fluxed with ether for several hours. The ethereal solution was filtered and the ether removed, when several Gm. of a mixture of fatty acids were obtained. They have not been identified.

Ether Extract of the Resin.—The ether extract of the resinous mass (B), after removal of the solvent and drying in a desiccator over sulfuric acid under reduced pressure, was a dark green, soft, resinous mass weighing 18 Gm. This was dissolved in hot alcohol, boiled with decolorizing charcoal and filtered. A black solution was obtained. The liquid was again boiled with decolorizing charcoal and a black solution was again obtained. The alcohol was removed; the resinous residue obtained dissolved in ether and extracted successively with ammonium carbonate, potassium carbonate and potassium hydroxide solutions. These alkaline solutions extracted only resinous material.

Chloroform Extract of the Resin.—This extract was a dark brown, resinous mass weighing 6 Gm. from which no pure substance could be obtained.

SUMMARY AND CONCLUSIONS.

1. The most important constituents of the leaves of *Ipomœa Pes-Caprœ* are: mucilage, a volatile oil, a complex resin, fat, a phytosterol, bitter substances and red coloring matter.

2. Neither the leaves nor extracts of the leaves appear to have any noticeable pharmacological activity.

3. Ointments prepared from the leaves and extracts of the leaves have no antiseptic action.

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A COMPARATIVE STUDY OF THE COLORIMETRIC, VITAMETER AND BIOLOGICAL TESTS FOR VITAMIN A.*

BY A. BLACK, R. D. GREENE, H. L. SASSAMAN AND C. SABO.[1]

Although the biological method of testing vitamin A is still considered by many to be the only absolutely reliable one, certain chemical tests and physical measurements have been very extensively used in recent years. Rosenheim and Drummond (1) reported in 1925 that arsenic trichloride gave a blue color with fish oils which was proportional to the vitamin A content. A little later Carr and Price (2) developed the antimony trichloride test. This latter procedure has been modified and improved by many and especially by Wokes and Willimott (3) and Norris and Danielson (4).

In 1928 Morton and Heilbron (5) reported that the absorption of light of 3285 Ängström units by fish liver oils and "A" concentrates ran closely parallel

* Scientific Section, A. PH. A., New York meeting, 1937.

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with the "A" content. Spectrophotometric tests have been developed and widely used. Hilger (6) developed a simple instrument, which was named a vitameter, to measure the absorption of light of about 3285 Ängström units. Although this instrument does not afford the precision of measurement of the spectrophotometer, it has been far more widely used, because of cost and convenience.

The literature on these chemical and physical tests is far too expansive to include in this report, but for the most part there has been fairly good agreement with the biological methods. However, it is felt that the data on the direct comparisons with the biological tests are limited. This laboratory has been making the antimony trichloride color test and vitameter tests along with the biological tests for a number of years and has accumulated a considerable amount of data which should be useful in determining the reliability and limitations of these various substitute methods.

Data on 115 samples of fish liver oils and concentrates are included in this study and all have been obtained since adoption of the new U. S. P. method. Earlier results have not been included because of the possible lower accuracy of the biological data. Furthermore, the results on all samples which have been tested biologically at dose levels suitable for accurate interpretations are included in this study.

EXPERIMENTAL.

Biological Method.—The U. S. P. XI (formerly U. S. P. X 1934 revision) procedure was followed in all cases. A master curve was used as an aid in interpreting the results and to increase the accuracy.

Antimony Trichloride Color Test.—In developing our method for making the color tests, advantage has been taken of the various improvements which have appeared in the literature, particularly the work of Norris and Danielson (4). The Rosenheim-Schuster tintometer was tried but found to be less satisfactory than standard color solutions, which are prepared from copper sulfate and cobalt chloride. Such color solutions were suggested by Arny and Taub (7).

The details of the method are given below:

Reagents: Chloroform—U. S. P. chloroform is treated with anhydrous calcium chloride and allowed to stand. The clear solvent is drawn off and kept under anhydrous conditions.

Antimony-Trichloride Reagent—C.P. Antimony trichloride is dissolved in anhydrous chloroform at 40° C. The solution is cooled to 20° C. and allowed to settle. The clear liquid is decanted off under as near anhydrous conditions as possible. This reagent is stored and may be used over a period of several months.

Color Standards—to 5-cc. portions of 0.5*M* $CuSO_4.7H_2O$ solution in 1% HCL, 0-, 0.1-, 0.2- and 0.3-cc. portions of 0.5*M* $CoCl_2$ in 1% HCl are added. These solutions are diluted to 10 cc. with 1% HCl. A series of color standards is prepared from these 5 solutions by adding 2-cc. portions to test-tubes of 1-cm diameter. These tubes are then sealed and may be used over a year. In fact, we have never detected any changes even after standing several years. These five tubes have different shades of red and blue and in making a test, the tube which matches the color of that produced by the sample is selected.

Procedure: Samples which contain more than 10,000 U. S. P. XI units of vitamin A are tested directly. The test is made on the unsaponifiable extract of all samples which fall below this figure. The unsaponifiable extract is prepared as follows: One gram of the oil is saponified with 10 cc. of 0.5*N* alcoholic KOH solution by boiling 5 to 10 minutes. Twenty cubic centimeters of water are added and the solution is extracted twice with 25-cc. portions of ether. The ether extracts are combined and washed once with 20 cc. of 0.5*N* KOH solution and with 10–20-cc. portions of water until free of alkali. The solution is then evaporated to dryness under anaerobic conditions and the residue is dissolved in anhydrous chloroform and is then ready for color test.

The oil or unsaponifiable fraction is diluted with anhydrous chloroform. Two-tenths cubic centimeter of the diluted sample is added to a test-tube of 1-cm. diameter and 1.8 cc. of antimony trichloride reagent is added very rapidly. A blue color develops immediately and reaches a maximum after a few seconds. This color is compared with the standard tube which best matches the shade. For most fresh oils or unsaponifiable fractions, the tube with the lowest amount of cobalt chloride gives the best match. The dilution of the sample is adjusted so that the blue color produced with antimony trichloride just matches the standard. One of these color units is equal to 6 U. S. P. XI units of vitamin A, a figure which is based on a great number of tests on the U. S. P. Reference sample.

Vitameter Test.—The regular procedure for the vitameter was followed for most of the tests. However, more recently a photographic attachment, patterned after that of Notevart (8) has been employed. Absolute alcohol and isopropyl alcohol were used as the solvent, but no differences were noted. As in the case of the color tests, the unsaponifiable fraction was used when the fish oil contained less than 10,000 U. S. P. XI units of A per Gm. For conversion of E values into U. S. P. XI units, a factor of 2000 has been used. This factor is based upon the results of a great number of tests on the U. S. P. reference cod liver oil.

DISCUSSION OF RESULTS.

Tables I to V, inclusive, contain the results of the tests on the different kinds of samples. Each table contains the vitamin A content, expressed in terms of U. S. P. XI units, as determined by the different methods, as well as the percentage differences. Table VI contains a summary of these differences and also shows the distribution of samples which show the poorest agreement with the biological tests.

TABLE I.—COD LIVER OIL.

Sample No.	U. S. P. XI Units Bio. Test.	U. S. P. XI Units Color Test.	U. S. P. XI Units Vitameter.	% Difference Color Test.	% Difference Vitameter.
957	2400	2670	2520	+11.2	+ 5
1008	2350	1990	1983	−15.3	−15.6
1017	2100	2340	2440	+11.4	+16.2
1020	2500	2560	2360	+ 2.4	− 5.6
1021	2450	2305	2320	− 5.9	− 5.3
1023	2940	2970	3050	+ 1.0	+ 3.7
1045	2640	3820	3800	+44.7	+43.8
1055	2300	1990	2100	−13.5	− 4.3
1056	3200	3170	3200	− 0.9	0
1074	6300	4170	4267	−33.8	−32.2
1097	1675	1785	1933	+ 6.5	+15.4
1107	2500	2360	2260	− 5.6	− 9.6
1109	2150	2090	2160	− 2.8	+ 0.4
1115	2300	2320	2560	+ 0.8	+11.3
1128	2300	2560	2560	+11.3	+11.3
1141	10,500	13,945	12,600	+32.8	+20
1242	1950	1985	1800	+ 1.8	− 7.7
1278	1950	1985	2000	+ 1.8	+ 2.5
1285	2075	1700	1600	−18.0	−22.8
1286	2050	1700	2000	−17.0	− 2.5
1288	1950	1700	2200	−12.3	+12.3
1290	2175	2000	1870	− 8.0	−14.0
1413	1725	1540	1600	−10.7	− 7.2
Av.				− 0.8	+ 0.6

TABLE II.—HALIBUT LIVER OILS.

Sample No.	U. S. P. XI Units Bio. Tests.	U. S. P. XI Units Color Tests.	U. S. P. XI Units Vitameter.	% Difference Color Test.	% Difference Vitameter.
958	12,000	14,720	13,700	+22.7	+14.2
966	156,000	155,560	155,650	− 0.3	− 0.3
967	76,000	67,050	57,760	−11.6	−24.0
971	130,000	125,160	113,200	− 3.7	−12.9
1002	116,000	107,280	97,020	− 7.5	−16.3
1003	116,000	110,260	94,380	− 4.8	−18.6
1009	32,250	38,740	38,800	+20.1	+20.3
1013	115,500	107,280	80,250	− 7.1	−30.5
1014	111,000	89,400	77,930	−19.4	−29.8
1058	175,000	146,615	163,680	−15.9	− 6.8
1078	202,000	177,600	156,920	−12.1	−22.3
1085	124,000	102,690	99,050	−17.2	−20.1
1089	56,300	49,010	49,820	−12.8	−11.5
1094	36,000	36,060	36,960	+ 0.1	+ 2.7
1106	65,000	53,040	44,350	−18.4	−31.7
1116	153,000	151,980	161,570	− 0.6	+ 5.6
1117	150,000	161,810	157,340	+ 7.8	+ 4.8
1166	62,500	66,750	62,000	+ 6.7	− 0.8
1251	187,500	180,580	202,000	− 3.6	+ 7.7
1252	123,000	135,290	135,000	+10.0	+ 9.7
1257	138,000	141,850	145,000	+ 2.8	+ 5.0
1258	54,000	45,830	56,000	−15.1	+ 3.7
1263	89,700	85,100	92,000	− 5.1	+ 2.5
1345	82,000	80,000	92,000	− 2.4	+12.2
1365	8,000	6,000	5,800	−25	−27.5
1399	200,000	222,480	287,500	+11.2	+43.7
1401	160,000	141,180	140,000	−11.8	−12.5
Av.				− 4.2	− 4.9

TABLE III.—FISH LIVER OIL CONCENTRATE TABTETS.*

Sample No.	U. S. P. XI Units Bio. Test.	U. S. P. XI Units Color Test.[a]	% Difference Color Test.
973	2520	2238	−11.2
975	2500	2300	− 8.0
978	2700	2200	0
987	3000	2770	− 7.7
988	3000	2450	−18.3
999	2400	2390	− 0.4
1001	3000	2380	−20.6
1007	3300	2330	−29.3
1015	2100	2290	+ 9.0
1019	2220	2450	+10.3
1030	2500	2580	+ 3.2
1041	1900	2510	+32.1
1048	2700	2600	− 3.7
1052	2350	2800	+19.1
1054	2650	2760	+ 4.1
1063	2500	2680	+ 7.2
1065	2660	2640	− 0.7
1069	2600	2480	− 4.6
1072	2300	2390	+ 3.9
1075	2550	2390	− 6.2
Av.			− 1.1

* Squibb Adex Tablets. [a] The color test gives low readings for these tablets and a correction factor of 1.2 is used in calculating these values.

TABLE IV.—FISH LIVER OIL CONCENTRATE TABLETS.*

Sample No.	U. S. P. XI Units Bio. Tests.	U. S. P. XI Units Color Tests.[a]	U. S. P. XI Units Vitameter.[b]	% Difference Color Test.	% Difference Vitameter.
1083	4500	4520	..	+ 0.4	..
1091	4300	4290	..	− 0.2	..
1098	3600	4290	..	+19.1	..
1100	4100	4400	..	+ 7.3	..
1133	4000	4290	..	+ 7.2	..
1139	4000	2760	3300	−29.3	−17.5
1151	3950	3900	..	− 1.2	..
1161	3600	3860	..	+ 7.2	..
1167	3460	3900	..	+13.0	..
1169	3850	3760	3590	− 2.3	− 6.7
1181	3700	3730	3500	+ 0.8	− 5.4
1187	4100	3900	3750	− 4.8	− 8.5
1212	3950	3750	3690	− 5.5	− 7.0
1222	4100	3900	3780	− 5.1	− 8.5
1233	4100	4290	4230	+ 4.6	+ 3.1
1238	3500	3900	4080	+11.4	+16.5
1267	3500	3400	4370	− 2.8	+24.8
1292	3600	3760	3870	+ 4.4	+ 7.9
1306	3970	3760	4080	− 5.2	+ 2.7
1307	4025	3760	4080	− 6.5	+ 1.3
1315	3780	4200	4200	+11.1	+11.1
1326	4100	4500	4000	+ 8.9	− 2.4
1331	3900	4500	4000	+15.4	+ 2.5
1344	4300	4930	4250	+14.6	− 1.2
1367	3850	3600	4060	− 6.5	+ 5.4
1374	3670	5150	5220	+40.3	+42.2
1383	3500	4500	4640	+28.5	+32.5
1396	4200	5220	4640	+24.3	+10.5
1407	4020	4230	4060	+ 5.2	+ 1.0
Av.				+ 4.8	+ 5.0

* Squibb Adex Tablets.

[a] A correction factor of 1.2 is used in calculating these values.

[b] A correction factor of 1.16 is used in calculating these values.

TABLE V.—MISCELLANEOUS FISH LIVER OILS.

Sample No.	Description.	U. S. P. XI Units Bio. Test.	U. S. P. XI Units Color Test.	U. S. P. XI Units Vitameter.	% Difference Color Test.	% Difference Vitameter.
1127	Tuna liver oil	73,000	77,480	80,250	+ 6.1	+ 9.9
1129	Tuna liver oil	109,000	96,250	89,760	−11.7	−17.6
1144	Tuna liver oil	80,000	85,225	78,140	+ 6.5	− 2.3
1171	Tuna liver oil	113,500	135,290	160,000	+19.2	+40.9
1173	Tuna liver oil	131,000	190,720	192,000	+45.6	+46.6
1253	Tuna liver oil	40,000	39,730	43,400	− 0.6	+ 8.5
1283	Tuna liver oil	53,500	47,680	51,500	+10.8	+ 3.7
1165	Tuna liver oil	39,960	39,760	.....	− 0.5	..
1215	Tuna liver oil	32,400	34,020	39,360	+ 5.0	+21.4
1369	Tuna liver oil	47,000	50,000	51,400	+ 6.4	+ 9.4
Av.					+ 8.7	+12.0

TABLE V.—*Continued from page 203.*

1136	Mixed fish liver oil	14,400	13,530	14,000	− 6.0	− 2.0
1062	Sword fish liver oil	96,700	93,570	81,310	− 3.2	−15.8
1025	Sword fish liver oil	236,000	247,930	240,130	+ 5.0	+ 1.7
995	Salmon liver oil	150,000	137,080	105,990	− 8.6	−29.3
994	Salmon liver oil	150,000	149,000	138,750	− 0.6	− 8.0
1112	Halibut liver oil concentrate	50,000	82,840	93,390	+65.6	+86.7

TABLE VI.—SUMMARY OF DIFFERENCES FROM BIOLOGICAL TESTS.

Product.	Color Test. No. of Samples.	Color Test. Greater Than ±10%.	Color Test. Greater Than ±20%.	Vitameter Test. No. Samples.	Vitameter Test. Greater Than ±10%.	Vitameter Test. Greater Than ±20%.
Cod liver oils	23	12	3	23	11	3
Halibut liver oil	27	13	2	27	16	7
Fish liver oil tablets	49	10	4	21	7	3
Other fish liver oils and concentrates	16	5	2	15	7	5
Total	115	40	11	86	41	18
%		35	9.5		47	20.7

Before making a comparison of the data it is well to consider the limits of error of the different methods. It is well known that biological tests in general are subject to considerable error. We have found that the results of twenty assays made on the same cod liver oil over a period of two years fell between limits which varied plus or minus 16 per cent of the mean. Furthermore, the color and vitameter results may vary within limits of plus and minus 4 or 5 per cent. Consequently, variations of 20 per cent may easily be explained on the basis of the errors of the methods.

On examining the data one finds very slight differences in the agreement between the chemical and physical tests and the biological tests of the different products. For the most part, however, these differences are not significant. In the case of the fish liver oil concentrate tablets, both the color and vitameter results were low, but when a correction factor was applied, the agreement becomes very good. We have no explanation of this phenomenon, but it is confirmed by the results of several hundred tests which were made before these data were collected. The biological tests invariably confirm the theoretical value, based upon the tests of the concentrates before incorporation into tablets.

With either the vitameter or the antimony trichloride test the agreement with the biological test is very good in all but a rather low percentage of cases. The color test agrees within limits of plus or minus 20% in 90 per cent of the samples while the vitameter agrees in about 80%. Our data show a slight but definite superiority in favor of the color test. Attention is called to the fact that the spectrophotometer was not used in any of the tests, and it is probable that this more sensitive instrument would have given more reliable results.

In general, we believe that our results show that the agreement between the different tests is very good. There are a few cases, regardless of the products studied, in which the variations are far beyond the limits of error of the methods. In a few of these cases the biological test has been repeated and the original results confirmed. In this connection, a sample of halibut liver oil concentrate number 1112, Table V, is very interesting. This concentrate was very old and considerable destruction had occurred. In earlier work we had noted that the color test did not give as reliable results with partially oxidized samples. In all of the other samples, however, relatively fresh fish liver oils or concentrates were tested.

There appear to be a few samples of fish liver oils or concentrates which give results either too high or too low when tested for vitamin A by either the vitameter or the color reaction with antimony trichloride. This possible lack of specificity throws doubt upon the advisability of acceptance of such tests as substitutes for the biological tests. In general the chemical and physical tests agree more closely with each other than with the biological test, and the use of both tests do not furnish a reliable index of what samples must be tested by the longer animal method.

The very good agreement in a high percentage of cases shows definitely that either test may be very useful when run in connection with the biological method. Many animals can be

saved by selection of proper doses, based upon the results of such tests. Furthermore, these results may often serve as a check upon the animal test.

SUMMARY.

1. The vitameter and antimony trichloride tests give results with a very high percentage of fish liver oils and concentrates which are in close agreement with the biological tests.

2. There are a few cases where the discrepancies are beyond the limits of error of the methods and indicate a lack of specificity of either the vitameter or color test. It is doubtful that either method can serve as an absolute substitute for the biological method.

3. Either the vitameter or color test serves a very useful purpose as a supplement to the biological test.

4. The antimony trichloride test gives results fully as reliable as the vitameter. In general both methods give results which are in close agreement with each other.

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DRUG EXTRACTION. XVI. THE EFFECT OF THE FORM OF THE PERCOLATOR ON THE EFFICENCY OF EXTRACTION.*[1]

BY WILLIAM J. HUSA[2] AND C. L. HUYCK.

Although a great many forms of percolators have been advocated in more than a century of percolation history there have been surprisingly few published reports of exact comparisons of the efficiencies of the different types. In 1878, J. U. Lloyd (1) measured the effect of varying the diameter and height of percolator tubes in the extraction of cimicifuga. He found that when maceration after packing was omitted a tenfold increase in the length of the tubes doubled the yield of extractive matter. Recently Büchi and Feinstein (2) carried out percolations of cinchona in order to determine the relative efficiencies of Oldberg percolators, funnels and cylindrical glass tubes of uniform diameter. The relative efficiencies varied somewhat at different stages of the percolation but from the standpoint of practical percolation the differences were surprisingly small. Hence it was concluded that the Oldberg percolator was as efficient as the other forms and was preferable from the standpoint of ease of packing and general convenience.

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[1] This paper is based on part of a dissertation presented to the Graduate Council of the University of Florida by C. L. Huyck, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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