REPORT OF THE VITAMIN ASSAY COMMITTEE OF THE AMERICAN DRUG MANUFACTURERS' ASSOCIATION—TWENTY-SIXTH ANNUAL MEETING—MAY 1937.

I—THE PRACTICAL APPLICATION OF THE SPECTROPHOTOMETRIC METHOD FOR ASSAY OF VITAMIN A.*

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The biological method (specified by U. S. P. XI) for the assay of the Vitamin A content of cod liver oil and related products is laborious, time consuming and very expensive. As a consequence, for a number of years investigators and analysts have been seeking for a more rapid, convenient and less expensive method for Vitamin A assays. This search resulted in the development of the so-called Carr-Price (1) antimony trichloride method. This method consists of a comparison of a blue color developed by the reaction of Vitamin A and antimony trichloride in a chloroform solution with standard blue color glasses. The antimony trichloride method for Vitamin A assay has many desirable features and it has been very useful for estimating Vitamin A potency but since other factors may give a blue color it has not been generally accepted as a reliable quantitative method for Vitamin A assay.

A number of investigators found in their studies of absorption spectra that Vitamin A gives a characteristic curve in the ultraviolet region with the peak at 3200 Angstrom units. Accordingly by measuring the absorption of ultraviolet light at 3280 Angstrom units an estimate could be made of the Vitamin A.

Unfortunately the usual spectrograph instruments on which these investigations were conducted are far too expensive, and besides their use necessitated highly experienced and capable operators. Consequently this type of determination of Vitamin A seemed unsuitable for general practical Vitamin A assay. However, in 1933 a specially devised and relatively inexpensive instrument, "Vitameter A," was manufactured by the Adam Hilger, Ltd., of London.

Since this instrument seemed to offer considerable promise for the rapid assay the Vitamin Committee of the American Drug Manufacturers' Association made a preliminary investigation of its value during 1935 particularly for the assay of cod liver oils. Three of the laboratories of the A. D. M. A. Vitamin Committee who made purchases of the instrument and two associate non-member laboratories participated in the study. The results of these 5 workers (2) made on 13 fish liver oils showed that assays could be made with a fair degree of accuracy within an hour or so. Compared with a month or more by the biological test the practical significance of the use of the Vitameter is self-evident—even if the test could be used only as a tentative or suggested index.

About the same time the Association of Official Agricultural Chemists also conducted a study of the value of the Vitameter for assaying cod liver oil. Five samples of cod liver oils previously bio-assayed for their Vitamin A potency were sent to eleven vitamin testing laboratories. The chairman, Dr. Irish (3), reported

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[1] The Vitamin Committee of the American Drug Manufacturers' Association.

that the results obtained by the different laboratories varied from 18% to 32% depending upon which one of the three procedures he outlined was followed.

Therefore, in view of the somewhat promising results obtained in this preliminary investigation with the Vitameter, the A. D. M. A. Vitamin Committee undertook at your recommendation a more extensive study during 1936. This year's program included the assay of thiry-four samples consisting of: 11 cod liver oils, 11 cod liver oil concentrates, 9 halibut liver oils, and 6 so-called "other fish liver oils," 2 halibut liver oil concentrates and 1 sardine body oil. These samples were supplied by different members of the committee and in nearly all instances they represented market products. Of particular interest throughout this report is the fact that Sample 11 is the U. S. P. Reference Cod Liver Oil which has an assigned biologic value of 3000 U. S. P. or International units per Gm.

Two factors, with regard to the equipment and experience of the laboratories, have entered into the problem and should be borne in mind. *First*, the manufacturers have been gradually improving the instruments throughout the past 2 to 3 years. Since the participants in this study have purchased their instruments at various times during this interval it is obvious that there are uncontrolled variations in the equipment. *Secondly*, the finesse in the technique of operating the instrument doubtless varies to a considerable extent with the amount of experience the operator acquires. That is to say no two laboratories have used exactly the same model of Vitameter or have they worked with it for exactly the same length of time. Furthermore, in order to amplify the project, it was decided to accept the coöperation of a few laboratories outside those represented on the committee. In so doing, it has been found that the data thereby contributed introduced in some instances values which were difficult to average into the groupings and therefore these had to be set out, as it were, for individual consideration. In order that these laboratories should be representative they were selected to include academic, federal government and industrial laboratories. The Vitamin Committee is deeply grateful for the generous coöperation extended to it by the Atlantic Coast Fisheries Corporation, Gorton-Pew Fisheries Company Ltd., Health Products Corporation, The Crookes Laboratories, The Norwich Pharmacal Company, The Ohio State University—Department of Chemistry, and the United States Department of Agriculture—Food and Drug Administration. Some of the laboratories were provided with both a Vitameter and a Spectrophotometer. Such laboratories assayed the samples with both instruments.

It is generally believed that the Vitameter gives more reliable results in the assay of oils of high Vitamin A potency than for oils of low potency. Accordingly the eleven cod liver oils were assayed by testing the fluid oil for its Vitamin content and by testing the Vitamin A content of the unsaponifiable portion of the oil.

It was originally intended that the laboratory procedure for conducting the Vitameter assays should be uniform for all the coöperating laboratories. Accordingly each laboratory was asked to conduct the assays as follows:

"It is hoped that, in each laboratory, two or more persons will make assays of all samples and report their results separately. Preferably use cyclohexane as the solvent but in any case report the solvent used. In preparing solutions add a weighed amount of the product under test to a measured volume of solvent."

"Preferably make Vitameter readings one hour after the solution has been prepared but

in any case report the time interval between preparation of solution and making Vitameter reading. The final 'reading' is to be the average of ten readings, except when the photographic method is used. In making dilutions of the dissolved sample adjust the strength of the solution so that the readings will be between 0.6 and 0.8 on the Vitameter scale. Make two separate assays of the cod liver oils (A) the cod liver oil as such and (B) the unsaponifiable portion of the cod liver oil. Follow Irish (3) method of saponification: 'One gram of oil is saponified with 10 cc. $N/2$ freshly prepared alcoholic KOH, by boiling until clear (time needed 5 minutes). Twenty cubic centimeters of water are added, the whole transferred to a small separator and extracted with two quantities of 25 cc. ether (peroxide free). The ethereal extracts are washed first with water (10–20 cc.) then with 10–20 cc. $N/2$ KOH and again with water, while rotating gently without shaking. The ethereal solution is then shaken thoroughly with two quantities of 10 cc. water, after which it is filtered into a flask, the ether evaporated almost to dryness and the residue dissolved in ethyl alcohol or cyclohexane and made up to the concentration required for the particular instrument in use. A preliminary test on the original oil will indicate the amount both of oil and of solvent which will be necessary.' "

Due to various unforeseen conditions it was necessary for some of the laboratories to modify this procedure somewhat. Laboratory No. 12, which was not provided with suitable electric current, modified their Vitameter by substituting for the copper arc a General Electric S-1 Therapeutic Lamp. It was very fortunate that this modification was attempted since a number of other laboratories have found it inconvenient to provide the proper current for the Vitameter.

Again Laboratory No. 2 found that the continuous use of the Vitameter caused considerable undesirable eyestrain to the operators. This tended to produce variable results. In order to eliminate this factor the instrument was equipped for making photographs of the spectrographic determinations. The procedure was a modification of the method recommended by Notevarp (4). This provides a permanent record of the results of the assays.

Further, the manufacturer of the Vitameter originally recommended chloroform as the solvent for preparing cod liver oils for Vitameter assay. Subsequently cyclohexane was recommended. During this study, tests were made with chloroform, cyclohexane, alcohol, absolute alcohol, isopropyl alcohol, a mixture of 80% alcohol and 20% cyclohexane, and a mixture of 90% alcohol and 10% cyclohexane as solvents. Satisfactory results were obtained with cyclohexane, isopropyl alcohol, alcohol and absolute alcohol but solutions prepared with chloroform tended to lose their Vitamin A potency on standing.

The results obtained from the spectrophotometric assay of the vitamin A content of over fifteen thousand solutions prepared from the samples under consideration are presented in the following six tables:

Table I: Results of Vitameter Assays—"E Values"—A. D. M. A. Laboratories.

Table II: Results of Vitameter Assays—"E Values"—Coöperating Laboratories.

Table III: Results of Spectrophotometric Assays—"E Values"—A. D. M. A. and Cooperating Laboratories.

Table IV: Comparison of Vitameter and Spectrophotometer Values from Same Laboratories—"E Values."

Table V: Deviation of the Respective Vitameter Values from the Average—A. D. M. A. Laboratories.

Table VI: Vitameter Data—Applying the Correction Factor for Each Laboratory.

TABLE I.—RESULTS OF VITAMETER ASSAYS—"E VALUES," A. D. M. A. LABORATORIES.

Sample.	Lab. No. 1.	Lab. No. 2.	Lab. No. 3.	Lab. No. 4.	Lab. No. 5.	Lab. No. 6.	Lab. No. 7.	Max.	Min.	Av.
					Cod Liver Oils.					
1	1.37	1.36	1.71	1.73	1.64	1.61	1.70	1.73	1.36	1.59
2	0.76	0.79	1.15	0.93	1.05	0.87	1.31	1.31	0.76	0.98
3	0.93	0.99	1.20	0.95	1.12	1.12	1.41	1.41	0.93	1.10
4	1.45	1.43	1.61	1.74	1.85	1.76	1.69	1.85	1.43	1.65
5	1.10	1.12	1.35	1.35	1.39	1.38	1.58	1.58	1.10	1.32
6	1.33	1.45	1.48	1.57	1.64	1.60	1.69	1.69	1.33	1.54
7	1.48	1.65	1.72	1.86	1.71	1.84	1.70	1.86	1.48	1.71
8	1.61	1.69	1.69	1.98	1.92	1.91	2.07	2.07	1.61	1.84
9	0.83	0.83	1.14	1.05	1.04	0.97	1.22	1.22	0.83	1.01
10	1.09	1.07	1.34	1.36	1.41	1.36	1.54	1.54	1.07	1.31
11	1.44	1.54	1.65	1.60	1.56	1.79	1.66	1.79	1.44	1.61
					Cod Liver Oil (Unsaponifiable Portion).					
1	1.16	1.06	1.44	1.34	1.54	1.46	1.68	1.68	1.06	1.38
2	0.74	0.69	0.91	0.79	0.94	0.76	1.30	1.30	0.69	0.88
3	0.88	0.84	0.93	0.93	1.09	1.02	1.48	1.48	0.84	1.02
4	1.34	1.22	1.31	1.38	1.59	1.69	1.64	1.69	1.22	1.45
5	0.98	0.75	1.24	1.06	1.24	1.20	1.52	1.52	0.75	1.14
6	1.24	1.32	1.25	1.37	1.51	1.51	1.68	1.68	1.24	1.41
7	1.24	1.38	1.27	1.40	1.51	1.59	1.70	1.70	1.24	1.44
8	1.43	1.38	1.55	1.65	1.67	1.73	2.08	2.08	1.38	1.64
9	0.74	0.77	0.85	0.92	1.04	0.85	1.22	1.22	0.74	0.91
10	0.98	0.91	0.89	1.06	1.34	1.16	1.52	1.52	0.89	1.12
11	1.37	1.38	1.40	1.21	1.54	1.60	1.68	1.68	1.21	1.45
					Cod Liver Oil Concentrates.					
12	23.99	22.22	21.4	28.18	26.30	32.20	40.00	40.00	21.40	27.75
13	18.56	18.48	16.9	25.78	22.80	25.94	30.00	30.00	16.90	22.64
14	18.32	20.27	19.1	23.98	24.00	26.19	30.50	30.50	18.32	23.19
15	64.46	63.52	62.7	81.69	73.40	102.65	82.00	102.65	62.70	75.77
16	16.25	28.50	16.5	19.50	18.10	23.76	23.00	23.76	16.25	20.80
					Halibut Liver Oils.					
17	37.56	34.90	34.0	43.40	37.60	46.46	44.70	46.46	34.00	39.80
18	32.13	30.38	28.6	35.08	34.10	38.25	42.50	42.50	28.60	34.43
19	17.68	16.50	15.5	19.10	18.27	22.67	20.25	22.67	15.50	18.57
20	49.56	51.60	50.1	59.56	57.55	60.29	75.00	75.00	49.36	57.67
21	66.25	59.76	70.5	87.30	73.30	85.55	100.00	100.00	59.76	77.52
22	15.65	16.59	14.6	21.79	17.10	22.80	21.50	22.80	14.60	18.57
23	24.03	28.03	28.4	41.50	31.02	34.70	37.00	41.50	24.03	32.10
24	43.87	41.66	45.1	55.12	45.87	53.66	67.50	67.50	41.66	50.39
25	53.68	52.63	58.1	70.90	59.72	78.00	80.00	80.00	52.63	64.72
					Other Fish Liver Oils.					
26	87.25	91.32	88.4	100.73	92.35	109.61	130.0	130.00	87.25	99.95
27	48.51	54.01	51.0	66.75	56.65	59.90	76.0	76.00	48.51	58.97
28	76.88	74.74	72.8	90.31	72.45	114.16	103.3	114.16	72.45	86.38
29	32.54	37.97	36.9	46.77	37.75	48.79	57.5	57.50	32.54	42.60
30	53.68	59.16	54.9	85.19	61.85	77.70	77.5	77.70	53.68	67.14
31	35.26	35.71	39.5	46.80	40.25	56.42	60.0	60.00	35.26	44.85

				Halibut Liver Oil Concentrate.						
32	562.00	724.50	581.3	912.00	742.00	779.00	725.0	912.00	562.00	717.96
				Cod Liver Oil Concentrate and Halibut Liver Oil Mixture.						
33	34.09	36.36	39.3	39.38	33.60	48.40	43.0	48.40	33.60	39.16
				Sardine Body Oil.						
34	0.41	0.48	0.4	0.63	0.58	0.66	1.3	1.30	0.40	0.64

TABLE II.—RESULTS OF VITAMETER ASSAYS—"E VALUES," COÖPERATING LABORATORIES.

Sample.	Lab. No. 8.	Lab. No. 9.	Lab. No. 10.	Lab. No. 11.	Max.	Min.	Av.	Lab. No. 12.
				Cod Liver Oils.				
1	1.07	1.48	1.57	1.33	1.57	1.07	1.36	0.37
2	0.60	0.95	0.85	0.76	0.95	0.60	0.79	0.18
3	0.68	1.18	1.26	0.92	1.26	0.68	1.01	0.24
4	1.08	1.72	1.55	1.55	1.72	1.08	1.48	0.38
5	0.75	1.76	1.18	1.06	1.76	0.75	1.19	0.31
6	1.13	1.92	1.52	1.51	1.52	1.13	1.42	0.41
7	1.27	1.95	1.45	1.84	1.95	1.27	1.63	15.40
8	1.37	2.08	1.64	1.78	2.08	1.37	1.72	0.37
9	0.72	0.97	1.05	0.97	1.05	0.72	0.93	0.18
10	1.00	1.31	1.42	1.19	1.42	1.00	1.23	0.32
11	1.06	1.04	1.46	1.56	1.56	1.04	1.28	0.37
				Cod Liver Oil (Unsaponifiable Portion).				
1			1.56	1.32				0.18
2			1.00	0.75				0.17
3			0.86	0.92				0.15
4			1.10	1.52				0.19
5			0.85	1.05				...
6			1.26	1.49				...
7			1.46	1.72				...
8			1.61	1.70				...
9			0.96	0.94				...
10			1.13	1.15				...
11			1.22	1.49				...
				Cod Liver Oil Concentrates.				
12	16.2	53.2	29.8	26.88	53.2	16.2	31.52	7.80
13	15.4	14.3	21.2	21.88	21.88	14.3	18.20	6.70
14	14.3	7.7	19.1	21.29	21.29	7.7	15.60	6.90
15	63.8	38.5	71.7	73.61	73.61	38.5	61.90	24.00
16	13.5	13.0	19.2	18.20	19.2	13.0	15.98	4.65
				Halibut Liver Oils.				
17	30.1	45.6	38.2	35.6	45.6	30.1	37.4	13.00
18	24.6	31.8	35.6	32.5	35.6	24.6	31.1	10.20
19	13.6	18.8	19.0	16.0	19.0	13.6	16.8	6.80
20	45.0	59.0	56.4	53.4	59.0	45.0	53.5	7.40
21	58.1	75.8	65.7	68.7	75.8	58.1	67.1	10.40
22	12.2	18.1	19.6	15.3	19.6	12.2	16.3	4.75
23	19.3	27.8	34.3	28.3	34.3	19.3	27.4	5.90
24	32.9	42.9	43.3	44.2	44.2	32.9	40.8	8.15
25	46.1	55.6	71.2	58.9	71.2	46.1	58.0	12.60

				Other Fish Liver Oils.				
26	75.1	89.1	90.0	89.1	90.0	75.1	85.8	14.35
27	42.2	49.9	46.8	50.9	50.9	42.2	47.4	13.00
28	61.2	75.9	75.3	80.4	80.4	61.2	73.2	21.00
29	27.2	34.3	44.2	40.3	44.2	27.2	36.5	8.15
30	48.1	65.0	63.4	62.1	65.0	48.1	59.7	21.50
31	28.7	38.8	49.3	40.4	49.3	28.7	39.3	9.80
				Halibut Liver Oil Concentrate.				
32	501.9	1064.0	559.0	748.0	1064.0	501.9	718.23	...
				Cod Liver Oil Concentrate and Halibut Liver Oil Mixture.				
33	28.4	42.0	38.4	44.57	44.57	28.4	38.34	9.10
				Sardine Body Oil.				
34	0.5	0.36	0.73	0.57	0.73	0.36	0.54	0.16

TABLE III.—RESULTS OF SPECTROPHOTOMETER ASSAYS—"E VALUES," A. D. M. A. AND COÖPERATING LABORATORIES.

Sample.	Lab. No. 3.	Lab. No. 9.	Lab. No. 10.	Lab. No. 13.	Lab. No. 14.	Max.	Min.	Av.	Av.*
					Cod Liver Oils.				
1	1.56	1.31	1.37	1.33	1.30	1.56	1.30	1.37	1.59
2	0.96	0.78	0.80	0.81	0.90	0.96	0.78	0.85	0.98
3	1.21	1.18	0.87	0.92	1.00	1.21	0.87	1.04	1.10
4	1.86	1.45	1.14	1.57	2.10	2.10	1.14	1.62	1.65
5	1.31	1.02	0.96	1.08	0.80	1.31	0.80	1.03	1.32
6	1.57	1.28	1.29	1.36	1.00	1.57	1.00	1.30	1.54
7	1.79	1.45	1.21	1.55	1.80	1.80	1.21	1.56	1.71
8	1.83	1.43	1.50	1.40	1.30	1.83	1.30	1.49	1.84
9	1.11	0.78	0.78	0.86	1.10	1.11	0.78	0.92	1.01
10	1.16	1.23	1.16	1.12	1.30	1.30	1.12	1.19	1.31
11	1.71	1.37	1.19	1.58	...	1.71	1.19	1.46	1.61
					Cod Liver Oil (Unsaponifiable Portion.)				
1	1.43	0.72	1.17	1.15		1.43	0.72	1.12	1.38
2	0.92	0.91	0.65	0.67		0.92	0.65	0.79	0.88
3	0.92	0.80	0.75	0.85		0.92	0.75	0.83	1.02
4	1.30	1.29	1.04	1.39		1.39	1.04	1.26	1.45
5	1.17	0.92	0.75	0.98		1.17	0.75	0.96	1.14
6	1.31	0.96	1.09	1.16		1.31	0.96	1.13	1.41
7	1.35	1.31	1.31	1.29		1.35	1.29	1.32	1.44
8	1.54	1.48	1.51	1.28		1.54	1.28	1.45	1.64
9	0.87	0.77	0.83	0.71		0.87	0.71	0.79	0.91
10	0.94	0.79	1.06	0.98		1.06	0.79	0.94	1.12
11	1.42	...	0.99	1.26		1.42	0.99	1.22	1.45
					Cod Liver Oil Concentrates.				
12	20.98	23.30	21.7	24.90		24.90	20.98	22.72	27.75
13	16.13	20.10	18.6	20.00		20.10	16.13	18.71	22.64
14	17.07	18.70	16.5	18.45		18.70	16.50	17.68	23.19
15	67.41	64.30	64.3	62.40		67.41	62.40	64.60	75.77
16	13.49	16.00	15.4	14.45		16.00	13.49	14.84	20.8

				Halibut Liver Oils.				
17	30.86	34.70	27.0	33.65	34.70	27.00	31.55	39.80
18	28.09	29.70	26.8	30.75	30.75	26.80	28.84	34.43
19	16.70	16.80	15.5	16.95	16.95	15.50	16.49	18.57
20	46.28	56.20	52.5	57.05	57.05	46.28	53.01	57.67
21	58.38	71.10	56.0	71.05	71.10	56.00	64.13	77.52
22	14.69	16.70	15.7	16.70	16.70	14.69	15.95	18.57
23	25.03	25.30	24.2	26.40	26.40	24.20	25.23	32.10
24	44.60	44.30	35.5	45.75	45.75	35.50	42.54	50.39
25	57.46	58.40	52.9	61.00	61.00	52.90	57.44	64.72
				Other Fish Liver Oils.				
26	86.12	87.40	80.9	88.90	88.90	80.90	85.83	99.95
27	49.03	51.20	45.5	52.85	52.85	45.50	49.65	58.99
28	72.72	73.40	76.9	76.25	76.90	72.72	74.82	86.38
29	32.94	33.25	33.3	35.05	35.05	32.94	33.64	42.60
30	50.79	59.30	59.8	57.25	59.80	50.79	56.79	67.14
31	36.51	36.70	31.8	37.00	37.00	31.80	35.50	44.85
				Halibut Liver Oil Concentrate.				
32	587.50	620.00	570.0	597.50	620.00	570.00	593.75	717.96
				Cod Liver Oil Concentrate and Halibut Liver Oil Mixture.				
33	39.03	37.60	32.7	34.45	39.03	32.70	35.95	39.16
				Sardine Body Oil.				
34	0.39	None	0.58	None	0.58	0.39	0.49	0.64

TABLE IV.—VITAMETER AND SPECTROPHOTOMETER BY SAME LABORATORY—"E VALUES," A. D. M. A. AND COÖPERATING LABORATORIES.

Sample.	Lab. No. 3. Vitameter.	Lab. No. 3. Spectrophotometer.	Lab. No. 9. Vitameter.	Lab. No. 9. Spectrophotometer.	Lab. No. 10. Vitameter.	Lab. No. 10. Spectrophotometer.	Av. Vitameter.	Av. Spectrophotometer.
				Cod Liver Oils.				
1	1.71	1.56	1.48	1.31	1.57	1.37	1.59	1.41
2	1.15	0.96	0.95	0.78	0.85	0.80	0.98	0.85
3	1.20	1.21	1.18	1.18	1.26	0.87	1.21	1.09
4	1.61	1.86	1.72	1.45	1.55	1.14	1.63	1.48
5	1.35	1.31	1.76	1.02	1.18	0.96	1.43	1.10
6	1.48	1.57	1.52	1.28	1.52	1.29	1.51	1.38
7	1.72	1.79	1.95	1.45	1.45	1.21	1.71	1.48
8	1.69	1.83	2.08	1.43	1.64	1.50	1.80	1.59
9	1.14	1.11	0.97	0.78	1.05	0.78	1.05	0.89
10	1.34	1.16	1.31	1.23	1.42	1.16	1.36	1.18
11	1.65	1.71	1.04	1.37	1.46	1.19	1.38	1.42
				Cod Liver Oil (Unsaponifiable Portion).				
1	1.44	1.43			1.56	1.17	1.50	1.30
2	0.91	0.92			1.00	0.65	0.96	0.79
3	0.93	0.92			0.86	0.75	0.90	0.84
4	1.31	1.30			1.10	1.04	1.20	1.17

* Average of Vitameter Assays—See Table I.

5	1.24	1.17			0.85	0.75	1.04	0.96
6	1.25	1.31			1.26	1.09	1.26	1.20
7	1.27	1.35			1.46	1.31	1.36	1.33
8	1.55	1.54			1.61	1.51	1.58	1.53
9	0.85	0.87			0.96	0.83	0.92	0.85
10	0.89	0.94			1.13	1.06	1.01	1.00
11	1.40	1.42			1.22	0.99	1.31	1.21
				Cod Liver Oil Concentrates.				
12	21.4	20.98	53.2	23.30	29.80	21.70	34.80	21.99
13	16.9	16.13	14.3	20.10	21.20	18.60	17.47	18.28
14	19.0	17.07	7.7	18.70	19.10	16.50	15.27	17.42
15	62.7	67.41	38.5	64.30	71.70	64.30	57.63	65.34
16	16.5	13.49	13.0	16.00	19.15	15.40	16.22	14.96
				Halibut Liver Oils.				
17	34.0	30.86	45.6	34.70	38.20	27.0	39.27	30.85
18	28.6	28.09	31.8	29.70	35.60	26.8	32.00	28.20
19	15.5	16.70	18.8	16.80	19.00	15.5	17.77	16.33
20	50.1	46.28	59.0	56.20	56.35	52.5	55.15	51.66
21	70.5	58.38	75.8	71.10	65.70	56.0	70.66	61.83
22	14.6	15.69	18.1	16.70	19.60	15.7	17.43	15.70
23	28.4	25.03	27.8	25.30	34.25	24.2	30.15	24.85
24	45.1	44.60	42.9	44.30	43.30	35.5	43.77	41.47
25	58.1	57.46	55.6	58.40	71.15	52.9	61.62	56.25
				Other Fish Liver Oils.				
26	88.4	86.12	89.1	87.40	89.95	80.9	89.15	84.81
27	51.0	49.03	49.9	51.20	46.80	45.5	49.23	48.58
28	72.8	72.72	75.9	73.40	75.30	76.9	74.67	74.34
29	36.9	32.94	34.3	33.25	44.15	33.3	38.45	33.16
30	54.9	50.79	65.0	59.30	63.40	59.8	61.10	56.63
31	39.5	36.51	38.8	36.70	49.30	31.8	42.53	35.00
				Halibut Liver Oil Concentrate.				
32	581.3	587.50	1064.2	620.00	559.00	570.0	734.80	592.50
				Cod Liver Oil Concentrate and Halibut Liver Oil Mixture.				
33	39.3	39.03	42.0	37.60	38.35	32.70	39.88	36.44
				Sardine Body Oil.				
34	0.4	0.39	0.36	None	0.73	0.58	0.50	0.49

TABLE V.—DEVIATION OF THE RESPECTIVE VITAMETER VALUES FROM THE AVERAGE—PER CENT, A. D. M. A. LABORATORIES.

Sample.	Lab. No. 1.	Lab. No. 2.	Lab. No. 3.	Lab. No. 4.	Lab. No. 5.	Lab. No. 6.	Lab. No. 7.
				Cod Liver Oils.			
1	−13.8	−14.4	+ 7.5	+ 8.8	+ 3.2	+ 1.3	+ 6.9
2	−22.5	−19.4	+17.3	− 5.1	+ 7.2	−11.1	+33.6
3	−15.4	−10.0	+ 5.5	−12.7	+ 1.8	+ 1.8	+27.3
4	−12.1	−13.3	− 2.4	+ 5.5	+12.1	+ 6.7	+ 2.4
5	−16.7	−15.2	+ 2.3	+ 2.3	+ 5.3	+ 4.6	+19.7
6	−13.6	− 5.8	− 3.9	+ 1.9	+ 6.5	+ 3.9	+ 9.9

	7	−13.5	− 3.5	+ 0.6	+ 8.8	0.0	+ 7.6	− 0.6
	8	−12.5	− 8.2	− 8.2	+ 7.6	+ 4.4	+ 3.8	+12.5
	9	−17.8	−17.8	+12.9	+ 3.8	+ 3.0	− 3.8	+10.9
	10	−16.8	−18.3	+ 2.3	+ 3.8	+ 7.7	+ 3.8	+17.6
	11	−10.0	− 3.7	+ 3.1	0.0	− 2.5	+10.6	+ 3.7
I	(*a*) Total (11)	−164.7	−129.6	+37.0	+24.7	+48.7	+29.2	+143.6
	(*b*) Average	− 15.0	− 11.8	+ 3.3	+ 2.2	+ 4.4	+ 2.7	+ 13.1
		Cod Liver Oil (Unsaponifiable Portion).						
	1	−16.0	−23.2	+ 4.4	− 2.9	+11.6	+ 5.8	+21.8
	2	−15.9	−21.6	+ 3.4	−10.2	+ 6.8	−13.7	+47.7
	3	−13.7	−17.6	− 8.8	− 8.8	+ 6.9	0.0	+45.0
	4	− 7.6	−15.8	− 9.7	− 4.8	+ 9.7	+16.5	+13.1
	5	−14.8	−33.2	+ 7.8	− 7.8	+ 7.8	+ 4.3	+32.2
	6	−12.0	− 6.3	−11.3	− 2.8	+ 7.0	+ 7.0	+19.0
	7	−13.9	− 4.2	−11.8	− 2.8	+ 4.9	+10.4	+18.1
	8	−12.9	−15.9	− 5.5	+ 0.6	+ 1.8	+ 5.5	+27.0
	9	−18.7	−17.6	− 6.6	+ 1.1	+14.3	− 6.6	+34.2
	10	−12.5	−18.7	− 2.1	− 5.4	+19.7	+ 3.6	0.0
	11	− 4.2	− 3.5	− 2.1	−15.4	+ 7.7	+13.1	+17.5
II	(*a*) Total (11)	−142.2	−177.6	−42.3	−59.2	+98.2	+45.9	+275.6
	(*b*) Average	− 12.9	− 16.1	− 3.8	− 5.4	+ 9.0	+ 4.2	+ 25.1
		Cod Liver Oil Concentrates.						
	12	−13.5	−19.8	−22.8	+ 1.6	− 5.2	+16.0	+40.2
	13	−18.1	−18.4	−25.4	+13.9	+ 0.7	+14.6	+32.6
	14	−21.0	−12.6	−17.6	+ 3.4	+ 3.5	+12.9	+31.5
	15	−15.0	−16.2	−17.3	+ 7.8	− 3.2	+35.5	+ 8.2
	16	−21.9	+34.0	−19.4	− 6.3	−13.0	+14.2	+10.6
III	(*a*) Total (5)	−89.5	−33.0	−102.5	+20.4	−17.2	+93.2	+123.1
	(*b*) Average	−17.9	− 6.6	− 20.5	+ 4.1	− 3.4	+18.6	+24.6
		Halibut Liver Oils.						
	17	−10.0	−11.7	−14.0	+ 9.8	− 4.9	+17.6	+13.4
	18	− 6.7	−11.8	−17.0	+ 1.9	− 1.0	+11.1	+23.5
	19	− 5.2	−11.5	−14.1	+ 2.5	− 2.0	+21.7	+ 8.6
	20	−14.3	−10.4	−13.0	+ 3.3	− 0.2	+ 4.6	+30.0
	21	−14.5	−22.9	− 9.1	+12.5	− 2.9	+10.4	+29.0
	22	−15.7	−10.7	−21.4	+17.3	− 7.9	+22.8	+15.8
	23	−25.2	−12.6	−11.5	+29.3	− 3.4	+ 8.0	+15.3
	24	−13.0	−17.3	−10.5	+ 9.4	− 9.0	+ 6.5	+34.0
	25	−17.1	−18.7	−10.2	+ 9.6	− 7.8	+20.5	+23.6
IV	(*a*) Total (9)	−121.7	−127.6	−120.8	+95.6	−39.1	+123.2	+193.2
	(*b*) Average	− 13.5	− 14.2	− 13.4	+10.6	− 4.3	+ 13.7	+ 21.4
		Other Fish Liver Oils.						
	26	−12.8	− 8.7	−11.6	+ 0.8	− 7.6	+ 9.6	+30.0
	27	−17.8	− 8.5	−13.5	+13.0	− 4.0	+ 1.5	+28.8
	28	−11.0	−13.5	−15.7	+ 4.6	−17.3	+32.2	+19.7
	29	−24.8	−10.8	−13.3	+ 9.8	−11.4	+14.5	+35.0
	30	−20.0	−11.9	−18.2	+26.9	− 7.9	+15.8	+14.4

	31	−21.4	−20.4	−11.9	+ 4.4	−10.3	+25.8	+33.8
V	(*a*) Total (6)	−107.8	−73.8	−84.2	+59.5	−58.5	+99.4	+161.7
	(*b*) Average	− 17.9	−12.3	−14.0	+ 9.9	− 9.8	+16.6	+27.0
				Halibut Liver Oil Concentrate.				
VI	32	−21.7	+ 0.9	−19.1	+27.0	+ 3.3	+ 8.5	− 1.0
				Cod Liver Oil Concentrate and Halibut Liver Oil Mixture.				
VII	33	−12.0	− 7.2	+ 0.5	+ 0.6	−14.3	+23.0	+ 9.9
				Sardine Body Oil.				
VIII	34	−36.0	−25.0	−37.5	− 1.6	− 9.4	+ 5.2	+103.0
IX	(*a*) Grand Total (45):	−696.3	−572.9	−368.6	+167.5	+11.7	+427.6	+1012.8
	(*b*) Average All (45):	−15.5	−12.7	− 8.2	+ 3.7	+ 0.3	+ 9.5	+22.5
				Calculated Correction Factor for Each Vitameter.				
X		1.155	1.127	1.082	0.963	0.997	0.908	0.775

TABLE VI.—VITAMETER DATA: APPLYING THE CORRECTION FACTORS—"E VALUES," A. D. M. A. LABORATORIES.

Sample.	Lab. No. 1.	Lab. No. 2.	Lab. No. 3.	Lab. No. 4.	Lab. No. 5.	Lab. No. 6.	Lab. No. 7.	Av.
				Cod Liver Oils.				
1	1.58	1.53	1.85	1.71	1.63	1.46	1.32	1.58
2	0.88	0.89	1.36	0.89	1.05	0.79	1.02	0.98
3	1.08	1.11	1.30	0.92	1.12	1.01	1.08	1.09
4	1.67	1.62	1.74	1.67	1.85	1.59	1.31	1.64
5	1.27	1.27	1.46	1.30	1.39	1.25	1.23	1.31
6	1.54	1.64	1.60	1.51	1.64	1.45	1.31	1.53
7	1.71	1.86	1.86	1.81	1.71	1.67	1.31	1.70
8	1.86	1.90	1.83	1.90	1.92	1.73	1.61	1.82
9	0.96	0.94	1.23	1.01	1.04	0.88	0.95	1.00
10	1.26	1.21	1.45	1.31	1.41	1.23	1.20	1.30
11	1.66	1.74	1.78	1.54	1.56	1.61	1.29	1.60
				Cod Liver Oil (Unsaponifiable Portion).				
1	1.34	1.20	1.56	1.25	1.54	1.32	1.30	1.36
2	0.85	0.78	0.99	0.76	0.94	0.69	1.01	0.86
3	1.01	0.95	1.01	0.89	1.09	0.93	1.15	1.00
4	1.55	1.37	1.42	1.32	1.59	1.54	1.27	1.43
5	1.13	0.85	1.34	1.02	1.24	1.09	1.18	1.12
6	1.43	1.49	1.35	1.31	1.51	1.37	1.30	1.39
7	1.43	1.55	1.37	1.34	1.51	1.44	1.32	1.42
8	1.65	1.55	1.68	1.58	1.67	1.57	1.61	1.62
9	0.85	0.87	0.92	0.88	1.04	0.77	0.95	0.90
10	1.13	1.02	0.96	1.02	1.34	1.05	1.18	1.10
11	1.58	1.55	1.51	1.16	1.54	1.45	1.30	1.44
				Cod Liver Oil Concentrates.				
12	27.7	25.0	23.2	27.1	26.3	29.2	31.0	27.0
13	21.4	20.8	18.3	24.8	22.8	23.5	23.3	22.1
14	21.2	22.9	20.6	23.0	24.0	23.7	23.6	22.7
15	74.5	71.5	67.9	78.5	73.4	93.2	63.6	74.6
16	18.7	32.1	17.9	18.8	18.1	21.6	17.8	20.7

				Halibut Liver Oils.				
17	41.0	39.4	36.8	41.7	37.6	42.0	34.7	39.0
18	37.1	34.3	30.9	33.7	34.1	34.7	33.0	34.0
19	20.4	18.6	17.3	18.4	18.3	20.5	15.8	18.5
20	57.0	58.3	54.1	57.2	57.6	54.7	58.3	56.7
21	76.4	67.5	76.1	83.8	73.3	77.5	77.6	76.0
22	18.1	18.7	15.8	21.0	17.1	20.7	16.7	18.3
23	27.8	31.7	30.6	39.8	31.0	31.5	28.8	31.6
24	50.7	47.1	48.7	53.0	45.9	48.6	53.5	49.6
25	62.0	59.5	62.8	68.0	59.7	70.7	62.1	63.6
				Other Fish Liver Oils.				
26	101.0	103.0	95.5	96.7	92.4	99.3	100.8	98.5
27	56.0	60.8	55.2	64.2	56.7	54.4	59.0	58.0
28	88.8	84.1	78.9	86.7	72.5	103.7	80.2	85.0
29	37.4	42.8	39.8	45.0	37.8	44.3	44.5	41.7
30	62.0	66.6	59.3	81.8	61.9	70.6	60.0	65.8
31	40.7	40.2	42.6	45.0	40.3	51.2	46.5	43.8
				Halibut Liver Oil Concentrate.				
32	649.0	815.0	607.0	875.0	742.0	707.0	562.0	708.0
				Cod Liver Oil Concentrate and Halibut Liver Oil Mixture.				
33	39.3	41.0	42.5	37.8	33.6	44.9	33.3	38.9
				Sardine Body Oil.				
34	0.47	0.54	0.43	0.61	0.58	0.6	1.0	0.6

DISCUSSION.

Inspection of Table I will reveal that the respective "E values," obtained by Vitameter, for the seven laboratories on the eleven cod liver oils varied materially. However, there was fairly good agreement between the results obtained by some of the laboratories. For instance, Laboratories 1 and 2 checked rather closely and the results reported by Laboratories 5 and 6 were in good agreement between themselves but much higher than the other two. The greatest difference between the maximum and minimum results was for Sample No. 2 the maximum value, 1.31, being 72% larger than the minimum value, 0.76. The best agreement in this series was for Cod Liver Oil No. 11. The maximum value, 1.79, was 23% larger than the minimum value, 1.44. The results obtained for sample No. 11 are of particular interest since this sample was the official U. S. P. Standard of Reference Cod Liver Oil. The average "E value," 1.61, for the U. S. P. Standard of Reference Cod Liver Oil is somewhat lower than the "E value," 1.74, reported by Morgan (5).

It will be noted that the "E values" determined on the unsaponifiable portion of the Cod Liver Oils are lower than those reported for the fluid oils except in the case of Laboratory No. 7. The values reported for the assays of the unsaponifiable portion of the Cod Liver Oils are not only lower but more variable than for the fluid oils. The greatest difference in this series is for Sample No. 5 for which the maximum value, 1.52, is 100% greater than the minimum value, 0.75. The smallest difference between the maximum and minimum value is for Sample 6 the maximum value, 1.68, being 35% larger than the minimum value, 1.24. These greater variations would seem to indicate that the cause lies in errors due to the separation of

the unsaponifiable portion of the oil. The average "E value," 1.45, for the unsaponifiable portion of the U. S. P. Standard of Reference Cod Liver Oil is lower than the "E value," 1.58, reported for similar determinations by Morgan (5).

Five samples, No. 12 to No. 16, of cod liver oil concentrates were assayed for their Vitamin A content. These samples were assumed to be typical of cod liver oil concentrates in general. Here it is also apparent that there is a grouping of data according to instrument, Laboratories 1, 2 and 3 having lower values while Laboratories 6 and 7 were highest. The greatest difference between the maximum and minimum values reported for the cod liver oil concentrates was for Sample No. 12 (Laboratory 7) for which the maximum value, 40.0, is 87% larger than the minimum value, 21.4 (Laboratory 3). The least difference between the results reported by the different laboratories was for Sample No. 16, the maximum value, 23.76, (Laboratory 6) is 46% greater than the minimum value, 16.25 (Laboratory 1).

The greatest deviation in the "E values" of the halibut liver oils was for Sample No. 23 in which the maximum value, 41.50 (Laboratory 4) is 73% greater than the minimum value, 24.03 (Laboratory 1). The least deviation in the halibut liver oil series was for Sample No. 17 for which the maximum value, 46.46 (Laboratory 6) is 37% larger than the minimum value, 34.0 (Laboratory 3). In selecting the halibut liver oils an attempt was made to secure samples possessing a considerable range of Vitamin A potency. This was apparently accomplished since the average "E values" varied from 18.57 to 77.52 which indicates that the Vitamin A potency of Sample No. 21 is over four times that of Samples No. 22 and No. 19.

In the next series, "other fish liver oils," the greatest deviation was for Sample No. 29 for which the maximum value, 57.5 (Laboratory 7) was 77% greater than the minimum value, 32.54 (Laboratory 1). The least deviation for this series was for Sample No. 30 for which the maximum value, 77.70 (Laboratory 6) was 45% greater than the minimum value, 53.68 (Laboratory 1). Again, for the Halibut Liver Oil Concentrate No. 32 there is a wide variation in the results. Laboratories 1 and 3 had the lowest value with Laboratory 4 running highest, and the other four agreeing quite closely. Finally, in the case of Sample No. 34, Sardine Body Oil, this oil had the lowest Vitamin A content of any. Comparing these results, we again find that they group themselves according to certain instruments with Nos. 1, 2 and 3 having the lowest "E values" and 6 and 7 the highest.

From the data in Table I, regardless of the nature of the product, in general one fact appears to stand out as far as Vitamin equipment is concerned, namely:

Comparing the data for the different laboratories for the same respective samples the variations are of such a magnitude and order, that they indicate the fluctuations are due mainly to inherent factors in the instrument, and perhaps to some extent to the particular laboratory technique. That is to say, regardless of the above fluctuations, the data indicate that the Vitameter is capable of quantitatively measuring Vitamin A for any one laboratory at a level, however, which should be readjusted to some standard.

The Vitameter assay results in Table II reported by coöperating laboratories are more limited and do not permit of the same comparison as for the results reported by the A. D. M. A. Laboratories. It will be noted (comparing Laboratories 8, 9, 10 and 11) that the average values reported for all these coöperating laboratories are consistently lower for practically all the thirty-four samples than the average

values for the seven A. D. M. A. laboratories. These lower averages are due principally to the data reported by Laboratory No. 8. Again interest in this series of results is perhaps principally centered in Sample No. 11—the U. S. P. Standard of Reference Cod Liver Oil. Laboratories 10 and 11 agree fairly well with the A. D. M. A. results. The average "E value" obtained by the four laboratories was 1.28 which is much lower than the "E value," 1.61, obtained by the seven A. D. M. A. laboratories. The impression gained here is that the same general effect is evidenced as with the instruments used by the A. D. M. A. Group, namely, a consistent trend to run in groups.

The values reported by Laboratory No. 12 are of particular interest since, as stated previously, they were obtained with a Hilger Vitameter which had been modified by replacing the copper arc with a General Electric S-1 Therapeutic Lamp. This was not done necessarily with the expectation that the values would agree with the corresponding data where the copper arc was used, but rather with the possible thought that the same relationship would exist between the different oils and some set standard. Inspection of the results for Laboratory No. 12 show that the "E values" are decidedly less than the corresponding data for any of the other eleven laboratories. That is, they were of a lower magnitude. Furthermore, comparing the variations between different oils, it would seem that the results for Laboratory 12 do not follow the same direction as those for the standard instrument. These results would seem, therefore, to indicate that the modified Vitameter gives values which are entirely different. For this reason the data were not averaged with those from the other four laboratories. Correspondence with the manufacturers of the Vitameter and the G. E. S-1 Therapeutic Lamp elicited from each a statement that Vitamin A assays could not be made with a Vitameter in which the copper arc was replaced by the ultraviolet lamp in question.

In its report, Laboratory No. 9 stated,

> "We found that the results obtained with the spectrophotometer were much more reliable than those obtained with our old Vitameter. In this connection attention should be called to the rather rapid deterioration of the Vitameter with use. This is particularly true of the silver film which is attached to the quartz plate to transmit the radiations near 3280 Angstrom units. This type of change tends to cause the Vitameter to produce other than strictly monochromatic light which in turn produces an inaccuracy in the 'E values,' for the logarithm of absorption is proportional to the concentration only for monochromatic light."

Fortunately, five laboratories were equipped with high-precision spectrophotometers. One, however, was able to complete only the assays on the series of cod liver oils. The results from those five laboratories are given in Table III together with the average of the Vitameter data for the A. D. M. A. Committee. The variation in the data on the liquid cod liver oils was greatest for Sample No. 5 of which the maximum value, 1.31 (Laboratory No. 3), is 64% greater than the minimum value, 0.80 (Laboratory No. 14). The least variation in the results reported by the five laboratories was for Sample No. 10 of which the maximum value, 1.30 (Laboratory No. 14), is 16% greater than the minimum value, 1.12 (Laboratory No. 13). The average "E value" obtained in the spectrophotometer assays of the U. S. P. Standard of Reference Cod Liver Oil was 1.46. This value is about 90% of 1.61, obtained by the A. D. M. A. Laboratories and over 114% of 1.28, reported by the coöperating laboratories for their Vitameter assays.

Four laboratories reported results of spectrophotometer assays of the unsaponifiable portion of the cod liver oil samples. In this series the greatest deviation was for Sample No. 1 of which the maximum value, 1.43 (Laboratory No. 3) is 99%, larger than the minimum value, 0.72 (Laboratory No. 9). The least deviation in this series was for Sample No. 7 of which the maximum value, 1.35 (Laboratory No. 3), was 5% greater than the minimum value, 1.29 (Laboratory No. 13). The "E value" for the unsaponifiable portion of the U. S. P. Standard of Reference Cod Liver Oil was 1.22 as compared with 1.45 obtained by the A. D. M. A. Laboratories on the Vitameter.

Four laboratories reported spectrophotometer assays for the cod liver oil concentrates. Of this series the greatest deviation in the results was for Sample No. 13 of which the maximum value, 20.10, was 25% greater than the minimum value, 16.13. The least deviation was for Sample No. 15 of which the maximum value, 67.41, was 8% greater than the minimum value, 62.40.

Referring to the results for the halibut liver oil series, the greatest deviation between the four different laboratories was for Samples No. 17 and No. 24, the maximum value reported for each of these being 29% larger than the minimum value. The least deviation was for Sample No. 19 of which the maximum value, 16.95, was only 9% greater than the minimum value, 15.50. Here also the average vitameter data for the corresponding 9 halibut liver oils ran definitely higher than those for the spectrophotometer.

In the case of the spectrophotometer assays of the 6 "other fish liver oils," the greatest deviation in this group was for Sample No. 30 of which the maximum value, 59.80 (Laboratory 9), is 18% greater than the minimum value, 50.79 (Laboratory 3). The least deviation in this series was for Samples No. 28 and No. 29, the maximum values reported for both of these samples were only 6% greater than the minimum values. Here again the Vitameter data for the corresponding oils ran appreciably higher than those for the spectrophotometer.

It is obviously of interest to compare the results obtained with the Vitameter and spectrophotometer under comparable conditions in the same laboratory. Three laboratories (Table IV) made such comparable assays. The average "E values" obtained with the Vitameter in the cod liver oil series are somewhat larger than the average results obtained with the spectrophotometer. However, the Vitameter results obtained by Laboratory No. 3 are higher than those obtained with the spectrophotometer for only five of the eleven cod liver oils. With two exceptions the results reported by Laboratories Nos. 9 and 10 for cod liver oils were uniformly higher for the Vitameter than with the spectrophotometer.

The spectrophotometer "E values" were lower for the unsaponifiable portion of the cod liver oil samples than for the liquid oils. However, the same general trends between the various oils occurred in the former as in the latter series.

The average Vitameter "E values" of the five cod liver oil concentrates were higher than those of the spectrophotometer for Samples No. 12 and No. 16, but the results obtained with the spectrophotometer were on the other hand larger than the Vitameter results for Samples Nos. 13, 14 and 15. The cause of this inconsistency is not known.

In the case of the Halibut Liver Oils the average of the Vitameter "E values" reported by the four laboratories is consistently greater than the average spectro-

photometer "E values" reported from the same laboratories. However, inspection of the results of the individual samples shows that the variations in the Vitameter results were greater than the spectrophotometer results.

To sum up, the general impression from the data, in Tables III and IV, is that comparing corresponding individual samples the Vitameter gives values that run higher than those with the spectrophotometer, irrespective of the type of product under test. On the other hand, it would seem in assaying the same oils (or concentrates) by the different spectrophotometers that the values showed a much wider variance than one would have expected or predicted. The fault would hardly seem to lie in the lack of uniformity of the samples sent out since all precautions were taken to avoid this. There is a tendency for certain samples to react peculiarly and this makes it difficult to evaluate them as precisely as desired. This happens with both the Vitameter and the spectrophotometer. There are instances on the other hand where the data for the same oils are in very close agreement when obtained by one type of instrument and at wide variance when determined with the other instrument. In general, the Vitameter results, as stated previously, show a definite indication of falling into groups.

Since the principal interest in this study was concerned with the possible practical usefulness of the Vitameter for making Vitamin A assays of cod liver oil and related products, a statistical study has been made of the results obtained with the Vitameter. Since a survey of the data revealed that there were probably fewer variables in the assay procedures of the seven A. D. M. A. Vitamin Committee Laboratories than in those of coöperating laboratories, it was decided to confine the statistical analyses to the data in Table I.

On comparing the values for each laboratory with the average for the seven, it will be seen that the data for individual laboratories tend to vary in a definite direction. For instance, in the case of Laboratories Nos. 1 and 2, their results are definitely below the average, while those for No. 7 are decidedly above the average. The values for the other 4 laboratories fall in between. In Table V, the data have been calculated to bring out this tendency more clearly by expression as plus or minus the per cent of deviation from the average. The results for the liquid cod liver oils show that the Vitameters for Laboratories Nos. 1, 2 and 7 gave distinctly different values than those for Laboratories Nos. 3, 4, 5 and 6. The averages for the eleven samples (Line Ib) for the respective laboratories are, -15.0, -11.8, $+3.3$, $+2.2$, $+4.4$, $+2.7$ and $+13.1$. If one compares the data for the other samples, non-saponifiable fraction, cod liver oil concentrates, halibut liver oils, etc., it is seen that each instrument has, within limits, a tendency to give a characteristic deviation. To obtain the most representative and fairest cross-section picture of this for each Vitameter when used under conditions as laid down here, one should consider the averages of all 45 samples as given in Line IXb. These data indicate pretty accurately how far below, or above, the average the true values lie. In other words, with these data it is suggested that each one apply a correction factor to his particular equipment and in this way bring his values more in line with the others. To illustrate, Table V, line X, Laboratory No. 1, the proposed factor is 1.155 and for Laboratory No. 7 it is 0.775.

The results in Table I have been recalculated by applying these respective factors. These recalculated results are given in Table VI. A careful comparison of

the corresponding values in the two tables shows that the variations have been smoothed out to a considerable degree, especially for the more potent oils. There are still some gross exceptions which would seem to be due to possible errors in making a thorough mixing of the dilutions, to contamination of the cell walls with oil film, or to other factors which are not obvious or easily detected.

This method of calculation would seem to show that the Vitameter in order to be capable of measuring vitamin A most accurately, for comparative purposes, should be checked or standardized against some oil or group of oils which have been carefully read on a standard spectrophotometer and preferably bio-assayed against a standard or suitable sub-standard oil, and also read on standard spectrophotometer.

The most logical oil to use for this purpose is the U. S. P. Reference Cod Liver Oil which has an assigned biological value of 3000 units per Gm. For a more satisfactory standardization of the Vitameter, it might be more feasible to use a high potency oil, like halibut liver oil, which had been biologically evaluated against the U. S. P. Reference Oil. The procedure would then call for each laboratory to determine its own "E value" of the standard oil and from this calculate its own conversion U. S. P. units per Gm. factor. In practice even a standard spectrophotometer should be calibrated by this procedure.

SUMMARY AND CONCLUSIONS.

The data from the various laboratories show that individual laboratories can obtain consistent results, and that the results of different laboratories bear a fairly constant relation to those of other laboratories.

It is quite apparent from the results of this study that in order to correlate the data of different laboratories it is essential to refer the data of all laboratories to a standard of reference. The U. S. P. Standard of Reference Cod Liver Oil, which is being widely used for biological assays, is well suited to this need. The average "E value" obtained for this oil by the Vitamin Committee was 1.61.

The Vitamin A potency of the U. S. P. Standard of Reference Cod Liver Oil has been officially designated as 3000 Vitamin A units per Gm. Hence, the tentative factor for converting Vitameter E values into U. S. P. XI Vitamin A units per Gm. is 1875.

It is apparent from the statistical discussion of the data obtained in this study, that in conducting Vitamin A assays each laboratory must apply a factor for converting its "E values" into U. S. P. Standard of Reference Cod Liver Oil biological Vitamin A units.

In practical operation the Vitamin A potency of a cod liver oil may be computed in terms of U. S. P. XI units per Gm. from the Vitameter "E value" by

$$\frac{1.61 \text{ times } 1875 \text{ times "E value" of oil being assayed}}{X}$$

where X = the "E value" obtained by the individual laboratory for the U. S. P. Standard of Reference Cod Liver Oil.

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