A COMPARISON OF SPECTROPHOTOMETRIC AND BIOLOGICAL ASSAYS FOR VITAMIN A.*

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A great many articles have appeared in the literature concerning the various advantages and comparative accuracy of the colorimetric, spectrographic and biological methods of assays for Vitamin A. However, attempts to confirm some of the published data by collaborated studies have met with only partial success.

As Vitamin A is a nutritional factor, it is the general opinion, that the logical measure of Vitamin potency is the biological response as determined by biological assay. The present U. S. P. Method of Assay for Vitamin A is a decided improvement over previous biological methods. Nevertheless, the inaccuracy due to variance in biological response, and other difficulties encountered in conducting a biological assay, as well as the time required and the expense involved, are sufficient reasons for the development and adoption of a satisfactory alternate for the biological assay.

Having available extensive facilities for conducting biological assays and a considerable experience in their interpretation, together with a large variety of Vitamin A bearing substances, physical equipment was installed so that this laboratory would be in a position to make the necessary correlations of biological assays with the physical estimations.

British and other workers claim that the Vitamin A content of Cod Liver Oil and other Fish Liver Oils can be determined by means of the absorption at 3280 Angstrom units. In view of these claims, we have conducted a comprehensive series of examinations of Cod Liver Oil, Fish Liver Oils and their Concentrates.

SPECTROMETRIC STUDIES.

Methods and Apparatus.—The results obtained in British Laboratories with the Hilger Vitameter were so promising and aroused so much interest that several laboratories in the United States purchased and studied this instrument. The Vitameter is supposed to be a simplified optical absorption meter for measuring the absorption at 328 millimicrons or 3280 Angstrom units. However, it was difficult to establish conditions which would insure any degree of accuracy in the readings obtained with this instrument. Certain inherent faults of the Vitameter caused it to give E values which could not be checked satisfactorily with results obtained in other places, or in repeated analyses of the same specimen. Therefore, it was decided to obtain a quartz spectrograph and place it in comparison with the Vitameter.

The Hilger small quartz spectrograph with "Spekker" photometer was chosen because of its quality of optical and mechanical design, its comparatively low cost and the facilities it offered in regard to convenience in operation and conservation of time in obtaining results. A quartz Direct Current mercury vapor arc was used to

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test from time to time the accuracy of the wave-length scale of the Spectrophotometer. A Bausch and Lomb photoelectric densitometer was installed to correct visual dotting of absorption curves and thus to make more accurate readings of the entire ultraviolet absorption curve of the plates. The densitometer was supplied with a Julius suspension for the Leeds and Northrop galvanometer, to minimize effects of vibration.

The work was housed in a building separated from factory operations, for it was felt that there was too much vibration in the main laboratory building for proper maintenance of adjustment of the delicate physical instruments. As it was found that large fluctuations of temperature, such as the diurnal changes of summer temperatures, caused serious aberrations of results, an air conditioning system was installed to insure a constant temperature of 20° C. (68° F.), the point for which all volume apparatus is calibrated.

Photographic Methods.—The plates used were Eastman 33 (Process) Plates. The developer was an Elon-Hydroquinone formula recommended by the Eastman Kodak Laboratory. Simple tray development was standardized as to time and temperature (4 minutes at 20° C.). The fixing bath was the chrome alum bath described in Eastman Kodak Company literature. Two plates were usually taken for each specimen. A first plate covered density settings of the photometer from 0.0 to 1.5 by steps of 0.1. This gave a plate from which the absorption curve of the material in the ultraviolet could later be read if desired. Finding on this plate (wet) the nearest to a matching strip, a second plate was taken covering densities 0.1 above and below this strip, with photometer settings 0.02 or 0.03 apart. From this 2nd plate the matching density at 3280 Angstrom units was determined.

Calibration of the Instrument.—The Spekker photometer was standardized by comparing the value obtained at 3130 Angstrom units for standard potassium chromate solution (read against distilled water in the standard cell) with the molar extinction at 3130 recorded in the literature (1).

Choice of Solvent.—The use of either ethyl alcohol or cyclohexane has been recommended for making dilutions in this type of work. Experiments were conducted to compare these solvents with isopropanol. Unsaponifiable fractions obtained by the A. O. A. C. method from U. S. P. Reference Oil were diluted with the three different solvents. Readings obtained immediately after dilution were compared with results obtained after allowing the solutions to stand at 20° C. for 24 hours or longer. Initially, all the solvents gave approximately the same results on any one instrument (Vitameter or Spectrophotometer). On standing, there is a definite decrease in the E value in cyclohexane and ethyl alcohol solutions. However, in isopropanol solutions, there is no diminution in the E value during one week's time. Dilute solutions of cod liver oil concentrates have been kept in this solvent for over a month without loss of E value. Isopropanol transmits a satisfactory band of light to 2300 Angstrom units.

It was found necessary to use 99% isopropanol since more dilute grades would not dissolve some of the samples tested. In view of the above-mentioned advantages and the low cost, this solvent was adopted as a diluent in all determinations.

Preparation of the Specimens.—It was the consensus of opinion that, in cases of oils which contain less than 10,000 Vitamin A units per Gm., the E value of the oil

should be determined on the unsaponifiable fraction. However, in cases where the determinations are made on the whole oil, approximately one Gm. of the oil is weighed directly into a 100-cc. volumetric flask and diluted to volume with 99% isopropanol. The E value is then determined on the proper aliquot dilution.

The method of saponification adopted was a slight modification of the procedure recommended by F. W. Irish (Associate Referee of the A. O. A. C.) which is as follows: Two grams of oil are saponified with 20 cc. of $N/2$ freshly prepared alcoholic KOH, by boiling until clear on a water-bath (time 7–10 minutes); forty cc. of distilled water are added, part being poured down the reflux condenser, the solution cooled, transferred to a Squibb type separatory funnel and extracted with two 50-cc. quantities of peroxide-free ether. The ethereal extracts are washed first with two 20-cc. portions of distilled water, then with two 20-cc. portions of $N/2$ KOH and again with two 20-cc. portions of water while rotating gently without shaking. Patience must be exercised to wait for breaking of any emulsion at the interface. The ethereal solution is then shaken thoroughly with two 20-cc. portions of distilled water, after which it is filtered through a dry paper into a flask, transferred with rinsings with ether into a distilling bulb in a bath not over 54° C. (130° F.) and the ether evaporated nearly to dryness. The residue is immediately dissolved in 99% isopropanol,[1] transferred with rinsing to a 100-cc. volumetric flask and made to volume. From this dilution representing two Gm. of the initial specimen as weighed out, aliquots are taken with accurately calibrated pipettes, to make the proper dilutions for satisfactory reading on the particular instrument in use.

The Vitameter reading was always taken first, ten observations being averaged, the eye error of the operator added and the $E^{1\%}_{1\,cm.}$ calculated from the optical density and the percentage strength of the dilution. The Vitameter read visually was found to give reasonable results only within the range 0.60 to 0.80 of density (without eye error). From the results obtained with the Vitameter, an estimate can be made as to the proper dilution to employ for the Spectrophotometer. The latter instrument gives most accurate readings at a density of 1.0 to 1.2. The calculations of the $E^{1\%}_{1\,cm.}$ are made in the same manner as for the Vitameter. For the above-mentioned reasons, it is our regular procedure to obtain a Vitameter E value and a Spectrophotometer E value for each specimen examined. The equivalent in biological U. S. P. units is calculated by multiplying the E values by the proper conversion factor for each instrument.

Determination of the U. S. P. Reference Oil Conversion Factors.—Ten different samples of the U. S. P. Reference Oil were saponified and the E values determined on the unsaponifiable fraction according to the method given above. The ten spectrophotometer and ten vitameter results were averaged as shown in Table I. The quotients obtained by dividing 3000 (the official number of units present in U. S. P. Reference Oil) by the E values obtained on each instrument are the conversion factors employed, namely, 1948 for the Vitameter and 2222 for the Spectrophotometer. As may be noted in the table, the two instruments do not give the same result.

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[1] The high coefficient of expansion of isopropanol makes temperature control an important factor.

TABLE I.—U. S. P. REFERENCE OIL FOR VITAMIN A, $E^{1\%}_{1\ cm.}$ AND FACTORS.

Vitameter.	Spectrophotometer.
1.51	1.34
1.54	1.35
1.50	1.39
1.52	1.35
1.52	1.32
1.54	1.33
1.57	1.32
1.54	1.32
1.56	1.37
1.59	1.37
1.54 = Average	1.35 = Average
3000/1.54 = 1948 = Vitameter factor.	3000/1.35 = 2222 = Spectrophotometer factor.

Difficulties Encountered with the Vitameter.—The chief difficulty with this instrument is that the filter is not truly a monochromator. It transmits a band of light ranging from about 2900 to 3900 Angstrom units. This is true in spite of the fact that its own Copper arc light source contributes strongest intensity at 3247 and 3274 Angstrom units.

The readings are markedly affected by the changing intensity of light due to the flickering of the arc. This flickering was reduced somewhat by installing a voltmeter in the circuit, maintaining constant voltage and setting the arc gap to the proper distance. The Vitameter is also affected by the aging of the filter, the uviol glass filter cover, the uviol glass cell walls, and the compensating glass. This condition may account for the variation in results obtained in different laboratories. In view of the above, it is obvious that the Vitameter cannot record the same absorption as does the Spectrophotometer at 3280 Angstrom units, as shown in Table I. This same deviation is shown when the Vitameter Standard Glass is measured in the Spectrophotometer. After observing the frequency of false results with the Vitameter, we decided to employ it only as an instrument for first approximation, for determining the correct dilution which will be in the readable range of the spectrophotometer, and thus save spectrum plates and time.

Advantages of the Spectrophotometer.—The Spectrophotometer gives results which can be confirmed in other laboratories. The Spectrophotometer plates afford a permanent record of the determination. Limits of error for determinations by this method at various levels of E values are cited in the 1936 Addendum to the British Pharmacopœia of 1932 (page 91). In repeated determinations of the same specimens, this laboratory has obtained results, which check with a maximum deviation of $\pm 4\%$.

Ultraviolet Absorption Curves of Various Fish Oils and Concentrates.—From the plates, we have read the absorption curves in the ultraviolet for various fish oils and concentrates. There was observed in the curves of Concentrates, or unsaponifiable fractions, a low point, or minimum of absorption around 3750 Angstrom units. Some fish oils read as whole oils show a plateau around the region of 3750 Angstrom units, while others, supposed to be from the same species, show a minimum. Occasionally, the curve of a whole oil will rise toward the far ultraviolet with no depression in the curve but only a slight inflection.

In many instances, the peak is not at 3280 Angstrom units, but may shift as far in the ultraviolet, as 3180 Angstrom units.

The maximum of absorption at the peak may be as much as 10% higher than the reading at 3280 Angstrom units. In view of the specific instructions given in the official methods of the League of Nations Vitamin Conference Committee and the British Pharmacopœia Addendum, that the absorption should be measured at the wave-length of 3280 Angstrom units, all our determinations were made at this specified wave-length.

The Vitameter E values and the Spectrophotometer E values of approximately 1000 specimens (representing a large variety of Vitamin A bearing substances) have been determined in this laboratory. However, in this particular study, we have only considered the results obtained with the specimens, the Vitamin A potencies of which were determined by means of both the Spectrophotometer and Biological assays. Furthermore, the only Spectrophotometric determinations considered were those conducted subsequent to the period of experimentation and after all the adverse conditions (such as electric current, light and temperature) in the Physics Laboratory had been corrected.

COMMENT ON BIOLOGICAL ASSAYS.

Under the most favorable conditions, biological assays of Vitamin A potency cannot be accurate, because of the many variable factors which may cause a deviation in the results. From the many opinions expressed in the literature, a conservative estimate of the error in biological assays is approximately ± 20%; although some laboratories claim they can check their own biological assays within a deviation of ± 10%.

There are many difficulties encountered by biological laboratories in conducting Vitamin A assays according to the method outlined in the U. S. P. XI. In discussions with bio-chemists representing a number of different laboratories, one question that invariably arises is: "When is a rat really suitable for the assay period?" The U. S. P. states that "a rat shall manifest evidence of Vitamin A deficiency characterized by declining weight and/or ophthalmia." In the event *Declining Weight* is the criterion employed, and the prescribed seven-day period, as outlined in the definition for *Declining Weight*, is strictly adhered to, disastrous conditions are likely to occur in that a great number of animals will not survive the assay period. Therefore, the opinion has been expressed, that the period should be shortened or the definition of *Declining Weight* modified.

Furthermore, the varying degrees in the pathological condition of the rats at the beginning of the assay period, affect the food consumption and the consequent weight response. For this reason there seems to be no definite agreement as to whether the gain in weight shall be measured according to the present U. S. P. specifications or from the point of resumption of growth.

It is also the opinion of those interviewed that the U. S. P., in specifying that the average weight gain of the assay group shall be equal to or greater than the average weight gain of the Reference group, does not permit or allow a sufficient tolerance for the variation in biological response.

In view of the foregoing, the results of biological assays employed in this study are based on our interpretation of the present U. S. P. method of assay for Vitamin

TABLE II.—COD LIVER OILS.

No.	Description.	Spectrophotometer. E Value.	Factor.	Equivalent in U. S. P. Units.	Units Determined by Biol. Assay.	% Difference.*
	Non-destearinated					
1	Whole oil	0.370	2222	822	800	+2.75
	Unsapon. fraction	0.351	2222	780		−2.50
2	Destearinated					
	Whole oil	0.460	2222	1,022	1,000	+2.20
	Unsapon. fraction	0.425	2222	944		−5.60
3	Non-destearinated					
	Whole oil	0.698	2222	1,551	1,500	+3.40
	Unsapon. fraction	0.660	2222	1,467		−2.20
4	Non-destearinated					
	Whole oil	0.797	2222	1,771	1,600	+10.69
	Unsapon. fraction	0.723	2222	1,607		+0.44
5	Non-destearinated					
	Whole oil	0.671	2222	1,491	1,600	−6.81
	Unsapon. fraction	0.703	2222	1,562		−2.38
6	Non-destearinated					
	Whole oil	0.674	2222	1,498	1,600	−6.38
	Unsapon. fraction	0.746	2222	1,658		+3.62
7	Non-destearinated					
	Whole oil	0.777	2222	1,726	1,700	+1.53
	Unsapon. fraction	0.798	2222	1,773		+4.29
8	Non-destearinated					
	Whole oil	0.764	2222	1,698	1,700	−0.12
	Unsapon. fraction	0.774	2222	1,720		+1.18

TABLE II.—*Continued.*

No.	Description.	Spectrophotometer. E Value.	Spectrophotometer. Factor.	Spectrophotometer. Equivalent in U. S. P. Units.	Units Determined by Biol. Assay.	% Difference.*
9	Non-destearinated					
	Whole oil	0.672	2222	1,493	1,700	−12.18
	Unsapon. fraction	0.759	2222	1,686		−0.82
10	Non-destearinated					
	Whole oil	0.764	2222	1,698	1,700	−0.12
	Unsapon. fraction	0.800	2222	1,778		+4.59
11	Destearinated					
	Whole oil	0.845	2222	1,878	1,750	+7.31
	Unsapon. fraction	0.765	2222	1,700		−2.86
12	Non-destearinated					
	Whole oil	0.790	2222	1,755	1,900	−7.63
	Unsapon. fraction	0.818	2222	1,818		−4.32
13	Non-destearinated					
	Whole oil	0.878	2222	1,951	2,000	−2.45
	Unsapon. fraction	0.798	2222	1,773		−11.35
14	Non-destearinated					
	Whole oil	0.824	2222	1,831	2,200	−16.77
	Unsapon. fraction	0.984	2222	2,186		−0.64
15	Non-destearinated					
	Whole oil	1.64	2222	3,644	4,000	−8.90
	Unsapon. fraction	1.63	2222	3,621		−9.45
16	Non-destearinated					
	Whole oil	4.53	2222	10,066	10,000	+0.66
	Unsapon. fraction	4.42	2222	9,821		−1.79
17	Reference oil					
	Whole oil	1.43	2222	3,177	3,000	+5.90
	Unsapon. fraction	1.30	2222	2,889		−3.70
	Average per cent difference of the whole oils				=	−1.58
	Average per cent difference of the unsaponifiable fractions				=	−1.97

* The figures in this column represent the per cent that the units, calculated from the Spectrophotometric E values, are more or less than the units determined by biological assay.

A. In instances where other laboratories assayed the same material, their results confirm our results within what may be considered a permissible biological variance.

COMPARATIVE DATA.

In Table II are shown the results of the Spectrophotometric and Biological assays for Vitamin A of 17 samples of Cod Liver Oil, representing shipments received from all parts of the world (England, Iceland, Japan, Newfoundland, Norway and Russia). It will be noted that the Spectrophotometric determinations are of the whole oils as well as of their unsaponifiable fractions, whereas the Bio-assays are of the whole oils only. The number of units as calculated from the Spectrophotometric E values averaged 1.58% less in the case of whole oils and 1.97% less in the case of unsaponifiable fractions, than those determined by Bio-assays.

The differences between the E values obtained with the whole oils and those obtained with the unsaponifiable fractions are not sufficient to warrant the practice of conducting the determinations with the unsaponifiable fractions.

The oils enumerated in Table II conformed with all the requirements of the U. S. P. and contained less than 1.2% Free Fatty Acids (calculated as Oleic Acid). However, with oils that were rancid or contained more than 2% Free Fatty Acids or impurities such as iron oleate, E values have been obtained with the whole oils which were as much as 25% greater than the E values obtained with their unsaponifiable fractions. Therefore, in cases similar to those mentioned, it would be advisable and more accurate to determine the E value of the unsaponifiable fraction.

In Table III are shown the results of the Spectrophotometric and Biological assays for Vitamin A of nine samples of Halibut Liver Oil representing shipments received from various suppliers. The number of units as calculated from the Spectrophotometric E values averaged 2.85% less than those determined by Bio-assays.

TABLE III.—HALIBUT LIVER OILS.

No.	E Value.	Spectrophotometer. Factor.	Equivalent in U. S. P. Units.	Units Determined by Biol. Assay.	% Difference.*
1	26.8	2222	59,550	60,000	− 0.83
2	36.5	2222	81,103	90,000	− 9.89
3	36.1	2222	80,214	90,000	−10.87
4	28.8	2222	63,994	60,000	+ 6.66
5	35.1	2222	77,992	80,000	− 2.51
6	29.5	2222	65,549	60,000	+ 9.25
7	38.2	2222	84,880	84,000	+ 1.05
8	86.0	2222	191,092	216,000	−11.53
9	33.5	2222	74,437	80,000	− 6.95
				Average per cent difference =	− 2.85

* The figures in this column represent the per cent that the units, calculated from the Spectrophotometric E values, are more or less than the units determined by biological assay.

In Table IV are shown the results of the Spectrophotometric and Biological assays for Vitamin A of 16 samples of High Potency Fish Liver Oil Blends representing shipments received from various suppliers. The number of units as calculated from the Spectrophotometric E values averaged 1.68% less than those determined by Bio-assays.

In Table V are shown the results of the Spectrophotometric and Biological assays for Vitamin A of 11 samples of Vitamin A and D Concentrates prepared from Cod Liver Oil and other Fish Liver Oils in this laboratory. The number of units as calculated from the Spectrophotometric E values averaged 4.03% more than those determined by Bio-assays.

TABLE IV.—HIGH POTENCY FISH LIVER OIL BLENDS.

No.	E Value.	Spectrophotometer. Factor.	Equivalent in U. S. P. Units.	Units Determined By Biol. Assay.	% Difference.*
1	17.5	2222	38,885	40,000	− 2.79
2	9.76	2222	21,687	20,000	+ 8.44
3	11.5	2222	25,553	25,000	+ 2.21
4	17.0	2222	37,774	40,000	− 5.57
5	29.3	2222	65,105	75,000	−13.19
6	11.2	2222	24,886	25,000	− 0.46
7	15.5	2222	34,441	32,000	+ 7.63
8	39.1	2222	86,880	80,000	+ 8.60
9	81.0	2222	179,982	175,000	+ 2.85
10	35.8	2222	79,548	90,000	−11.61
11	8.27	2222	18,376	20,000	− 8.12
12	11.9	2222	26,442	28,000	− 5.56
13	59.5	2222	132,209	137,000	− 3.50
14	70.7	2222	157,095	170,000	− 7.59
15	60.1	2222	133,542	136,000	− 0.18
16	89.5	2222	198,869	195,000	+ 1.98
			Average per cent difference	=	− 1.68

* The figures in this column represent the per cent that the units, calculated from the Spectrophotometric E values, are more or less than the units determined by biological assay.

TABLE V.—CONCENTRATES.

No.	E Value.	Spectrophotometer. Factor.	Equivalent in U. S. P. Units.	Units Determined by Biol. Assay.	% Difference.*
1	44.3	2222	98,435	100,000	− 1.57
2	79.3	2222	176,205	170,000	+ 3.65
3	69.1	2222	153,540	140,000	+ 9.67
4	30.5	2222	67,771	60,000	+12.95
5	9.42	2222	20,931	20,000	+ 4.66
6	30.6	2222	67,993	60,000	+13.32
7	22.3	2222	49,551	60,000	−17.41
8	28.5	2222	63,327	60,000	+ 5.55
9	75.8	2222	168,428	180,000	− 6.43
10	29.4	2222	65,327	60,000	+ 8.79
11	18.0	2222	39,996	36,000	+11.10
			Average per cent difference	=	+ 4.03

* The figures in this column represent the per cent that the units, calculated from the Spectrophotometric E values, are more or less than the units determined by biological assay.

The difference (as shown in Tables II, III, IV and V) between the number of units calculated from the Spectrophotometric E values and those determined by Bio-assays is not significant when compared with the variation in biological response.

In calculating the equivalent in International units from the Spectrophotometric E values, the Addendum 1936 to the British Pharmacopœia 1932 has accepted provisionally 1600 as the conversion factor. Some laboratories in the United States employ 1600 as a conversion factor in calculating the equivalent in U. S. P. units from the E value. Whether 1600 is the correct figure to employ as a conversion factor is a controversial subject in England and United States. In this connection, it is apropos to quote Pritchard, *et al.* (2):

> "The value of 1600 recommended by the International Conference of 1934 represents a useful compromise, but careful estimates of the conversion factor on different preparations and in different laboratories may vary over the range 700–2500 and it remains uncertain how far the discrepancies are due to inaccuracy in the

biological assays and how far to other contributory causes of a more fundamental nature. The discrepancies appear, however, to be outside any known experimental error."

CONCLUSIONS.

In view of the data submitted in this paper, the suggested procedure which will yield more significant figures and tend to obviate the variances in the results obtained in different laboratories, is:

Each laboratory should determine the E value of the U. S. P. Reference Oil. The quotient obtained by dividing 3000 (the official number of units in one Gm. of Reference Oil) by the E value of the Reference Oil, would be the correct conversion factor for the particular instrument employed. The product obtained by multiplying the E value by the proper conversion factor is the equivalent in biological U. S. P. units in one Gm. of the material examined.

Sufficient data are presented to warrant the adoption (by the U. S. P.) of a Spectrophotometric method for the determination of the Vitamin A content of Cod Liver Oil and other Fish Liver Oils, as an alternate for the Biological assay. The obvious advantages of the Spectrophotometric determinations over Biological assay are:

Greater degree of accuracy with consequent closer agreement in the results obtained in different laboratories
Greater convenience
Conservation of time and expense.

REFERENCES.

(1) Weigert, "Die Optische Methoden der Chemie," Leipsig, page 234 (1927).

(2) Pritchard, Harry, and Wilkinson, Harry, "A Discrepancy between Biological Assays and Other Methods of Determining Vitamin A," *Biochem. J.*, Vol. 31, No. 2, 259 (February 1937).

AMERICAN INSTITUTE OF CHEMISTS.

The American Institute of Chemists held its annual meeting May 15th, in the Chemists Club, New York, followed by a dinner at which the medal of the American Institute of Chemists, awarded annually for outstanding service to the science of chemistry, was presented to Dr. James F. Norris, director of the research laboratory in organic chemistry at the Massachusetts Institute of Technology.

The Willard Gibbs Gold Medal of the Chicago section of the American Chemical Society, one of the highest distinctions in chemical science, was conferred on Dr. Herbert Newby McCoy, internationally known for his achievements in radioactivity. Dr. Paul Van Cleef, chairman of the section, presided and delivered an address on "The Inspiration of Willard Gibbs." The presentation speech was by Dr. Edward R. Weidlein, director of the Mellon Institute of Industrial Research, and president of the American Chemical Society.

The American Chemical Society sponsored the Colloid Symposium which was held June 10th to 12th at the University of Minnesota and Mayo Clinic. The committee on colloid science of the National Research Council participated.

HOSPITAL PROGRESS.

The special directory number of *Hospital Progress*, which is the official organ of the Catholic Hospital Association of the United States and Canada, has issued a very valuable special directory number. The foreword is "The Catholic Hospital and School of Nursing at the End of 1936." The tables and directories take up 89 pages of the special number.

"The Story of an Old Book Cover," by Sister Mary E. Xavier, of Mount Mary College, Milwaukee, is a leading article of *The Science Counselor* of the June number; the *Foreword* is by **Dr. Edward Kremers.** The old volume was printed in 1573, and is an interesting translation of the parchment.