavored oils were materially different since the addition of 0.5% of essential oil to cod liver oil of known viscosity had no appreciable effect upon the viscosity. While the above data shows some variation in the viscosity of different market brands the differences are not material since several persons were unable to distinguish any difference in viscosity upon swallowing comparative doses of the above oils showing maximum (5.80) and minimum (5.32) viscosities.

Successive batches of medicinal cod liver oil from a single source of manufacture appear to vary in viscosity among themselves to an even lesser degree than do market brands from various sources. Ten successive batches from a single source of manufacture showed viscosities of 5.55, 5.58, 5.64, 5.67, 5.58, 5.80, 5.64, 5.70, 5.80 and 5.61, respectively.

Oxidation is a well-known factor operating to increase the viscosity of cod liver oil. It is a familiar fact that when cod liver oil is exposed to the air it becomes progressively more viscous and finally forms a tacky, gelatinous mass. Three lots of cod liver oil of known viscosity by the U. S. P. IX viscosity test for Liquid Petrolatum, showed definitely increased viscosities, when tested 3 months later, after having been stored in screw-capped bottles, the bottles being uncapped occasionally to simulate the treatment they would likely undergo as bulk stock containers in the retail pharmacy (see "Treatment No. 1" in table). When these oils were stored for 3 months in wide-mouthed bottles, the mouth of each bottle being covered with muslin, to illustrate the effect of exaggerated exposure to the air, the resultant respective viscosities of the 3 oils were much more greatly increased (see "Treatment No. 2" in table). In the latter series, surface oxidation was plainly visible (surface film and shred formation) and the taste of the oils seriously impaired. The U. S. P. reminder to preserve cod liver oil in well-closed containers is pertinent and displacement of the air in the container by an inert gas is of additional precautionary value.

TABLE SHOWING EFFECT OF SPONTANEOUS OXIDATION UPON THE VISCOSITY OF COD LIVER OIL.

Oil No.	Initial Viscosity.	Viscosity after Treatment No. 1.	Viscosity after Treatment No. 2.
1	5.64	5.90	7.03
2	5.61	5.78	7.12
3	5.67	5.74	6.23

As pointed out by the writer in JOUR. A. PH. A., 22 (1933), 109–112, stearin content ordinarily found in market brands of medicinal cod liver oil has no appreciable effect upon the viscosity of the oils at room temperature, although at much lower temperatures and especially around congealing temperatures the proportion of stearin very greatly affects the viscosity, the viscosity being increased by increase in the stearin content.

The viscosity of cod liver oil is very materially influenced by the temperature, high temperatures reducing the viscosity and low temperatures greatly increasing it.

As also pointed out by the writer in the above-mentioned article in JOUR. A. PH. A., 22 (1933), 109–112, medicinal cod liver oil is often thickened or congealed by precipitation of stearin at temperatures of about minus 8° C. or lower and even at refrigerator temperature (10° C.) its viscosity is greatly increased (from 5.6 to 8.1, on the average). "As a consequence, when it is desired to minimize the influence of viscosity upon the taking of cod liver oil it is well to direct that the dose be taken from a small bottle of the oil kept at room temperature, the main supply being preserved in the refrigerator or other cool place."

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COD LIVER OIL—STABILITY OF VITAMIN A CONTENT UNDER CONDITIONS OF COMMERCIAL DISTRIBUTION.*

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The value of cod liver oil resides in its vitamin content. Consequently, consideration of the degree of stability of the vitamin content of cod liver oil is of importance. The following data concern the stability of the vitamin A content of cod liver oil, consideration of the vitamin D content being reserved for a possible future communication. Poulsson (1) found that a sample of cod liver oil, 23 years old, promoted growth in rats kept on a deficient diet when fed with 3 to 5 mg. daily of the oil. Evers (2) reported that cod liver oil, when properly stored, retains a considerable proportion of its vitamin A activity for long periods (up to 26 years), and that exposure to light or oxidation lowers its vitamin A activity, the chief cause of loss of activity being the action of light. Sunlight, especially, was found by Evers to destroy vitamin A rapidly and in this respect it appeared to be more active than ultraviolet radiation from a mercury vapor lamp. He suggested that the oil be preserved in amber bottles with as little exposure to the air as possible. Holmes and Pigott (3) found that exposure of cod liver oil in flint-glass bottles to direct sunlight transmitted through ordinary glass windows as much as possible during 16–24 months resulted in marked loss of potency. Exposure to diffused sunlight during 14–26 months showed no detectable detrimental effect except in an excessively warm location. The experiments were controlled by parallel samples enclosed in cartons to shut off the light. Holmes and Pigott concluded that light-proof containers, such as amber bottles or flint bottles wrapped in paper or cartons should be used in storing and distributing cod liver oil.

* Scientific Section, A. PH. A., Madison meeting, 1933.