COMMITTEE REPORTS

REPORT OF THE VITAMIN ASSAY COMMITTEE OF THE AMERICAN DRUG MANUFACTURERS' ASSOCIATION—TWENTY-FIRST ANNUAL MEETING—APRIL 1932.

BY ARTHUR D. HOLMES, CHAIRMAN.

Immediately following the 1931 annual meeting of the American Drug Manufacturers' Association the Vitamin Assay Committee attended a special meeting called by the United States Pharmacopœia Revision Committee. This meeting was arranged by the Chairman of the Revision Committee to develop methods for the assay of vitamin A and vitamin D. The meeting was attended by twenty-five of the leading vitamin investigators in this country. Early in the meeting it was decided to prepare tentative vitamin A and vitamin D methods to be presented at the special League of Nations meeting which was to be held in London, June 17th. The vitamin A and vitamin D methods outlined in detail in your Committee's May 1931 report served as the basis of consideration. During the meeting the methods suggested by your Committee were discussed in detail from the viewpoint of investigators in academic, industrial, consulting and medical laboratories. After full discussion the United States Pharmacopœia Vitamin Advisory Committee concluded that your Committee's vitamin A method, modified in certain respects, and the vitamin D method, practically unchanged, should be presented by the American representatives, Prof. E. V. McCollum and Prof. Harry Steenbock, at the London meeting of the League of Nations.

During the consideration of vitamin methods and standardizations by the League of Nations Permanent Commission on Biological Standardization it was agreed that International vitamin units should be defined and adopted. Since it is at present believed that Carotene is the parent substance of Vitamin A and since it is possible to prepare this material in pure form it seemed logical to use Carotene as the basis for defining an International vitamin A unit. Consequently it was recommended at the League of Nations meeting that the Vitamin A activity of one gamma (0.001 mg.) of the International standard Carotene be considered as one vitamin A unit.

The Permanent Commission on Biological Standardization defined a vitamin D unit as follows:

"The unit of vitamin D recommended for adoption is defined as the vitamin D activity of 1 milligram of the international standard solution of irradiated ergosterol."

The further consideration of vitamin methods and vitamin standards by the Permanent Commission on Biological Standardization may be summarized by the following excerpts from its report.

"It was decided not to recommend any one particular method of conducting the biological assay, but to invite members of the Conference to submit to the League of Nations Health Organization their observations on the value of the methods they have been using."

..."The Conference recommends that Carotene be accepted as an international provisional standard of reference for vitamin A and that a selected sample of cod liver oil be held in view as a possible secondary standard."

..."The Conference recommends that the standard solution of irradiated ergosterol at present issued from the National Institute of Medical Research, London, be adopted as international vitamin D standard for the next two years."

Subsequent to the London meeting of the League of Nations the Chairman of the U. S. P. Revision Committee sent a report of the conclusions adopted at the London meeting to the members of the U. S. P. Vitamin Advisory Committee. He then issued invitations for a second Conference on vitamin methods for January 8, 1932. This Conference was held in New York and was attended by thirty-three persons interested in vitamin assay methods. On January 7th your Committee met and gave careful consideration to the various details of vitamin A and vitamin D assay methods. In the light of additional data which had been assembled and recent developments in the knowledge of the nature of vitamin A and D your Committee prepared and recommended the following vitamin A and vitamin D methods for consideration by the special U. S. P. vitamin conference held on January 8th.

ASSAY METHODS RECOMMENDED BY THE AMERICAN DRUG MANUFACTURERS' ASSOCIATION VITAMIN COMMITTEE FOR CONSIDERATION BY THE UNITED STATES PHARMACOPŒIA VITAMIN ADVISORY COMMITTEE, JANUARY 8, 1932.

VITAMIN A AND D ASSAY FOR COD LIVER OIL AND RELATED PRODUCTS.

I. Vitamin A.—Cod liver oil shall be assayed for its vitamin A by the following process or method: It is recommended that a cod liver oil of known potency assayed by the assay method herein described be accepted as a provincial Standard of Reference for vitamin A, and that a selected sample of carotene be held in view as a possible secondary standard. When so assayed, the oil shall contain at least 400 vitamin A units per Gm. or the equivalent in international units.

This assay is based upon the estimation of the minimum amount of cod liver oil necessary to meet specific growth-promoting as well as relative anti-ophthalmic requirements in a standard test animal kept under definite control. The test animals shall be rats from known source and bred preferably under the direction of the experimenter.

The vitamin A potency of the cod liver oil shall be expressed in units per Gm. of oil, the unit to be the minimum daily amount (in mg.) of cod liver oil required to cause, in sixty per cent of the animals in any one group, a gain in weight of at least twelve Gm. within a period of 28 days under the conditions of growth and diet specified in this assay. The maximum weight must be attained at the end of the test, and the eye condition must be corrected by an amount of cod liver oil not to exceed three times the minimum growth factor.

The vitamin A content per Gm. of cod liver oil is computed by dividing one thousand mg. (1 Gm.) by the determined minimum daily amount of oil, in mg., required to induce the requisite growth recovery.

Method for Vitamin A.—It is desirable that each laboratory should establish its own breeding colony or else secure rats under known conditions as to age and dietary history.

In the breeding program, the males and females should be kept on a well-balanced diet which is not too excessive in the vitamins A and D. The following diet has been tried by several laboratories and is suggested:

BREEDERS' DIET.

	Per Cent.
Wheat meal (entire kernel)	33.0
Yellow corn meal (entire kernel)	34.0
Whole milk powder	21.0
Old process linseed oil meal	7.0
Alfalfa leaf flour (green color)	2.0
Liver (vacuum dried)	2.0
Calcium carbonate	0.5
Sodium chloride	0.5

Such diet should be supplemented daily by giving each rat some fresh green leafy vegetable, preferably lettuce or spinach, about five Gm. per animal.

When the females are about one hundred days old, they are mated. The litters are reduced to seven rats each when the young are five to seven days old. On the tenth to twelfth day, the green stuff is discontinued. At weaning (21–23 days old) the mother is taken away and placed on the regular diet.

When the young are 21–26 days of age, weighing 38–45 Gm. they are fed the Vitamin A Free Diet, but adequate in other respects, so as to deplete them for the vitamin A test.

VITAMIN A FREE DIET.

	Per Cent.
Casein (freed from vitamin A)	18
Salt mixture (Osborne & Mendel,[1] or McCollum,[2] No. 185)	4
Agar (finely ground)	2
Yeast (dried) (1)	8

[1] *Jour. Biol. Chem.*, 37 (1919), 572.

[2] *Ibid.*, 47 (1921), 121.

	Per Cent.
Cornstarch	63
Hydrogenated vegetable oil (vitamin free)	5
Vitamin D (2)	
(The water supply should be distilled water)	

Animals fed such a diet should manifest, in due course, one or all of the following: decline in weight, or stationary weight, or xerophthalmia in varying degrees. Tentatively, it has been agreed to accept the weight criteria for the beginning of treatment with cod liver oil (or related products). Preferably, the ophthalmic condition should also be manifest, but in case it is not, then as a check, similar control test rats must show eye symptoms not later than 10 days after starting the treatment. The weight criteria are either a declining or stationary weight for 5 to 7 days. In order to insure more comparable conditions, the beginning of the administration of the cod liver oil should preferably not occur before the 25th day or after the 40th day of the vitamin A free preparatory period.

The distribution of rats, when ready, should be so adjusted that there will be at least six in each group, having one group as a negative control, and that the doses vary from group to group so that one can judge the potency of the oil above and below the unit value sought. To dilute the cod liver oil (or product) use vitamin A and D free peanut or corn oil. Check tests should be made on this diluent from time to time. The dilution should be made so that the dose at any time is not too large to affect the animal's condition. It is suggested that 0.1 cc. be used. The dilution should be made at least once a week, and kept in a cool, dark place. The Standard of Reference should be run parallel with the product under test. The doses should be given at regular intervals, separate from the diet, preferably daily.

A record should be made of the body weights and eye condition at least twice a week. The eye condition may be designated as normal, watery, sensitive to light, edema, bloody exudate, pustules and opacity of cornea.

II. Vitamin D (Antirachitic).—Cod liver oil (or related products) shall be assayed for its vitamin D content by the following method: It is recommended that a solution of irradiated ergosterol of definite potency, or a cod liver oil of known antirachitic potency, as assayed by this method be used as Standard of Reference. When so assayed, the oil shall contain at least 100 vitamin D units per Gm. or the equivalent in international units.

This assay is based upon the estimation of the minimum amount of cod liver oil necessary to initiate a specific degree of recalcification in the leg bones of rachitic rats reared and fed under definite control as specified hereinafter.

The vitamin D potency of cod liver oil shall be expressed in units per Gm. of oil, the unit to be the minimum average daily amount (in mg.) of cod liver oil required to produce, in 60% of the animals in any one group, a degree of recalcification represented by a narrow continuous "line" across the metaphysis of the leg bones of the rats which have been kept and fed under the conditions as specified in the assay.

The vitamin D content per Gm. of cod liver oil is computed by dividing 1000 mg. (1 Gm.) by the determined minimum average daily amount of oil in mg., required to induce the requisite degree of recovery. The average daily dose is understood to be the total amount of cod liver oil given divided by the length of the test period, 10 days.

Method for Vitamin D.—Use rats of a known dietary history as described under vitamin A method. When the rats are 25–30 days old, they should weigh 50–60 Gm. They are then fed either the Steenbock Ricket-Producing Diet, No. 2965, or the McCollum Diet, No. 3143.

> After the experimental animals have been on the rachitogenic diet for a suitable period (18–21 days) examine the leg bones of 4 rats by the "line test" as described later, to ascertain whether a satisfactory stage of rickets has been attained.

NOTES: (1) The yeast should have a vitamin B complex potency such that not more than 150 mg. daily is required for the recovery test on the Sherman and Spohn basal vitamin B-complex ration.

(2) The vitamin D content of the diet, may be derived from any suitable source free from vitamin A, and should be equivalent to the vitamin D content of 3% of the Standard of Reference cod liver oil.

If so, the remainder are considered as satisfactory for the test, and should be distributed into groups of at least 7 animals each, reserving one group as a negative control. The doses should be varied from group to group so that one can judge the potency of the cod liver oil above or below the unit value sought. The Standard of Reference should be run parallel with the product under test.

STEENBOCK RICKET-PRODUCING DIET, NO. 2965.[1]

	Per Cent.
Whole yellow corn (freshly ground)	76
Wheat gluten	20
Calcium carbonate	3
Sodium chloride	1

McCOLLUM RICKET-PRODUCING DIET, NO. 3143.[2]

Corn (yellow)	33
Wheat (soft winter)	33
Wheat gluten	15
Gelatin	15
Calcium carbonate	3
Sodium chloride	1

The rats should receive distilled water.

From the beginning of the administration of the cod liver oil, and the Standard of Reference, the rats should be kept in individual cages and a record should be made of the daily food consumption.

The cod liver oil (or related product) and the Standard of Reference should be diluted with a vitamin A and vitamin D free oil, such as peanut or corn oil, so that the total daily volume of oil consumed by each rat shall not exceed 0.1 cc. These dilutions should be prepared as often as once a week. The dilutions should be fed separate from the diet. The negative control group should receive an equivalent amount of the diluent.

The dilutions should be given over a period of 8 consecutive days. The rats are then continued on the rachitogenic diet for the balance of the ten-day test period to allow for the latent effect.

Any animal which has lost weight continuously during the test period, has eaten less than two Gm. of food on two consecutive days, or has averaged to eat less than four Gm. of food per day should be excluded from the final interpretation.

At the end of the 10th day of the test, the animals should be killed, and the femur and tibia (or ulna and radius) removed from the right leg and preserved in formalin (10%) for examination essentially as follows: rinse in water, split, place in acetone for 3 minutes, dry, treat with silver nitrate (2%) for 3 minutes, place under a bright light until the "line" is clearly evident, immerse in sodium thiosulfate solution (5%) for 3 minutes, and examine under binocular microscope.

Early in the January 8th Conference the U. S. P. Vitamin Advisory Committee agreed to adopt the international vitamin A and vitamin D units recommended at the London Conference. The Vitamin Advisory Committee also agreed to adopt cod liver oil as a Reference Standard for vitamin A assays and a solution of irradiated ergosterol or cod liver oil as a Reference Standard for vitamin D assays—the Reference Standards to be prepared, evaluated and distributed by the Food and Drug Administration, Department of Agriculture, Washington, D. C.

Following these decisions a number of those present contended that since vitamin units and vitamin standards had been adopted there was no occasion for preparing or adopting vitamin methods. When it had been definitely established that it was essential for the Pharmacopœia to contain assay methods the vitamin methods recommended by your Committee were utilized as a basis for consideration in the preparation of official U. S. P. methods. Early in the discussion of vitamin methods it was decided not to include in the Pharmacopœia methods any details concerning the nature or management of the breeding colony from which the experimental animals would

[1] *Jour. Biol. Chem.*, 64 (1925), 263.

[2] *Ibid.*, 47 (1921), 507.

be obtained. Much discussion developed concerning the essential details of official vitamin methods. Eventually, however, vitamin A and vitamin D methods were prepared for recommendation to the U. S. P. Revision Committee. These methods which have not yet become official are very similar in form and substance to the vitamin A and vitamin D methods reported above.

During the year which has elapsed since your Committee's last report the Executive Committee of the American Drug Manufacturers' Association has voted that the activities of your Committee which have been confined to a consideration of vitamin A and D methods are extended to include a study of methods of assay of other vitamins. Obviously this action is to the interest of the American Drug Manufacturers' Association since a number of its members are manufacturing and distributing products containing vitamins other than vitamin A and vitamin D. It is needless to say that it is desirable in such instances that the member be in a position to make claims for the vitamin activity of his product and that he be in a position to substantiate his claims by submitting the results of a vitamin assay of his product made by approved or official methods.

It is the feeling of your Committee that very definite progress has been made during the current year. The Committee feels that it has contributed in no small way to the development of forthcoming U. S. P. Assay methods for vitamins A and D, and it desires at this time to express its appreciation of the support and encouragement which the American Drug Manufacturers' Association has constantly extended to it.

NEW JERSEY ANNOUNCEMENT REGARDING THE FOUR-YEAR COURSE IN PHARMACY.

Secretary Robert P. Fischelis announces that the Board of Pharmacy of the State of New Jersey desires to call attention to the following resolution bearing on the type of four-year course which will be approved by this Board as meeting the college graduation requirement. This resolution was passed at a meeting of the Board held in Trenton, March 1, 1932.

WHEREAS the Board of Pharmacy of the State of New Jersey on September 25, 1928, adopted a resolution making it mandatory for all applicants for registration as pharmacists in New Jersey who begin their college course in pharmacy any time after January 1, 1932, to submit proof of graduation from a four-year course in a College of Pharmacy approved by this Board; and

WHEREAS the Board of Pharmacy of the State of New Jersey on January 27, 1930, adopted a resolution declaring it to be the sense of this Board that approval of the four-year course in Pharmacy shall be confined to such courses and institutions as will recognize the need for fundamental training in Physics, Chemistry and Biology as a basis for the course in Pharmacy, as well as the need for the addition of so-called cultural subjects to the curriculum, such as English, some phases of History, Economics and one or more Modern Languages, and

WHEREAS the time has arrived for a more specific statement as to the character of the four-year course in Pharmacy to be approved by the Board of Pharmacy of the State of New Jersey, be it

Resolved, that the following rules and regulations, in addition to the general rules for approval of courses in Pharmacy promulgated by this Board, July 1, 1926, shall govern the approval of four-year courses in Pharmacy to be accepted by this Board as meeting the college requirement under the New Jersey Pharmacy Act:

1. Courses totalling less than 800 clock hours of instruction per college year for each of the four years of the course and which do not include at least 40% of laboratory work (in clock hours) will not be approved.

2. Courses offered on a three- or four-day per week schedule will not be approved.

3. Courses leading to any other degree than a fully recognized Bachelor of Science degree of University grade will not be approved.

4. Courses in which a degree such as Ph.G. or Ph.C. is awarded at the completion of the third year of the course to be followed by an additional year leading to a Bachelor's degree will not be approved.

5. The type of four-year course which is most apt to receive approval of this Board will be a well-rounded course in which Pharmacy will play the predominating rôle. Courses which are worked out on the basis of a Freshman year of general college work and which actually amount to a three-year course in pharmacy based upon four years of high school work and one year of a liberal arts or science college course will be approved for the time being; but courses in which the