

COMMITTEE REPORTS

REPORT OF THE VITAMIN ASSAY COMMITTEE OF THE AMERICAN DRUG MANUFACTURERS' ASSOCIATION—NINETEENTH ANNUAL MEETING—APRIL 1930.

BY DR. ARTHUR D. HOLMES, CHAIRMAN.

Historical.—The Vitamin Assay Committee was created at the 1927 Convention of the American Drug Manufacturers' Association and provision was made that it should function as a Sub-Committee of the Scientific Section. The members of the original Vitamin Assay Committee were Eli Lilly & Co. (Dr. H. W. Rhodehamel); Parke, Davis & Co. (Dr. A. D. Emmett); E. R. Squibb & Sons (Mr. H. A. Holaday); and the E. L. Patch Company (Dr. A. D. Holmes, Chairman). The first meeting of the Committee was held in Detroit, September 1927. Dr. E. H. Volwiler, Chairman of the Scientific Section, and all members of the Sub-Committee were present.

At the first conference of the members, a laboratory procedure for the study of the U. S. P. X vitamin A method was agreed upon. After several months of intensive work, the Committee met in New York and correlated and summarized the results that had been obtained. A report of those results was presented before the Scientific Section at the American Drug Manufacturers' Association Convention, April 1928. In accepting the report, the Association made provision for the continuance of the Committee and increased the scope of its activities to include a study of method for assaying cod liver oil for vitamin D. The association also accepted the recommendation of the Committee to increase the minimum vitamin A (U. S. P. X) value from 50 to 250 units per Gm.

At this time, the Committee felt that it was desirable to correlate its activities with the investigations of the officials of the Drug Control Laboratories of the Food and Insecticide Administration. Accordingly, a conference of the Committee and the Drug Control officials was held in Washington, July 1928. A program of research was outlined which was to be carried on by the members of the Vitamin Committee and by one or more of the Federal Laboratories. Unfortunately, due to lack of appropriations, the Federal Laboratories were unable to cooperate.

In order to coordinate the interest of as large a group as possible in this project, the Contact Committee invited the Vitamin Assay Committee to attend its December 1928 meeting in Washington. The Vitamin Committee summarized its investigations to that date and at the request of the Contact Committee reported the nature of its investigations to the three organizations constituting the Contact Committee, namely: The American Drug Manufacturers' Association, The American Pharmaceutical Manufacturers' Association and the officials of the Drug Control Laboratories. At this time the Contact Committee voted that beginning with September 1929, all member firms of the A. D. M. A. and the A. Ph. M. A. adopt uniform statements for expression of the Vitamin A content of cod liver oil on labels, namely, units per Gm. of oil.

Investigations were continued until April when the Committee met at Columbus, Ohio, to coordinate the results obtained by the various laboratories represented. A report of the year's investigation was submitted at the Annual Meeting held in Asheville, N. C., April 1929. Inasmuch as the results that had been obtained indicated the need for further study, the Association reappointed the Vitamin Assay Committee. Subsequently, the Frederick Stearns Company kindly offered to cooperate and a representative of their Research Laboratory (Mr. R. L. Jones) was added to the Committee.

At the Annual Meeting, the Association authorized the Committee to solicit assistance from any member firms who were in a position to contribute to this problem. Thereupon, the Committee addressed a letter to member firms maintaining Research Laboratories equipped for vitamin studies. As a consequence, the Abbott Laboratories (Mr. C. Nielsen) has been associated with the Committee during the past year.

Discussion.—As the investigation progressed, it became evident that certain modifications should be made in the above-outlined procedure. Due to the extensive study that has been made by this committee during the past three years on the development of a better vitamin A method and the formation of a vitamin D method, the changes introduced have been felt desirable and of a definite constructive nature. In other words, as a result of our efforts, at the final conference of the Vitamin Assay Committee, which was attended by representatives of all labora-

tories participating in the investigation, it was agreed that the procedure which is outlined below be recommended as a satisfactory method for determining the vitamin A and vitamin D content of cod liver oil and related products. Accordingly, your Vitamin Assay Committee suggests the adoption of the following vitamin A and vitamin D methods realizing, of course, that as time goes on, further modifications will be necessary.

VITAMIN A AND D ASSAY FOR COD LIVER OIL AND RELATED PRODUCTS—APRIL 1930.

I. Vitamin A.—Cod liver oil may be assayed for its vitamin A content by the following process or method. When so assayed the oil shall contain at least 250 vitamin A units per Gm.

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 antiophthalmic requirements in a standard test animal kept under definite control as regards source, age and food supply. The test animal shall be albino rats from known source and bred preferably under the direction of the experimenter.

The vitamin A potency of 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 15 Gm. within a period of thirty-five 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 1000 mg. (1 Gm.) by the determined minimum daily amount of oil, in mg., required to induce the requisite growth recovery.

Method.—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.¹

To obtain suitable test rats for vitamin A and D assay, females from the colony should be used to rear the actual test animals. These females should be taken off the colony diet as soon as weaned and fed the Breeders' Diet below. Later, they are placed on a restricted diet as stated elsewhere.

BREEDERS' DIET.

Wheat meal (entire kernel)	33.0%
Yellow corn meal (entire kernel)	33.0%
Whole milk powder	21.0%
Old process linseed oil meal	7.0%
Alfalfa leaf flour (green color)	4.0 %
Calcium carbonate (CaCo ₃)	0.5%
Sodium chloride (NaCl)	0.5%

¹ The following well-balanced diet, rich in vitamins, has been found satisfactory as a colony diet:

COLONY DIET.

Wheat meal (entire kernel)	33.0%
Yellow corn meal (entire kernel)	33.0%
Whole milk powder	21.0%
Old process linseed oil meal	7.0%
Alfalfa leaf flour (green color)	2.0%
Liver (boiled and dried)	2.0%
Calcium carbonate (CaCo ₃)	0.5%
Sodium chloride (NaCl)	0.5%
Cod liver oil (of high vitamin assay)	1.0%

The ingredients of all diets should be ground fine enough to insure a uniform mixture. The diet, with the exception of the whole milk powder and the cod liver oil, may be mixed in large quantities and the milk powder and cod liver oil should be added to such portions as will be consumed in about one week.

This Colony Diet must be supplemented daily by the addition of fresh green leafy material. An approximate allowance of about 5 Gm. daily per rat of this material should be sufficient. Boiled bone or other hard material should be supplied occasionally for gnawing.

This diet must be supplemented daily by the addition of fresh green leafy material preferably lettuce or spinach, about five Gm. being sufficient per rat per day.

When about 100 days old, the females are mated with males which are continuously maintained on the Breeders' Diet. When the young are five to seven days old, the litters should be adjusted to seven animals for each female. On the tenth to twelfth day the mothers are given the Vitamin Restricted Diet, without any green stuff.

VITAMIN RESTRICTED DIET.

Wheat meal (entire kernel)	33.0%
Yellow corn meal (entire kernel)	34.0%
Skimmed milk powder	21.0%
Old process linseed oil meal	7.0%
Yeast (dried)	4.0%
Calcium carbonate	0.5%
Sodium chloride	0.5%

Later when these rats are weaned (21-23 days old), the mothers are then returned to the Breeders' Diet (not the Colony Diet). The young are continued on the Vitamin Restricted Diet until they are twenty-five to twenty-nine days old at which time they should weigh thirty-eight to forty-eight Gm. They are then fed the Vitamin A Free Diet so as to deplete them for the vitamin A test.

VITAMIN A FREE DIET.

Casein (Vitamin A Free)	18%
Salt mixture (Osborne-Mendel or Mc-	
Collum No. 185)	4%
Agar (finely ground)	2%
Yeast (dried) (1)	8%
Dextrinized corn starch	63%
Hydrogenated vegetable oil (Vitamin	
A Free)	5%
Vitamin D (2)	

(1) The vitamin "B" value of the yeast should be such that not more than 150 mg. of the yeast is required for a daily-rat-weight recovery dose when animals are on the Sherman Basal Vitamin B Free Ration.

(2) In order to insure sufficient antirachitic activity, one-fourth of the yeast should be irradiated before it is incorporated in the diet. To accomplish this, the yeast in a layer not exceeding one-sixteenth of an inch in depth is irradiated for fifteen minutes at a distance of 12 or 18 inches with a quartz mercury vapor lamp. The yeast should be stirred during exposure. If desired, this antirachitic factor may be supplied by other materials of known potency.

VITAMIN A TEST.

The animals should be fed the Vitamin A Free Diet until there is either a decline in weight or a stationary weight for at least seven days. Preferably xerophthalmia should be manifest at this time but declining weight or stationary weight for seven days and definite eye conditions do not necessarily occur simultaneously. In any case, the weight criteria should be used for the administration of cod liver oil to the test animal. As a check, the control test rats must show both symptoms not later than 10 days after the administration of the cod liver oil to the test animals has begun. In order to insure more comparable conditions the beginning of the administration of the cod liver oil should preferably not occur before the 30th day or after the 45th day of the vitamin A free preparatory period.

The experimental animals should be divided into groups of six rats each, one group to serve as a control and the others to be treated with varying amounts of the cod liver oil under test, each animal within the respective groups being given the same dosage.

Cod liver oil being so potent, should be diluted with a vitamin A and D free oil such as peanut oil or corn oil. The total daily volume of the diluted oil for each rat shall be 0.1 cc. This

diluted cod liver oil should be fed separate from the diet. The control animals should receive 0.1 cc. daily of the oil used as a diluent. The time for its administration should be judged by the same criteria as that for the cod liver oil.

The experimental test period should be of thirty-five days' duration. Any animal that dies in less than two weeks after the beginning of treatment should not be included in judging the results of the test. A record should be made three times a week of the body weight and the condition of the eyes during the test period. The condition of the eyes may be recorded as normal, watery, sensitive to light, œdema, bloody exudate, pustules and opacity of cornea.

*II. Vitamin D (Antirachitic).—*Cod liver oil may be assayed for its vitamin D content by the following method. When so assayed the oil shall contain at least 60 vitamin D units per Gm.

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.

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 sixty per cent 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, ten days.

Method.—Experimental animals for vitamin D test, obtained from mothers which have been reared and bred on the Breeders' Diet (see page 617) are fed the Vitamin Restricted Diet until they are twenty-eight to thirty-two days old and weigh fifty to sixty Gm. They are then fed the Steenbock Ricket-Producing Diet, No. 2965, or the McCollum Ricket-Producing Diet, No. 3143.

STEENBOCK RICKET-PRODUCING DIET, No. 2965.

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

MCCOLLUM RICKET-PRODUCING DIET, No. 3143.

Corn (yellow)	33%
Wheat (soft winter wheat)	33%
Wheat gluten	15%
Gelatin	15%
CaCO ₃	3%
NaCl	1%

During the period of preparation for the test and while on test the animals should receive distilled water.

After the experimental animals have been on the rachitic diet for a suitable period (18 to 21 days) examine four rats, by the "line test" as described later, to see what stage of rickets has been attained. If these animals are found to have developed definite rickets, the remainder are considered as satisfactory for the test and should be divided into groups of seven animals each. It is desirable to have at least four groups, one as control, and three for treatment with the cod liver oil in varying doses so that one can judge the potency of the oil above and below the unit value sought.

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

The cod liver oil should be diluted with a vitamin A and D free oil such as peanut oil or corn oil so that the total daily volume of oil consumed by each rat shall be 0.1 cc. The oil should be fed separate from the diet. A control group of representative animals should receive 0.1 cc. daily of the diluent.

The diluted cod liver oil (or the diluent) should be given for eight consecutive days. The rats are continued on the rachitic diet for two remaining days of the test period to allow for the latent effect of the cod liver oil.

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 conclusion of the tenth day of the test, the animals should be killed and the femur and tibia bones (or ulna and radius) removed from the right leg and preserved in formaldehyde (10%) for examination. When ready for staining, the bones should be thoroughly rinsed in water, split, placed in acetone for three minutes, dried on a blotter, placed in silver nitrate (2%) three minutes, intensified under bright light until the "line" is clearly evident, placed in sodium thiosulfate solution (5%) for three minutes and examined under a microscope. Reserve the bones from one other leg in formaldehyde for possible confirmation test.

INVESTIGATIONS CONDUCTED DURING THE CURRENT YEAR.

Representatives of the six laboratories, assisting in the study of Vitamin Assay Methods, met at Detroit, March 1930, summarized and correlated the results which have accumulated during the current and preceding years.

The Committee feels that it would be unwise at this time to undertake to discuss in detail all the scientific studies that have been conducted in connection with the investigation of methods for vitamin A and vitamin D assay of cod liver oil. On the other hand, the Committee feels very strongly that the members of the Association should be informed concerning the general nature of the investigation and the extent to which attention has been given to the numerous details that have a definite bearing on the efficiency of the methods of assay which have been developed. Accordingly, it is proposed to discuss briefly the investigation that the Vitamin Committee has conducted.

PROGRAM FOR THE FURTHER STUDY (1929-1930) OF METHODS TO DETERMINE THE VITAMIN A AND VITAMIN D CONTENT OF COD LIVER OIL AND RELATED PRODUCTS.

I. Study of Methods for Vitamin A Assay.

Obviously in a study of this character, it was desirable that the experimental conditions in all laboratories should be as nearly identical as possible. Thus it was recommended that each laboratory should establish its own breeding colony from a common stock and that this colony should be maintained on a balanced diet rich in vitamin A and vitamin D. It was next proposed that in order to obtain gradual depletion of vitamins, the young females obtained from this colony should be used to rear the rats intended as test animals. It was provided that these females should be reared and maintained on a Breeders' Diet. The Breeders' Diet was standardized for all laboratories participating. The composition of the Breeders' Diet was as follows:

BREEDERS' DIET (1929-A).

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)	4.0%
Calcium carbonate (CaCO ₃)	0.5%
Sodium chloride (NaCl)	0.5%

NOTE: The ingredients should be ground fine enough so as to insure a uniform mixture.

This diet must be supplemented daily by the addition of fresh green leafy material such as lettuce, clover or spinach. Lettuce is preferable. About five Gm. daily per rat of this material should be sufficient.

In order to start breeding colonies from common stock, each laboratory arranged to purchase male and virgin female rats approximately five weeks old from the same source. These animals were fed the Breeders' Diet until approximately 100 days old when they were mated. In order that the size of litters should be comparable for the different laboratories, it was agreed

that when the young were five to seven days old, all litters should be adjusted to seven animals for each female. In order that the young should not have an opportunity to partake of the Vitamin A and D Rich Breeders' Diet, it was arranged that when the young were ten to twelve days old, the diet of the mothers should be changed by replacing the Breeders' Diet with a diet designated as the Vitamin Low Diet. Its composition was as follows:

VITAMIN LOW DIET (1929-M).

Wheat meal (entire kernel)	33.0%
Yellow corn meal (entire kernel)	34.0%
Skimmed milk powder	21.0%
Old process linseed oil meal	11.0%
Calcium carbonate	0.5%
Sodium chloride	0.5%

When the young were ready for weaning, the mothers were removed from the young and returned to the Breeders' Diet. The young were continued on the Vitamin Low Diet until they were twenty-five to twenty-nine days old. At this time a record was made of their weight and they were transferred to a diet designated as Vitamin A Free Diet which consisted of the following ingredients:

VITAMIN A FREE DIET (1929-B).

Casein (extracted, Vitamin A Free)	18%
Salt mixture (Osborne-Mendel or Mc-Collum No. 185)	4%
Agar (finely ground)	2%
Yeast (high vitamin assay ¹)	8%
Starch (corn autoclaved)	63%
Crisco	5%

METHOD OF VITAMIN A ASSAY OF COD LIVER OIL.

From the twenty-fifth to the twenty-ninth day, the experimental animals were maintained on the Vitamin A Free Diet until symptoms of vitamin A malnutrition developed. A record was made concerning the date of occurrence of xerophthalmia, and the date when the animals arrived at stationary or declining weight. A careful record was made of the nature of the eyes at that time. In making the vitamin A test, the experimental animals were divided into eight groups of six animals each and subjected to the following procedure:

1st group (pathological controls) received the Vitamin A Free Diet with the addition of 0.1 cc. peanut oil daily.

2nd group received the Vitamin A Free Diet (1929-B) supplemented with 1.2 mg. cod liver oil daily.

3rd group received the Vitamin A Free diet (1929-B) supplemented with 1.6 mg. cod liver oil daily.

4th group received the Vitamin A Free Diet (1929-B) supplemented with 2.0 mg. cod liver oil daily.

5th group received the Vitamin A Free Diet (1929-B) supplemented with 2.5 mg. cod liver oil daily.

6th group received the Vitamin A Free Diet (1929-B) supplemented with 3.2 mg. cod liver oil daily.

¹ The vitamin value of the yeast should be such that not more than 150 mg. of the yeast is required for a daily recovery dose. In order to insure vitamin D activity, one-fourth of the yeast may be irradiated before it is incorporated in the diet. To accomplish this, the yeast in a layer not exceeding one-sixteenth of an inch in depth is irradiated for fifteen minutes at a distance of 12" or 18" with ultraviolet light produced by a mercury vapor quartz lamp. There should be some slight movement of the yeast during exposure. Or if desired, this vitamin D may be supplied by vitamin D products of known potency.

7th group received the Vitamin A Free Diet (1929-B) supplemented with 4.0 mg. cod liver oil daily.

8th group received the Vitamin A Free Diet (1929-B) supplemented with 5.0 mg. cod liver oil daily.

The same composite sample of cod liver oil (from a common source) was used by all laboratories for the vitamin A studies. It was desired that each experimental animal should receive the same amount of oil. Accordingly, it was provided that the cod liver oil should be diluted with a vitamin A and D free oil such as peanut oil or corn oil so that the total daily volume of oil consumed by each rat was 0.1 cc. The diluted oil was fed separate from the diet. The experimental period, as in the U. S. P. X method, was of thirty-five days' duration. However, observations were made at the end of twenty-eight days to ascertain whether a twenty-eight day period would be equally as efficient as a thirty-five day period. A record was made three times a week of the body weight and eye conditions of the animals during the experimental period. It was suggested that the condition of the eye should be reported as normal, watery, sensitive to light, œdema, bloody exudate, pustules and opacity of cornea.

VITAMIN A UNIT.

It was agreed that the vitamin A potency of cod liver oil should be expressed in the same manner as in the U. S. P. X method, namely, in units per Gm. of oil. The vitamin A unit was assumed to be the minimum daily amount of cod liver oil required to cure induced symptoms of vitamin A starvation in young albino rats and to cause a gain in weight of from ten to twenty Gm. within a period of thirty-five days specified in this assay. The vitamin A content of cod liver oil was computed in terms of units per Gm. by dividing 1000 mg. (1 Gm.) by the determined minimum (in mg.) required daily growth recovery dose of oil as indicated by at least four rats out of six.

II. Study of Methods for Vitamin D Assay.

The experimental animals used by the different laboratories for making the vitamin D study were reared as described under the vitamin A assay method. They were continued on the Vitamin Low Diet until they were twenty-eight to thirty-two days of age and presumably at that time weighing fifty to sixty Gm. Then they were transferred to the Steenbock Ricket-Producing Diet, No. 2965 or to the McCollum Diet, No. 3143.

STEENBOCK RICKET-PRODUCING DIET, No. 2965.

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

MCCOLLUM RICKET-PRODUCING DIET, No. 3143.

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

During the period of the test the animals received distilled water. When the experimental animals had been on the rachitic diet twenty-one days, they were examined to determine the stage of rickets attained. If the experimental animals had developed definite rickets, the remaining rats were considered as satisfactory for the test. These were divided into groups of seven animals each and were given cod liver oil in accordance with the following procedure.

1st group (four animals-controls) received Steenbock Diet No. 2965 supplemented with 0.1 cc. peanut oil daily for six consecutive days.

2nd group received the rachitic diet supplemented with 7.0 mg. cod liver oil daily for six consecutive days.

3rd group received the rachitic diet supplemented with 9.0 mg. cod liver oil daily for six consecutive days.

4th group received the rachitic diet supplemented with 11.0 mg. cod liver oil daily for six consecutive days.

5th group received the rachitic diet supplemented with 14.0 mg. cod liver oil daily for six consecutive days.

The experimental animals were kept in individual cages and a record was made of the daily food consumption of each animal.

All laboratories used the same cod liver oil for all vitamin D studies. The cod liver oil was diluted with a vitamin A and D free oil such as peanut oil or corn oil so that the total daily volume of oil consumed by each rat was 0.1 cc. The diluted oil was fed separate from the diet. The diluted oil was given for six consecutive days and the rates were then continued on the rachitic diet for four additional days. (Incidentally, various laboratories modified this procedure so that information was accumulated concerning the administration of oil for different periods.) Any animal that lost weight continuously; that ate less than two Gm. of food during two consecutive days; or that averaged to eat less than four Gm. per day was excluded from the test.

At the conclusion of the tenth day of the experimental period, the animals were killed and the femur and tibia bones (ulna and radius) were removed from the right leg and preserved in formaldehyde (10%) for examination. When ready for staining, the bones were thoroughly rinsed in water, split, placed in acetone for three minutes, dried on a blotter, placed in silver nitrate (2%) three minutes, intensified under bright light, placed in sodium thiosulphate solution (5%) for three minutes and examined under a microscope. The bones from another leg were preserved in formaldehyde for possible confirmation test.

VITAMIN D UNIT.

It was agreed that the vitamin D potency of cod liver oil should be expressed in units per Gm. The unit was defined as the minimum average daily amount of cod liver oil required to produce a continuous narrow line across the metaphysis of the leg bones in four out of six rats of each group prepared under the conditions specified in this assay.

The average daily dose was determined by taking one-tenth (number of days in experimental period) of the total amount of cod liver oil given.

The vitamin D content of cod liver oil was computed in terms of units per Gm. by dividing 1000 mg. (1 Gm.) by the determined minimum (in mg.) average daily corrective dose of oil.

THE FOURTH ANNUAL SYMPOSIUM OF THE GENUS MENTHA.

F. J. BACON, CHAIRMAN.*

The Fourth Annual-Symposium on the Genus *Mentha* was held May 8th at the Emerson Hotel, Baltimore. The Symposium is gaining more and more recognition in the scientific group. This year the attendance numbered about thirty men interested in the subject of mints. Representatives from all the mint-growing sections of the United States were present.

This Symposium was originated in 1927 at St. Louis for the purpose of studying at a round-table discussion all problems connected with the scientific, technical and trade aspects dealing with mints.

At the fourth meeting of the group the following papers were presented and discussed:

1. "Cytological and Genetical Studies on the Genus *Mentha*," by Mabel Louise Ruttle, Geneva Experiment Station, Geneva, New York. Eleven species of the genus *mentha* were collected in Germany and England and examined cytologically. The chromosome number of the pollen-mother-cell, egg-mother-cell and the root tips was determined. The results of the work will probably offer a scientific explanation of the confusing number of forms and types found in the mints.

2. "Another Question about Mints," by B. V. Christensen, University of Florida. A supposedly true type of *Mentha biperita* L. grown in the Florida medicinal plant garden in 1929 yielded

* Professor of Pharmacognosy, Western Reserve University, Cleveland, Ohio.