partially absorbed by the glycerin, acting as a diluent. But contrary to theory the results of the Experiments II and III, of which the latter actually contained much larger an excess of solute, conformed closely. This would show that the tension of $MgSO_4.7H_2O$ to retain its water of crystallization is greater than the tension of glycerin to absorb water.

SOME ANALYTICAL ASPECTS OF COD LIVER OIL.*

A. E. BRIOD, R. VAN WINKLE, A. E. JURIST AND W. G. CHRISTIANSEN.

The important place which cod liver oil has attained in the prevention or treatment of certain deficiency diseases is now uncontested.

Little is to be found in the literature, however, on some of the lesser known properties of this oil and a study of a few of these properties brought out some interesting facts which form the basis of this paper.

All results listed below were obtained on good medicinal grade cod liver oil, from which the "Stearin" had been removed.

SPECIFIC GRAVITY AND VISCOSITY, IODINE AND SAPONIFICATION VALUES.

A preliminary examination of an average sample of Newfoundland cod liver oil gave the following results:

Specific gravity at $\frac{25^\circ\ C.}{25}$	0.9221
Hanus Iodine Value	157.2
Viscosity at 100° F. (Saybolt Universal Viscosimeter)	160 sec.
Saponification Value	186.1

UNSAPONIFIABLE MATTER.

The method used for the determination of the unsaponifiable residue was the official one given in the "Methods of Analysis of the A.O.A.C.," 1925 edition and gave for per cent of unsaponifiable matter: 0.88.

During this test care must be exercised to prevent oxidation after evaporation of the alcohol, especially since a double saponification is nearly always advisable if accurate results are desired. In this respect, the U. S. P. method is not believed to yield sufficiently exact figures and should be amended to include resaponification of the residue obtained after hydrolysis of the oil.

ACETYL VALUE.

The acetyl value, defined as the number of mg. of potassium hydroxide required for the neutralization of the acetic acid obtained on saponifying 1 Gm. of the acetylated fat, was determined next, the filtration method described by Lewkowitsch (1) being used.

The figures obtained were: Apparent acetyl value: 11.1; true acetyl value: 8.7.

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* Scientific Section, A. PH. A., Portland meeting, 1928.

It must be remembered that the acetyl value, in addition to being a measure of the hydroxy-acids present, also indicates free alcohols, oxidized acids, mono and diglycerides and, as Lewkowitsch points out, further includes the number of milligrams of potash required to saponify the acetylated cholesterol.

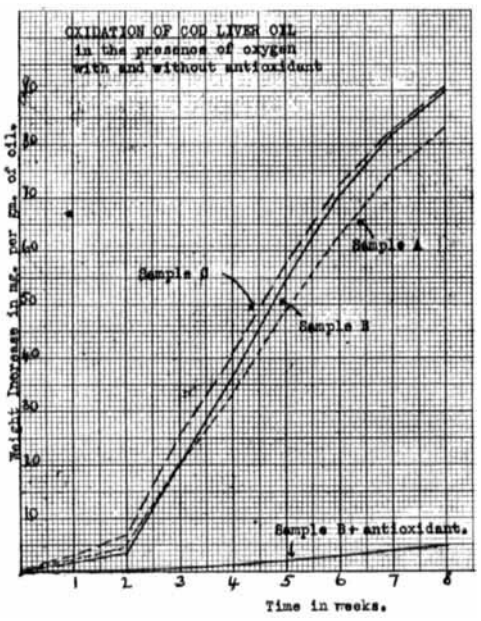

Fig. 1.

OXIDIZED FATTY ACIDS.

Fahrion's (2) method was used for this determination, with the only modification that the test was carried out under nitrogen protection. The figure thus obtained was: Per cent of oxidized fatty acids: 0.50.

OXIDIZABILITY VALUE.

The oxidizability value represents the number of milligrams of oxygen required to oxidize the water-soluble constituents of 100 Gm. of the oil and was determined by following Kerr's (3) modification of Issoglio's (4) original method. The values thus obtained on four different samples are given in the following table:

TABLE I.—OXIDIZABILITY VALUE OF COD LIVER OILS.

Sample.	Oxidizability value.
A	12.2
B	16.0
C	18.7
D	16.4

In his work on vegetable oils and lard, Kerr regards an oxidizability value of 15.0 or more as strong confirmatory evidence of rancidity. This does not appear to be the case, however, as far as cod liver oil is concerned. We have repeatedly found that many samples would give a positive Kreis test for rancidity, even though known to be fresh.

The above factors prompt us to believe that cod liver oil contains one or more compounds, probably aldehydes, which often give a positive Kreis reaction and induce a high oxidizability value without giving the oil any rancid odor or taste.

Such a belief is in agreement with the findings of Powick (5), who gives epihydrin aldehyde or another derivative of the action of H_2O_2 on acrolein as responsible for a positive Kreis test, and heptylic aldehyde as primarily responsible for the rancid odor.

Under these conditions, it would appear that both Kreis test and oxidizability value have only a very limited usefulness when applied to cod liver oil.

PEROXIDES IN COD LIVER OIL.

Oxidation of cod liver oil is believed to start through a peroxidation of its unsaturated glycerides and fatty acids, among which the best known is clupanodonic acid, and a study of many tests was undertaken for the detection of peroxides in cod liver oil:

a. The *hydriodic acid test,* used by Yoder (6), was found to be unspecific and absorption of iodine by the oil militates against it.

b. Schöne's (7) *titanium sulphate test* was not found sufficiently delicate for the detection of small amounts of peroxides.

c. The *potassium dichromate* and *aniline test* described by Bach (8) was also eliminated on account of its lack of sensitiveness.

d. The *potassium iodide and starch test* used by Powick (5) was tried next and discarded on the same account.

e. Schimkus' *iodoform test,* as described in his paper on catalytic activity of light-exposed cod liver oil (9), could not be applied to the detection of small traces of peroxides, because of the unstability of the reagent.

The same author's *potassium iodide and sodium thiosulphate test* was also found to lack sensitiveness.

f. Stamm's *phenolphthalein reagent* (10), used for the determination of peroxides in ether, was tried on the peroxides of cod liver oil but gave only negative results, even in the case of oils known to be strongly peroxidized.

g. The only test which finally gave us entirely satisfactory results was a *guaiacum and hemoglobin test,* based on C. F. Schönbein's (7) original guaicum test for hydrogen peroxide. It was applied to cod liver oil as follows:

Two reagents' solutions are freshly prepared: 0.1% hemoglobin in water; 0.5% guaiacum in absolute alcohol.

Approximately 10 cc. of the oil to be tested are placed in a test-tube, well shaken with an equal amount of absolute alcohol and set aside for a short time until the two layers have again separated.

In another test-tube 5 cc. each of the guaiacum and hemoglobin solutions are mixed together and 5 cc. of the alcoholic extract obtained in the upper layer of the first test-tube are finally added and well shaken with the reagents. In the presence of peroxides, a blue color will develop within a few minutes, the rate of development and depth of color being closely proportional to the amount of peroxidation in the oil.

Tested in this way, crude cod liver oil will generally yield a positive test, which is quickly intensified if the oil is left exposed to the air for any length of time.

The ease with which cod liver oil thus becomes oxidized suggests that ways and means be found to stop or retard the process of oxidation, which impairs both its taste and its vitamins' potency.

ANTIOXIDANTS AND COD LIVER OIL.

The action of many negative catalysts or antioxidants (11, 12, 13, 14, 15) on the rate of oxidation of cod liver oil was studied with the double purpose of preserving both the flavor and the potency of the oil.

Among the substances which were used in these experiments, representatives of the following types of compounds were studied: aromatic amines, mono and poly hydroxyphenols, aminophenols and two antioxidants secured (*a*) from the Goodyear Tire and Rubber Co. and (*b*) from the duPont Laboratories. A variety of experimental conditions were used.

A striking example of some of the results obtained, however, is given in the following graph, which represents a set of experiments performed at room temperature in an atmosphere of oxygen.

IRON AND SULPHUR IN COD LIVER OIL.

The percentage of iron and sulphur present in cod liver oil were also determined.

The method used for the iron determination was as follows: 80 to 100 Gm. of the oil were weighed to the third decimal place in a fused quartz dish. The oil was then ashed, using great care to burn off all the carbon without permitting any ash to be blown out of the dish or carried out by the flames produced during carbonation of the oil. The ash was taken in aqua regia, diluted with water, filtered and the resulting clear solution tested for iron by the method of E. Lyons (16), using thioglycollic acid in slightly alkaline solution. This test has been found accurate to within one part in ten million. The results which were obtained are given in the following table:

TABLE II.—IRON CONTENT OF COD LIVER OIL.

Sample.	Iron content. Parts in 10,000,000.
A	4.7
B	1.3
C	3.9
D	3.4

SULPHUR CONTENT OF COD LIVER OIL.

The literature on cod liver oil contains references to the fact that sulphur compounds are present in cod liver oil (17). The latter statements are purely on a qualitative basis and the form in which the sulphur is present is little understood. It was our purpose to determine first the sulphur content of various cod liver oils quantitatively.

The various methods of sulphur analysis were reviewed in order to obtain one which might be adaptable to cod liver oil.

Lamp Method (18).—Used for low boiling petroleum fractions. Low combustibility of cod liver oil eliminated any adaptation in this case.

Bomb Methods.—The use of a Parr bomb was not feasible due to the large amount of oil sample necessary for each analysis—two grams. An oxygen bomb would most likely be very satisfactory but was not available for this work.

Other Oxidation Methods.—Sanders (19) combines a previous oxidation using nitric acid with the bomb method. Pattern (20) uses metallic sodium and bromine to carry out his analysis. Smith (21) oxidizes the sulphur in coal, using potassium chlorate and sodium peroxide. Andrews (22) oxidizes with nitric acid

in the presence of magnesium oxide. Heslinger (23) burns the sample in a quartz furnace, the sulphur dioxide liberated is absorbed in hydrogen peroxide and the sulphur determined by titration with standard alkali. Pregl (24) oxidizes the SO_2 catalytically with platinum. Osborne fuses with a mixture of sodium carbonate and sodium peroxide. The above methods were considered carefully and in several cases experimented with, but in every instance were found objectionable for adaptation to cod liver oil.

Trotman and Bell (25) modified the Benedict-Davis method so that it would be applicable for determining sulphur in wool. We were able to modify their method so that it would also be adaptable to cod liver oil.

Two grams of oil are taken and saponified by heating two hours on the steam-bath with 6 cc. of 10 *N* sodium hydroxide and 10 cc. of water. One hundred cc. of a solution containing (25 Gm. $Cu(NO_3)_2$, 25 Gm. NaCl and 10 Gm. NH_4NO_3/-100 cc.) are added and the mixture evaporated to dryness on a steam-bath. It is then ignited over a Bunsen flame, slowly at first, until all of the copper is in the form of CuO. The ignited mass is digested in water containing 40 cc. of concentrated hydrochloric acid, filtered if necessary and 15 cc. of 10% $BaCl_2$ solution added to the filtrate. The solution is then warmed on the steam-bath and allowed to stand twenty-four hours before filtering. The precipitate is filtered off, washed thoroughly, ignited and weighed as $BaSO_4$. It is extremely important that the same reagents and the same amounts of each reagent be used in each set of analyses. This is due to the small sulphur contents of the reagents. Blank determinations on our method with different sets of reagents showed their sulphur content to vary from 0.0002–0.0258 Gm. of $BaSO_4$ per analysis.

TABLE III.—SULPHUR CONTENT OF CRUDE OIL.

	Wt. of oil, Gm.	Wt. of $BaSO_4$, Gm. (corr.).	Per cent S.
American (*a*)	2	0.0073	0.050
(*b*)	2	0.0083	0.057
Norwegian (*a*)	2	0.0070	0.048
(*b*)	2	0.0126	0.086

SULPHUR CONTENT OF DESTEARINATED OIL.

American, no.	Wt. of oil, Gm.	Wt. of $BaSO_4$, Gm. (corr.).	Per cent S.
1	2	0.0156	0.107
1	2	0.0125	0.086
2	2	0.0128	0.088
2	2	0.0163	0.112
3	2	0.0136	0.093
3	2	0.0126	0.087
4	2	0.0148	0.101
4	2	0.0130	0.089
5	2	0.0196	0.134
5	2	0.0174	0.119
6	2	0.0162	0.109

In order to check our sulphur method we added a known amount of sulphanilic acid to our oil and then determined the total sulphur content. The oil used was No. 6 above.

	Gm.		Gm.
Wt. of oil	2	Sulphur found	0.0064
Wt. of pure sulphanilic acid	0.0235	Sulphur calculated:	
Wt. of $BaSO_4$	0.0468	From cod liver oil	0.0022
		From sulphanilic acid	0.0039
		Total	0.0061

This shows that our method is determining the total sulphur content.

Our results indicate that sulphur is present in destearinated cod liver oil to the extent of 0.1% whereas the few results on undestearinated oil show only one half of this amount. We were not absolutely certain that the American destearinated and undestearinated represented identical oil samples. Assuming for the moment that they are identical the data would indicate that sulphur is picked up in the destearinating process. Such might be possible if the oil came in contact with rubber in any way. It is to be expected that the sulphur content will go up slightly in destearinating because the latter process removes a portion of the volume as stearine without diminishing the sulphur content. The latter per unit volume of destearinated oil will be slightly higher than in the case of undestearinated oil. To illustrate this some cod liver oil was destearinated in the laboratory, taking special precautions that it would come in contact with nothing which might introduce sulphur into it.

	Wt. of oil, Gm.	Wt. of $BaSO_4$ (corr.).	Per cent S.
Undestearinated	2	0.0071	0.049
Destearinated	2	0.0076	0.052

It is at once apparent that this lessening of the volume by removing the stearine does not account for the greater variation found in our former analyses. To investigate this matter further we obtained various oils from the cod liver oil rendering plants. The history of the oils obtained was thoroughly known, thereby increasing the value of the following results.

TABLE IV.

American Oil.

Oil.		Wt. of sample, Gm.	Wt. of $BaSO_4$, Gm. (corr.).	Per cent S.
283-A—Oil removed from No. 1 livers immediately	(*a*)	2	0.0127	0.087
	(*b*)	2	0.0092	0.063
283-B—Oil removed from No. 1 livers 12 hours or more later	(*a*)	2	0.0117	0.080
	(*b*)	2	0.158	0.108
283-D—Oil obtained by pressing after A and B were removed	(*a*)	2	0.0254	0.174
	(*b*)	2	0.0248	0.170
283-C—Oil removed from No. 2 livers (24 hours old)	(*a*)	2	0.0183	0.125
	(*b*)	2	0.0205	0.140

From our results on these two sets of oils we are led to believe that the variation in sulphur content of cod liver oils is due to the length of time which may exist before the oil is removed from the liver.

A second set of oils was obtained from a different source.

TABLE V.

American Oil.

Oil.	Wt. of oil, Gm.	Wt. of $BaSO_4$, Gm. (corr.).	Average per cent S.
Crude Oil	2	0.0053	
		0.0045	0.034
Skimmings	2	0.0052	
	2	0.0047	0.034
Pressed Chum	2	0.0120	
	2	0.0112	0.086
No. 2 Livers	2	0.0161	
	2	0.0162	0.110
Rotted Livers	2	0.0231	
	2	0.0187	0.143

SULPHIDE SULPHUR IN COD LIVER OIL.

Experiments were made to determine whether any of the sulphur present in cod liver oil was present as sulphide sulphur. J. H. Almy (26) developed a colorimetric method for the latter substance dependent upon the blue color obtained with *p*-amino-dimethyl-aniline-hydrochloride. Adapting this method to cod liver oil we were able to demonstrate that if sulphide sulphur was present in cod liver oil its concentration was less than 0.00004%. Seven different oil samples were analysed. To demonstrate that the method was applicable to cod liver oil provided sulphide sulphur were present, we added a known amount of sulphide sulphur to the oil and got colorimetric results comparable with our standards to the amount of S^- added.

SUMMARY.

A number of the physical and chemical characteristics of cod liver oil have been studied.

The peroxide content of cod liver oil has been examined and the applicability of a number of the known tests for peroxides has been tested.

The retardation of oxygen absorption of cod liver oil has been studied both qualitatively and quantitatively.

Cod liver oil has been found to contain 1.3 to 4.7 parts of iron per 10,000,000.

It has been shown that cod liver oil may contain from 0.03–0.2% sulphur.

Sulphur has been quantitatively found in both American and European oils.

The sulphur content of the oil appears to be dependent upon the length of time existing between the time when the fish is killed and the time when the oil is removed from the liver tissue.

The sulphur present in cod liver oil is not present as sulphide sulphur.

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STUDIES IN BIOASSAYS: "THE PROPOSED INTERNATIONAL STANDARD FOR DIGITALIS."*,[1]

BY CHARLES W. EDMUNDS, M.D.,[2] HAROLD W. LOVELL, A.B.,[3] AND SPENCER BRADEN.[3]

In U. S. Pharmacopœia IX biological assays were first adopted as an optional method of evaluating certain drugs for which chemical means were not available. In this group of drugs was the digitalis series including digitalis itself, strophanthus and squills. As a standard for these drugs, crystalline gratus-strophanthin or ouabain was adopted and the strength of each preparation was adjusted to a certain equivalent of ouabain as measured by its effect on frogs when tested by the prescribed method. Thus ouabain served as a check upon the frogs, the susceptibility of which to digitalis is known to vary at different seasons of the year.

During the years which followed the publication of this Pharmacopœia (IX), the standard ouabain apparently proved satisfactory because in the various discussions incident to the recent revision (X), no objection was made from any source to its continuance as a standard for the digitalis group, although it was suggested that a strophanthin isolated from Kombé seeds might be substituted for the *g*-strophanthin. This, however, was not done and ouabain was continued as the official standard.

On the other hand at the conference on Biological Standardization held in Edinburgh in 1923, it was recommended that a preparation of powdered digitalis leaves should be used as a standard for digitalis itself and that ouabain should be

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