COD LIVER OIL EXTRACTS—I.*

CHEMICAL COMPOSITION AND PROPERTIES.

BY ALBERT K. EPSTEIN AND B. R. HARRIS.

In a preliminary survey of the literature pertaining to extracts of cod liver oil in preparation for an investigation of their merits, it became evident that there was dearth of published information on the subject. The "United States Dispensatory" and "Merck's Index" give a little space to these preparations but barring these, practically no data could be found on this subject. The manufacturers and firms offering such materials for sale had very little of chemical information regarding them, other than the amount of cod liver oil which their extracts "represented." By this they mean the inverse ratio of the weight of extract, obtained by treating cod liver oil with a volatile solvent such as ethyl alcohol or ether, to the weight of the oil treated. To take a concrete example, if 100 pounds of cod liver oil give, on extraction, 4 pounds of extract, then the resulting product is spoken of as "representing" or "being equivalent to" 25 times its own weight of cod liver oil. The writers have no reason to take exception to such a description of cod liver oil; it has its place. Yet it is easy to see that such a characterization might easily be abused or misinterpreted. For example, if it were desired, a solvent might be chosen which is capable of extracting only a very minute proportion of the cod liver oil. In such a case, the product might truthfully be represented, on this basis, as being equivalent to several hundred or even several thousand times its own weight of cod liver oil. Such a characterization would be correct and yet might not be a fair representation of the merits of the extract. It is quite obvious that the quantity of extract obtained cannot, alone, be taken as a measure of the value of a product of this sort. The nature of the solvent used and the procedure by which the extract is made and preserved must necessarily play an important part in determining the merits of the extract.

Judging from the experience gained thus far and recorded in the scientific literature, in connection with the estimation of the therapeutic value of cod liver oils, it appears that the evaluation of cod liver oil extracts, as well, will have to rest on some kind of animal assay; on one kind or other of suitably controlled feeding experiments.

However, before proceeding with a biological assay, it was thought desirable to gain some insight into the chemical composition of these cod liver oil extracts. It was with this object in view that the work reported herein was undertaken.

The extracts, described in Table I, are said to be available on the American market. The alcohol extract, "Gaduol" is described as representing 20 to 25 times its own weight of cod liver oil and the ether extract, "Jecorrol," 15 to 20. Both preparations were similarly tested by the usual analytical methods. The results are recorded in Table I.

The extracts are extremely viscous, highly colored, almost black liquids with a distinctly fishy odor. The ether extract has a specific gravity of 0.840 and the alcohol extract, 0.920.

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TABLE I.—COMPOSITION OF COD LIVER OIL EXTRACT.

(All numerical figures represent percentages.)

	Loss in weight on 3 hours' heating at 110° C.	Total nitrogen.	Volatile ammoniacal nitrogen.	Ratio of volatile to total nitrogen.	Free fatty acids in terms of oleic acid.	Saponifiable matter in terms of triolein.	"Unsaponifiable" matter.	Total phosphorus.	Ash.
Ether extract	0.20	0.439	0.0334	7.62%	37.1	20.2	42.5	0.128	0.238
Alcohol extract	23.5	3.18	0.840	26.4%	54.8	10.4	29.9	trace	4.91

Comments:—Though claims are frequently made for the iodine content of these preparations, careful analysis failed to show the presence of either iodine or bromine. This agrees well with statements to be found in the literature in connection with cod liver oils, to the effect that the iodine containing substances are not extractable by alcohol.

It will be noticed that more than half of the extract consists of therapeutically inert material such as free fatty acids and saponifiable fat. Of course, the "unsaponifiable" is sufficiently high to represent an ample proportion of such therapeutically active matter as vitamins and the like.

Considerable difficulty was experienced in determining the "unsaponifiable matter" by the usual technic, owing to the formation of exceptionally troublesome emulsions. The figure given in Table I, above, is a calculated figure obtained by subtracting from 100 the sum of the "free fatty acid," "saponifiable fat" and "ash" figures. While this, no doubt, is not accurate, it nevertheless, furnishes a good approximation.

It was to be expected that an ether extract would contain more phosphorus than an alcohol extract since phospholipoids and similar substances are much more soluble in ether than in alcohol. However, the differences between these preparations cannot be attributed wholly to the solvents used, since that would be rigorously justifiable only if two portions of one batch of oil had been separately treated with ether and alcohol and the resulting extracts then compared. Since, in all likelihood, these extracts were not prepared from the same oil, some of the differences at least are apt to be due to differences in composition of the original materials subjected to extraction.

Calculation of the "free fatty acid" and "unsaponifiable" figures back to a whole oil basis on the assumption that the extracts represent approximately twenty times their own weight of oil, sheds some light on the composition of the oils.

TABLE II.—PROBABLE COMPOSITION OF THE COD LIVER OILS.

	Free fatty acid.	Unsaponifiable.
Ether	1.9%	2.1%
Alcohol	2.7%	1.5%

The above figures tend to indicate that the extracts were prepared not from the pale oil used in pharmaceutical preparations, but rather from the brown oil obtained from livers which had been allowed to attain a more or less advanced stage of putrefaction or from some other oil of inferior grade. Indeed it is difficult to understand just how an ether extract might be prepared, since ether is miscible with cod liver oil. It is therefore more likely that the "ether extract" is an extract of the residual liver material remaining after the expulsion of the liver oil.

The most striking difference, perhaps, is in the proportion of material volatile at 110° C. A distillate obtained from 100 grams of the alcohol extract, between

110° and 150°, consisted largely of amines with no significant amounts of water or alcohol. This distillate may also have contained some low boiling esters of the lower fatty acids reported to be present in cod liver oil.[1]

While, as pointed out above, comparisons between the extracts can have only a limited value, since they were in all likelihood, made from different oils, a little more reliance may be placed on comparisons of ratios within the extracts. For example, the alcohol extract contains approximately eight times as much of total nitrogen as the ether extract. The solvent or the composition of the oil form which the extract was made, or both, may be responsible for this and the validity of the comparison suffers from this uncertainty; however, a comparison of the respective ratios of volatile to total nitrogen is not open to quite as serious an objection. It will be observed that in the case of the alcohol extract this ratio is almost four times as great as for the ether extract.

It should be stated at this point—that the volatile ammoniacal nitrogen was determined by treating a weighed quantity of extract with saturated sodium carbonate solution (and a small amount of capryl alcohol, to minimize frothing), aerating the mixture at room temperature for five hours into a known volume of standard tenth normal sulphuric acid and titrating back, the excess of acid with standard sodium hydroxide solution. The result expressed in terms of nitrogen is a fair index of the content of volatile, basic, nitrogenous substances.

The solubilities of the extracts are of interest. Both are completely soluble in xylene, chloroform, carbon disulphide and glacial acetic acid. Acetone dissolves the ether extract almost completely, whereas the alcohol extract is only partially dissolved by this solvent.

The chloroform and carbon disulphide solutions were treated dropwise with concentrated sulphuric acid, with shaking, to ascertain the presence of the lipochromes and biliary coloring matters which are said to cause cod liver oil to respond to this test.[2] In no case could the characteristic violet tint be obtained, as compared with an authentic cod liver oil control. Addition of sulphuric acid did cause a change in color to a brownish red but the preliminary violet shade was never detected. Since there was some difficulty in observing the reaction because of the masking effect of the colors of the extracts, themselves, in chloroform or carbon disulphide solution, these solutions were treated with absorbent charcoal to decolorize them as far as possible and were then subjected to the sulphuric acid test. This treatment, however, did not materially alter the results of the tests. From these observations it may be concluded that the ether and alcohol extracts herein considered do not contain at all or do not contain in their original condition those substances which cause cod liver oil to respond to the sulphuric acid test.

As indicated in the early part of the paper, the writers look upon the chemical study of these extracts as merely of accessory interest; the determination of their actual therapeutic value will no doubt have to rest on a suitable biological assay.

In closing, the authors should like to reiterate, as stated above, that a systematic, comparative study of an ether and alcohol cod liver oil extract would consist of treating two portions of the same batch of material and analyzing the respective

[1] Lewkowitsch and Warburton, "Chemical Technology and Analysis of Oils, Fats and Waxes," Macmillan & Co., London, 1922, Vol. II, p. 441.

[2] *Ibid.*, Vol. II, pp. 435, 453.

products. However, that is not the purpose of this paper. It was the aim of the authors to report, in the JOURNAL literature, an analysis of pharmaceutical cod liver oil extracts which are being offered in the American market for the manufacture of so-called cod liver oil tablets, wines, elixirs and other proprietary preparations, so that the industry may be acquainted with the composition of these extracts.

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EPSTEIN & HARRIS, CONSULTING CHEMISTS,
CHICAGO, ILLINOIS.

NEW LIGHT ON COD LIVER OIL.*

BY BERNARD FANTUS, M.D.

There is one disease in which the special value of cod liver oil is now well established. This disease is rickets. Though the opinion of clinicians regarding its curative value in this disease had long been well crystallized, it remained for experimental demonstration upon laboratory animals to furnish not only unequivocal proof of this but also the key to its mode of action.

VITAMIN D CURES RICKETS.

The recent rapid development of our knowledge in this field we owe to the fact that lower animals, and among them rats, are susceptible to rickets; and that large numbers of experiments can so easily be conducted upon rats. These experiments have shown not only that cod liver oil is curative of rickets; but also that a concentrate could be prepared representing all the antirachitic activity of the original oil (Funk, 1924). They have shown that the antirachitic principle is entirely stable to saponification, in other words that the oil can be boiled with strong alkali so as to destroy the fats completely without impairment of the antirachitic potency. This points to the desirability of subjecting to intensive study the unsaponifiable substances isolated from this fat. It has been suggested, but not yet proved, that sterols, cholesterin in animals and the phytosterols of plants, carry the activity.

In the present state of our ignorance of the essential chemical nature of this antirachitic factor, it is classed among the vitamins; and recent studies have forced the designation "Vitamin D" upon it. At first, the antirachitic factor was not distinguished from vitamin A, as both are oil-soluble; and both are contained in cod liver oil. More recently it was shown that they differ from each other. Thus, while butter fat promotes growth and prevents xerophthalmia—in other words, contains vitamin A—it is of little use in the prevention of rickets. Coconut oil, on the other hand, can prevent rickets, but does not promote growth or prevent xerophthalmia. Furthermore, it has been shown by E. A. Park and his associates at Johns Hopkins that, by limited oxidation of cod liver oil, one can destroy vitamin A without destroying the antirachitic factor. Hence, the antirachitic factor is different from vitamin A. It has provisionally been designated vitamin D.

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