Report of the Vitamin Committee February, 1950

THE Vitamin Committee was organized in 1945, and its first assignment was to improve the analytical methods for vitamin A, a problem of serious importance to manufacturers of fish liver oils, animal feeds, margarine, and pharmaceuticals. The original membership, which has changed very little during the ensuing years,* was picked primarily from chemists who had served on government and industrial committees concerned with analyses and standards for vitamin A, along with chemists experienced with feed and margarine.

At the time the committee was organized, the only official method for vitamin A was the biological assay which required two months to complete and which had inherently a variation of 10 to 20%; the standard for comparison was a reference cod liver oil which deteriorated on standing and had to be replaced every few years. Many commercial transactions were based upon physicochemical assays, but these were not standardized and as a rule did not protect either the buyer or seller from the possibility of violating governmental regulations.

The main objective of the Vitamin Committee was to persuade and assist quasi-legal groups, such as the U. S. Pharmacopoeia, to adopt reliable physicochemical assay methods. If this was found not to be feasible, the committee was requested at least to standardize the shorter and more precise methods that were being used commercially. As it turned out, the officials of the U. S. Pharmacopoeia had already appreciated the need for new methods and were very willing to receive any information and suggestions.

In 1946 the Vitamin Committee recommended (1) a new standard based on a pure crystalline vitamin A derivative, along with a physicochemical method based upon the ultraviolet absorption measurements and upon the antimony trichloride reaction.

The standard recommended was a capsulated solution containing 3.44 mg. of vitamin A acetate (corresponding to 3.0 mg. of vitamin A alcohol) per gram of oil in refined deodorized cottonseed oil; and it was suggested that a small amount of tocopherol be added as an antioxidant. The U. S. Pharmacopoeia adopted and distributed such a standard in the summer of 1947. The only difference from our recommendation was that no tocopherol was added since the natural content of properly refined cottonseed oil seemed adequate.

While the new standard was gratifying from a scientific point of view, its adoption precipitated the same commercial crisis which attended the adoption of each new batch of reference cod liver oil during the past. The biological conversion factors against the new standard turned out to be considerably lower than those obtained on the last deteriorated samples of the previous reference cod liver oil.

To assist the various industries involved the Vitamin Committee held a large open meeting during the 1947 fall session of the A.O.C.S. Over 100 chemists and industrialists attended and exchanged enough information so that the transfer to the new basis went quite smoothly on the deadline, January 1, 1948.

THE new standard was put to use by a large number of laboratories because it could serve as a physical and chemical reference point as well as a biological one. A new batch of standard was required in 1948, and, as expected, the properties were identical with the first.

The International Standard for vitamin A since 1934 has been a solution of β-carotene. While this material was more reproducible than our U.S.P. reference oils, it was less satisfactory from many points of view because the material was not actually vitamin A, and its biological correlation with vitamin A varied with diet, strains of animals, and possibly other factors. The usefulness of the new U.S.P. Standard was appreciated in many other countries, and recently a portion of the stocks of the U.S.P. Standard was set aside and designated as the International Standard for vitamin A.

The availability since 1947 of a reliable and reproducible vitamin A standard made it possible to investigate the reliability of physicochemical methods with much greater certainty than had been possible in the past.

To assemble information to carry out collaborative assays and to make recommendations on this subject, the U. S. Pharmacopoeia set up an informal committee consisting of S. H. Fox, chairman, J. B. Wilkie, O. D. Bird, B. L. Oser, Kenneth Morgareidge, E. F. Week Jr., and N. D. Embree, which has been assisted by R. W. Lehman. The last five men named are members of the A.O.C.S. This committee will publish its information and recommendations elsewhere. They proposed and the U. S. Pharmacopoeia accepted a method that is not greatly different from that originally proposed by the Vitamin Committee in 1946. The new method, which will become official when published early in 1950 in the 14th edition of the United States Pharmacopoeia, is briefly as follows:

a) The ultraviolet absorption of the unsaponifiable fraction will be used as the basic measurement with impurities corrected by a modification of the Morton and Stubbs procedure, using extinction coefficients at 310 and 334 mμ. The Vitamin Committee's original recommendation was similar except that the purity of the spectrum was to be judged by the extinction measurements at 300 mμ and 350 mμ.

b) The antimony trichloride blue color produced from the unsaponifiable fraction (measured against that from the standard) must indicate a relative "blue color" potency of from 100 to 130% of that obtained by the ultraviolet method. The original recommendations called for confirmation by the blue color procedure, but it was not appreciated at that time that the presence of neo-vitamin A in natural and synthetic commercial preparations would cause the relative blue color to be somewhat higher when compared against that of the standard which does not contain any of the neo- form.

The Association of Official Agricultural chemists will publish in 1950 a method based on the U.S.P.

* H. J. Deuel Jr. replaced Harry Steenbock in 1946.

[1] Report of the Vitamin Committee of the A.O.C.S. N. D. Embree, Oil & Soap, *23*, 275 (1946).

procedure for the determination of vitamin A and fish liver oils for animal feeds.

While the vitamin A assay methods for official pharmaceutical products will cover most commercial oils and concentrates, it would be impossible to expect them to be directly suitable for margarine, feeds, and certain other vitamin A containing materials. However the new U.S.P. Standard and the adoption by the U.S.P. of physicochemical methods should facilitate the adoption of physicochemical methods for other products. One of the Vitamin Committee members, E. E. Rice, is the chairman of a committee of margarine chemists who have already worked out good chemical methods for the assay of vitamin A in margarine that should be adaptable for official use. Another member of the committee, H. C. Schaefer, has been appointed an associate referee of the Association of Official Agricultural Chemists with the assignment of investigating official physicochemical methods for feeds.

Since a reliable and reproducible vitamin A standard has been officially adopted and since official physicochemical assays have been or will be adopted in fields of interest to the Oil Chemists' Society, the present membership of the Vitamin Committee has carried out its original assignment. We recommend that a new problem be assigned to this committee and that new members be chosen who are especially qualified to work out that problem.

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Report of the Refining Committee April, 1950

DURING the course of the year E. Handschumaker, working in the laboratories of Lever Brothers Company, has carried on an extensive study of the Servall method of contrifuge refining. His work has been done with two main objectives in view:

a) An attempt to develop a reproducible refining method at a single temperature.
b) An investigation of the multipaddle machine developed in 1948 by Charles Cole and exhibited to the Refining Committee at the meeting in New York in 1948.

Mr. Handschumaker has written a complete report of his investigations, and this has been mailed to the various members of the Refining Committee. In brief, its conclusions are that the single temperature method of refining is practicable and reproducible and that the Cole multipaddle machine, while its use is practicable, needs certain refinements.

His recommendation is that further collaborative work on the centrifuge method would be futile unless all members of the committee were prepared to equip themselves with multipaddle machines similar to those constructed by Mr. Cole. It is his opinion that most of the differences reported among the collaborators in the past were due to the use of single agitators and minor modifications in technique. It is essential to follow every step of the method outlined as exactly as possible in order to obtain concordant results.

AT THE meeting in Chicago in November 1949 a subcommittee consisting of F. R. Earle, chairman, G. A. Crapple, and N. F. Kruse was appointed to make a thorough review and study of the whole question of centrifuge refining and to recommend further action to the committee as a whole.

This subcommittee first reviewed the files in detail and then sent out a questionnaire to the Refining Committee to obtain their views.

Fourteen members of the Refining Committee answered the questionnaire and the consensus was:

a) That the present standard A.O.C.S. Methods of refining were functioning well.
b) That the use of the centrifuge to consolidate foots should not be made a part of the official procedure for refining degummed oil.
c) That the 200-gram centrifuge method was of no particular interest and should be not studied further.
d) On the question as to whether the centrifuge method of refining, using the Servall machine, should be investigated further, the committee was equally divided.
e) Despite the answer to d, a majority of the committee indicated that they would not be interested in a centrifuge method, even though the standard deviation was markedly improved over the present cup method.
f) On questions concerning problems needing further study there was no agreement among the members of the committee, and seven of the 14 reported that they had no suggestions for collaborative work.

As a result of the answers to their questionnaire, the subcommittee reported to the Refining Committee that the greater number of members felt that the present cup method is functioning adequately and recommends that no further collaborative work be done by the committee as a whole until such time as there is agreement on the need for the study of specific problems. At their request the subcommittee has been discharged.

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