Work upon this fraction, which contains the saponaceous glycoside, will be reported in detail later.

Water Extract.—The water extract was a brown, granular solid. An aqueous solution of the substance foamed when shaken. The presence of reducing substances was indicated by the production of cuprous oxide upon boiling with Fehling's solution. The ferric chloride test was negative. A deep blue color was produced when a solution of iodine in potassium iodide was added to an aqueous solution of the extract.

SUMMARY

A preliminary chemical investigation of the rhizome and roots of Helonias (*Chamælirium luteum* A. Gray) is reported. No volatile oil or alkaloid could be detected. The nondrying, fixed oil was shown to contain oleic, linoleic and stearic acids. The presence of other saturated fatty acids and sterols was indicated. No tannic or gallic acid could be detected.

REFERENCES

(1) Grieve, M., "A Modern Herbal," Jonathan Cape, London, 1931.

(2) Youngken, H. W., "A Textbook of Pharmacognosy," Blakiston, Philadelphia 1936.

(3) Moser, J., *Am. J. Pharm.*, 89 (1917), 291.

(4) Greene, F. V., *ibid.*, 50 (1878), 250.

(5) Greene, F. V., *ibid.*, 50 (1878), 465.

(6) Kruskal, N., Cited in *Jahresber. Pharm.*, 27 (Neue Folge) (1892), 570.

(7) Pilcher, J. D., *J. Pharmacol.*, 8 (1916), 110.

(8) Clevenger, J. F., JOUR. A. PH. A., 17 (1928), 345.

(9) Rosenthaler, R., "The Chemical Investigation of Plants," translated from the Third German Edition by S. Ghosh, G. Bell, London, 1930.

(10) Jenkins, G. L., and DuMez, A. G., "Quantitative Pharmaceutical Chemistry," Second edition, McGraw-Hill, New York, 1937.

(11) Jamieson, G. S., "Vegetable Oils and Fats," Chemical Catalog Company, New York, 1932.

(12) Lettre, H., and Inhoffen, H. H., *Sammlung chem. u. chemish.-tech. Vortraege*, 29 (Neue Folge) (1936), 16.

(13) Lewkowitsch, J., "Chemical Technology and Analysis of Oils, Fats, and Waxes," Vol. I, Fifth edition, MacMillan, London, 1913.

Comparative Stability of Vitamin A in Cod-Liver Oil and in Oleovitamin A and D*

By Arthur D. Holmes and Madeleine G. Pigott

When it was foreseen that the supply of cod-liver oil would not be adequate for the country's needs, the Revision Committee of the United States Pharmacopœia provided for a substitute product which would possess the same vitamin A and vitamin D value as official cod-liver oil and designated it oleovitamin A and D. The official specifications (1) for this product provide that natural vitamin A and vitamin D may be dissolved in edible vegetable oils and the mixture shall have a U. S. P. vitamin potency of not less that 850 but no more than 1100 vitamin A, and not less than 85 and no more than 110 vitamin D units per gram. Obviously oleovitamin A and D does not contain any iodine (2), arsenic (3) or other substances which are found in cod-liver oil and its therapeutic value resides wholly in its vitamin content.

In the commercial distribution of oleovitamin A and D, this material, like other pharmaceutical products, is likely to remain in the hands of the wholesaler or upon the druggist's shelf for indefinite periods. Therefore a question naturally arises as to the relative permanency of the fat-soluble vitamins as they naturally occur in cod-liver oil and in solution in vegetable oils. Earlier studies in this laboratory have shown that the permanency (4) of vitamin A in cod-liver oil is influenced by the exposure of the oil to light. In other words, the rate of destruction of vitamin A in cod-liver oil packed in Flint glass containers depends upon both the intensity of the light or direct sunshine and the length of time that the oil is exposed to it. Accordingly, it seemed of inter-

* Received October 29, 1942, from the Research Laboratories, The E. L. Patch Company, Stoneham, Mass.

est to compare the permanency of vitamin A in natural cod-liver oil and in oleovitamin A and D when stored under identical conditions.

EXPERIMENTAL

The cod-liver oils were medicinal cod-liver oils taken from stock and were believed to be typical of commercial cod-liver oil. One oil sample, No. 4, had a vitamin A potency of approximately 1000 U. S. P. vitamin A units and sample 5 had a potency of approximately 4000 U. S. P. vitamin A units/Gm. Sample 4 was chosen because its potency was essentially the same as that specified for oleovitamin A and D. While medicinal cod-liver oils with a vitamin A potency of 4000 units/Gm. are not common, sample 5 was selected as representing cod-liver oils of high vitamin A potency.

The oleovitamin A and D oils, samples 1, 2 and 3, were prepared by dissolving a vitamin A concentrate in edible cottonseed, peanut and corn oils, respectively. The three vegetable oils were subjected to the vitameter assay procedure and all were apparently free from substances which would influence the vitameter assays of oleovitamin A and D oils prepared with the vegetable oils as diluents. Vitamin A concentrate designated as "winterized" was obtained from the Distillation Products Company. It was prepared by molecular distillation from natural sources and had a potency of 200,000 vitamin A units/Gm. To eliminate any possible destruction of vitamin A during the preparation of the oleovitamin A and D samples, the vitamin A concentrate was mixed with the vegetable oils while in direct contact with nitrogen.

Since Flint glass bottles are frequently used for distributing cod-liver oil, it was decided to package the samples of cod-liver oil and the oleovitamin A and D in 2-oz. Flint glass bottles with screw cap closures. The samples were placed in direct sunshine on the southwest side of the building. The daytime temperature of the atmosphere surrounding the samples was approximately 86° F. During the night the temperature dropped to approximately 50° F. The five samples were stored under these conditions for a period of 11 days during the last of November and first of December. Of this time between 4 and 5 days were cloudy or raining and the samples were exposed to only skyshine. In other words, the samples were exposed to early winter sunshine for something over 6 days. This exposure to light was somewhat similar to but very much more severe than that to which cod-liver oil and oleovitamin A and D products might be exposed in drugstores. At the end of this time there had been a noticeable fading of the characteristic cod-liver oil color of the cod-liver oil samples. The color of the oleovitamin samples was also less intense than at the beginning of the period of exposure.

Attention was centered upon vitamin A since it is very generally believed that vitamin A is less stable than vitamin D. The vitamin A potencies of the cod-liver oils and of the mixtures of edible oils and vitamins were determined with the Hilger vitameter. The assay samples were dissolved in isopropyl alcohol. A specially purified, 99% isopropyl alcohol was used. The vitameter readings were made within 15 min. or so after the samples were prepared. These readings were expressed as *E* values (extinction coefficient). The results of the vitameter assays at the beginning of the experiment, at the end of the experiment, the decrease in the *E* values during the period of exposure and the estimated vitamin potency at the end of the experiment are reported in Table I.

It will be noted that at the start of the exposure of the samples to light the *E* values of the three oleovitamin A and D samples were in close agreement, being 0.526, 0.521 and 0.529, respectively, for the cottonseed, peanut and corn oil mixtures. The vitamin A content of cod-liver oil, sample 4, was slightly higher than that of the oleovitamin oils since it possessed an *E* value of 0.652. The *E* value for sample 5 reported to have a vitamin A potency of 4000 vitamin A units/Gm. was 2.088. After 11 days' exposure to light the vitameter readings for the three oleovitamin A and D oils were 0.101, 0.098 and 0.104 respectively, for the cottonseed, peanut and corn oil, solutions. The vitameter values for the cod-liver oil samples were 0.329 and 1.175.

For purposes of comparison, the decrease in *E* values during the 11 days' exposure are reported on a percentage basis. The decrease in *E* values for the oleovitamin A and D oils were 80.80, 81.19 and 80.34%, respectively, for the cottonseed, peanut and corn oil solutions. The average decrease was 80.8% However, the decrease in the *E* values for the two cod-liver oil samples was definitely less than that of the oleovitamin A and D samples, being 49.55% and 43.73%, respectively, for the samples having reported original potencies of 1000 and 4000 vitamin A

TABLE I.—COMPARATIVE STABILITY OF VITAMIN A IN COD-LIVER OIL AND IN OLEOVITAMIN A AND D

Sample No.	Vitamin A Containing Oils	Vitameter Reading: At Start, *E* Value	Vitameter Reading: After 11 Days' Storage, *E* Value	Decrease During Storage: *E* Values, Per Cent	Decrease During Storage: Vitamin A, U. S. P. Units
1	Oleovitamin A and D (cottonseed oil)	0.526	0.101	80.80	808
2	Oleovitamin A and D (peanut oil)	0.521	0.098	81.19	814
3	Oleovitamin A and D (corn oil)	0.529	0.104	80.34	802
4	Cod-liver oil	0.652	0.329	49.54	375
5	Cod-liver oil	2.088	1.175	43.73	1767

units, respectively. The estimated decrease in vitamin A potency of the oleovitamin A and D oils during the exposure test were practically identical, being 808, 814 and 802 units, respectively, for the cottonseed, peanut and corn oil preparations The decrease in potencies of the cod-liver oils was estimated to be 375 and 1767 units/Gm., respectively, for samples 4 and 5. The difference in the vitamin A loss in the two cod-liver oil samples is doubtless influenced by the marked difference in the vitamin A potency of the samples. However, the average loss of vitamin A, 46.64%, for the cod-liver oil samples is only about half the loss, 80.80%, for the oleovitamin A and D products. Apparently the vitamin A in cod-liver oil is protected to some extent by an antioxidant in the cod-liver oil or it is in a more stable form than that which was dissolved in the vegetable oils.

SUMMARY

Samples of oleovitamin A and D prepared with cottonseed, peanut and corn oils and samples of medicinal cod-liver oils were exposed to early winter sunshine and skyshine for 11 days to compare the permanency of their vitamin A content.

Vitameter assays were made of the vitamin A content of the oleovitamin A and D oils and of the cod-liver oil samples before and after the exposure test. The results of these tests showed that there was approximately 80% loss of the vitamin A content of the oleovitamin A and D, regardless of whether cottonseed, peanut or corn oil was used as a diluent. The loss of vitamin A from the cod-liver oils was only about one-half the vitamin A loss from the oleovitamins. Possibly cod-liver oil may contain some natural antioxidant or its vitamin A content may be in a more stable form than that of the vitamin A concentrate incorporated in the vegetable oils, used for preparing the oleovitamin oils employed in this study.

REFERENCES

(1) "United States Pharmacopœia, XII," 1942, p. 311.

(2) Holmes, A. D., and Remington, R. E., *Amer. J. Dis. Child.*, 49 (1935), 94.

(3) Holmes, A. D., and Remington, R. E., *Ind. Eng. Chem.*, 26 (1934), 573.

(4) Holmes, A. D., and Pigott, M. G., *Boston Med. Surg. J.*, 195 (1926), 263.

Zinc Peroxide*,†

By Roland H. Noel and E. V. Lynn

The substances called zinc peroxide have been known and used in medicine for years. Since Thenard, in 1818, first produced (1) his "deutoxyde de zinc" by the action of zinc hydroxide and hydrogen peroxide, many others have prepared similar materials and by like processes, but almost always to yield products with somewhat differing composition. All of them contained zinc, oxygen and hydrogen in variable proportions, but hardly any two yielded the same results in elementary analysis, and this has been true often in two substances made by almost identical methods. This may perhaps explain the markedly inconsistent results in therapeutic application since introduction into practice 40 years ago. However, more recent reports would seem to indicate that, providing a zinc peroxide is used that is proved to contain maximum activity, excellent results can be obtained in arresting lesions caused by all types of anaerobic organisms.

* From the Department of Chemistry, Massachusetts College of Pharmacy, Boston, Mass.

† Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.

EXPERIMENTAL

Because no one here has ever prepared a compound of the formula ZnO_2 or any other peroxide that contained no hydrogen, the present experiments were instituted to learn if such a compound can exist. In an extensive series, pure zinc oxide was subjected to oxygen at temperatures of 100° to 1000° C. but in every case the oxide was found unchanged analytically after treatment, and no evidence of active oxygen could be found in the residue.

The attempt was next made to attain peroxida-