THE PRESERVATION OF HALIBUT LIVER OIL WITH HYDROQUINONE.*[1]

BY W. S. JONES AND W. G. CHRISTIANSEN.

It has been shown (1), (2) that the absorption of oxygen by cod liver oil is greatly retarded by the presence of an antioxidant, thus preventing loss of Vitamin A and development of mal-flavors in especially prepared oils. H. N. Brocklesby and O. F. Denstedt (3) have recently shown that the rate of absorption of oxygen by pilchard oil can also be decreased greatly by the addition of an antioxidant such as hydroquinone or pyrogallol.

Halibut Liver Oil is not only a very potent and highly concentrated source of Vitamin A, but also an oil which requires special refining in order to free it from natural objectionable odor and taste; hence an oil in which such protection should be of utmost importance. Therefore a study was made to determine the degree of protection afforded by an antioxidant such as hydroquinone in preventing vitamin and other deterioration. The present work shows that when halibut liver oil is exposed to both air and oxygen, the absorption of oxygen[2] and the loss of Vitamin A[3] is greatly retarded by the presence of hydroquinone.

EXPERIMENTAL.

A. Type of Oils Studied.—Two samples (A and B) of refined halibut liver oil differing slightly in Vitamin A potency were used; into a portion of each of these oils was incorporated 0.03% hydroquinone.

B. Preparation of Samples.—Four 25-Gm. portions of each of the four oils were weighed into 100-cc. tared beakers. Two weighed portions of each group were allowed to stand exposed to the air in the laboratory. (A paper covering was placed over samples to protect them against dust and yet not to exclude free access of air.) The remaining two weighed portions of each group were placed in a ten-inch vacuum desiccator. By means of inlet and outlet tubes in the cover of the desiccator, the air in the desiccator was swept out with oxygen. The inlet and outlet tubes were then closed off by means of pinch clamps.

C. Testing of Samples.—One sample of each pair was tested weekly by the Vitamin A color test; the second of each pair was weighed weekly. In case of samples exposed to oxygen the space in the desiccator was reflooded with oxygen and sealed from the air after each weekly observation.

D. Vitamin A Color Unit.—As a relative index of the Vitamin A potency of the oils, our laboratories have adopted a color standard (developed by the Biological Laboratories of E. R. Squibb and Sons) prepared as follows:

Five-cc. portions of 0.5 *M*-$CuSO_4.5H_2O$ (made up in 1% HCl) are measured out; 0.1-, 0.3-, 0.4-, 0.5- and 0.6-cc. portions of 0.5 *M*-$CoCl_2$ in 1% HCl are added, respectively, to different portions of copper sulphate solution. The mixtures are then diluted with 1% HCl to exactly 10 cc. and exactly 2 cc. of each solution is transferred to a 1-cm. bore test-tube and sealed. The cobaltous chloride imparts a red tint to the blue copper sulphate solution and the gradation in red obtained by the addition of increasing amounts of the cobalt solution makes it possible to match accurately the shades of blue produced by different oils. One cm. of thickness of this solution approximates six Lovibond blue units.

E. Technique of the Antimony Trichloride Vitamin A Color Test.—One-tenth gram of oil is weighed out and dissolved in chloroform to make a volume of 50 cc. A measured volume of the chloroform solution is diluted with more chloroform, so that the addition of 1.8 cc. of a saturated

---

* Section on Practical Pharmacy and Dispensing, Madison meeting, 1933.

[1] Research Department of the Chemical and Pharmaceutical Laboratories, E. R. Squibb and Sons, Brooklyn, N. Y.

[2] Determined by increase in weight of the oil.

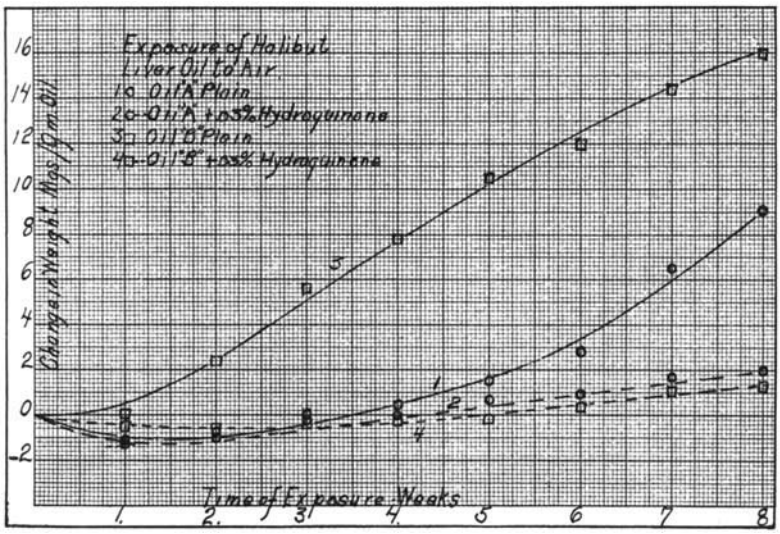
[3] Determined by color test and confirmed by biological assay.

chloroform solution of antimony trichloride to 0.2 cc. of the chloroform solution of oil in a 1-cm. bore test-tube produces a blue color which matches one of the copper sulphate standards. The Vitamin A color units per Gm. of oil would then be

$$\frac{50 \times A}{\text{Wt. of oil} \times 0.2}$$

A is the dilution factor; *i. e.*, the degree to which the 50 cc. of $CHCl_3$ solution must be diluted in order to match the standard blue. Thus, if 5 cc. is diluted to 20 cc., A will be 4.

F.—The results of the successive color tests are recorded in Table I.



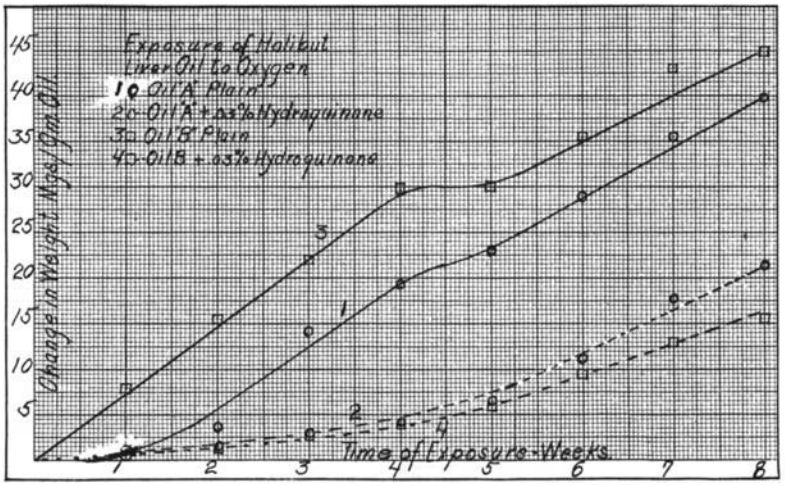
Graph 1.

TABLE I.

Initial Color Test.	Sample No.	Condition of Exposure.	Antioxidant.	Vitamin A Color Units per Gm. First Week.	Second Week.	Third Week.	Fourth Week.	Fifth Week.	Sixth Week.	Seventh Week.	Eighth Week.
	1	Air	None	10600	10900	10530	9000	9090	6000	Released for Bio. Assay	
Oil A	5	Air	Hydroquinone	10700	10800	10070	9250	10300	9500		
11,100 cu./Gm.	3	Oxygen	None	10500	7500	*	..	...	..		
	7	Oxygen	Hydroquinone	10700	10400	9000	7500	5450	*	..	..
	9	Air	None	7250	5200	3000	*	...	..	..	..
Oil B	13	Air	Hydroquinone	7270	7700	7500	7850	7500	7300	6550	6410
8700 cu./Gm.	11	Oxygen	None	3230	*	...	..	...	..	..	..
	15	Oxygen	Hydroquinone	8700	6820	6900	5680	5600	4500	2700	*

* At this point the color produced by the $SbCl_3$ has become so violet that it cannot be matched with the standards.

It was noted during the course of the work that as the Vitamin A was being destroyed (as indicated by the color test) the color of the oil gradually changed from a light amber to a bright yellow. Further the color produced in the antimony trichloride test differed from the copper sulphate blue (it became reddish purple), so that it could not be matched by any of our standards. The difference was slight in the beginning and became more noticeable as the experiment progressed. This change was very rapid and pronounced in samples without antioxidant and exposed to oxygen; the change becomes slower when an antioxidant is used and the exposure is less severe, *i. e.*, air instead of oxygen.



Graph 2.

These color tests show that exposure of halibut liver oil to oxygen as such or as air causes Vitamin A deterioration and that these losses are retarded greatly by the addition of hydroquinone.

G.—In order to confirm the results of the color tests, one pair of samples (Oil A without and with hydroquinone—Nos. 1 and 5 in Table I) were assayed biologically after they had been exposed to air for six weeks. The results are contained in Table II and show the pronounced protection afforded by Hydroquinone.

TABLE II.

	Vitamin A Potency in U. S. P. X Units.*
Original Oil	76,750
After 6 weeks' exposure to air without hydroquinone	41,500
Protected by addition of 0.03% hydroquinone and exposed to air for 6 weeks	61,250

* Not to be confused with new units (1934 Revised). To convert to 1934 Revised Units use factor 1.4.

H.—The change in weight of oils in mg./Gm. of oil, due to exposure to air and oxygen, are recorded in Table III and the data are plotted in curves on Graphs 1 and 2. Due to the fact that the increases in weight of oils exposed to pure oxygen were so much greater than those of the oils exposed to air the data of the two groups were plotted on separate plates. No explanation can be given at present for the sudden, temporary leveling of Curve 3, Graph 2.

It will be noted from Graphs 1 and 2 that

(*a*) Oils A and B without hydroquinone (Curves 1 and 3) differ in susceptibility to oxidation; this is particularly noticeable in the "air" experiment (Graph 1).

(*b*) Oils A and B with hydroquinone (Curves 2 and 4) do not differ appreciably in susceptibility to oxidation.

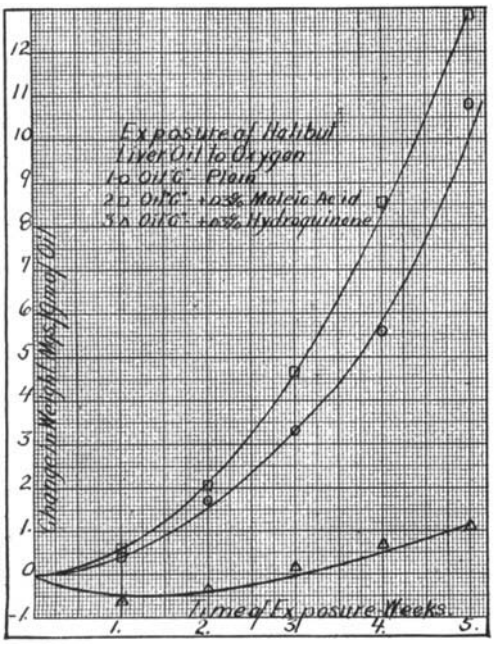
(*c*) An oil containing 0.03% hydroquinone is quite resistant to oxidation as compared with unpreserved oil.

TABLE III.

Sample No.	Exposed to	Antioxidant.	Change in Weight—(Mg. per Gm. of Oil). First Week.	Second Week.	Third Week.	Fourth Week.	Fifth Week.	Sixth Week.	Seventh Week.	Eighth Week.
						Oil A.				
2	Air	None	−1.1*	− 0.85	+ 0.02	+ 0.43	+ 1.5	+ 2.8	+ 6.5	+ 9.1
4	Oxygen	None	+1.2	+ 3.7	+14.2	+19.2	+23.1	+29.0	+35.5	+39.8
6	Air	0.03% hydroquinone	−1.2	− 1.0	− 0.3	− 0.05	+ 0.7	+ 0.9	+ 1.7	+ 2.0
8	Oxygen	0.03% hydroquinone	+0.5*	+ 1.5	+ 3.0	+ 4.3	+ 6.5	+11.1	+17.7	+21.4
						Oil B.				
10	Air	None	+0.1	+ 2.4	+ 5.6	+ 7.8	+10.5	+12.0	+14.4	+16.0
12	Oxygen	None	+8.0	+15.6	+21.8	+29.7	+29.9	+35.5	+40.6	+44.8
14	Air	0.03% hydroquinone	+0.5	− 0.6	− 0.3	− 0.3	− 0.2	+ 0.35	+ 1.1	+ 1.3
16	Oxygen	0.03% hydroquinone	+1.2	+ 1.6	+ 2.8	+ 4.1	+ 5.8	+ 8.5	+13.1	+16.5

* − Decrease in weight.
\+ Increase in weight.

I.—According to Greenbank (U. S. Patent 1,898,363) maleic acid inhibits the oxidation of unsaturated fats, oils, fatty acids and substances which contain fatty material and tend to become rancid. In order to study the antioxidant effect, if any, of maleic acid in halibut liver oil, an experiment, similar to that described above for oils "A" and "B" was set up, in which samples of a halibut liver oil, containing, respectively, (*a*) no antioxidant, (*b*) 0.03% hydroquinone, and (*c*) 0.03% maleic acid, were exposed to pure oxygen for 5 weeks. As before, one series of samples was weighed weekly and a second series was tested colorimetrically for Vitamin "A" weekly. The oil containing maleic acid was no more resistant to oxidation than the plain halibut liver oil and, according to the color test, lost its Vitamin "A" potency at the same rate as the plain oil. The oil containing the hydroquinone showed the usual resistance to oxidation and loss of Vitamin "A." The change in weight in mg./Gm. of oil is recorded graphically in Graph 3.



Graph 3.

CONCLUSIONS.

1. Hydroquinone retards the absorption of oxygen by refined halibut liver oil from air and an atmosphere of pure oxygen.

2. Hydroquinone, as indicated by the Vitamin A color test retards the deterioration of Vitamin A of halibut liver oil upon exposure to air or pure oxygen.

3. Hydroquinone, as shown by the biological test retards the deterioration of Vitamin A of Halibut Liver Oil upon exposure to air.

4. Maleic acid does not act as an antioxidant in halibut liver oil.

Biological assays reported herein were made in the Biological Research Laboratories of E. R. Squibb and Sons and we gratefully acknowledge their assistance; also that of Mr. E. Beaman of Research Laboratories of E. R. Squibb and Sons who assisted in our experimental work.

REFERENCES.

(1) Christiansen, *et al.*, JOUR. A. PH. A., 18, 771 (1929).
(2) Christiansen, Chappel, Briod, U. S. Patent 1,745,604.
(3) Biological Board of Canada, Bull. No. 37, 23 (1933).

ACCURACY AND SPEED FACTORS IN HAND-FILLING CAPSULES.*

BY JOHN W. LEE.[1]

The primary purpose of this paper is to compare two of the usual methods ordinarily employed in hand-filling capsules. One of the first problems that confronts us in either the establishment of a tolerance limit or the comparison of methods in hand-filling capsules, is the method or manner in which the contents of an individual capsule is determined.

Some of the methods that have been used in determining the contents of an empty capsule are:

(1) Dissolving the contents of the capsule in a suitable solvent and subsequent evaporation of the solvent.[2]

(2) Assay of the ingredients by the Official Process.

(3) The emptying of the contents and weighing directly.[3]

(4) Using individual capsules of the same size as a counterpoise.[2]

(5) Weighing a number of filled capsules at the same time using an equal number of empty shells as a counterpoise, changing the empty shells for different ones after one or two operations.[3]

The first method has the disadvantage of requiring too long a time, and it is not always possible to find a suitable solvent, especially, when the capsule contains a mixture of powders.

The second method mentioned also requires too much time and is not practical enough for use by the practicing pharmacist.

The third method, consisting of emptying the contents of the capsule and weighing directly is better suited for general use, but here again too much time is consumed and in the case of adhesive powders it is almost impossible to remove all of the powder from the shell.

The fourth method, using the empty shell as a counterpoise, introduces the error caused by the variance in weight of the individual shells.

---

* Section on Practical Pharmacy and Dispensing, A. PH. A., Washington meeting, 1934.

[1] Assistant Professor in Pharmaceutical Chemistry. The George Washington University School of Pharmacy. In collaboration with Prof. W. P. Briggs.

[2] Private communication.

[3] Mathews, Norris W., JOUR. A. PH. A., 22, 321 (1933).