COMPARISON OF SPECTROMETRIC METHOD AND ANTIMONY TRICHLORIDE TEST FOR ESTIMATION OF VITAMIN A POTENCY.*

BY W. S. JONES AND W. G. CHRISTIANSEN.[1]

For determining the Vitamin A potency of cod liver oil the U. S. P. prescribes a biological assay. Any vendor, therefore, who markets this product as U. S. P. oil must be able to show, by the official method, that it complies with the specifications for potency. We know that there may be some differences between human response and the response obtained with different species of animals, and that the selection of the test animal is therefore a matter of great importance, but we realize that products of complex composition, used to produce biological effects, should be fundamentally controlled by a biological assay. Even though Vitamin itself is a definite chemical compound, in cod liver oil it is associated with many other compounds which may be present in varying amounts and which may modify the biological effect.

Thus, for the present, the biological assay must be considered the primary test of potency, and other methods secondary ones, supplying preliminary estimates. We present, in this paper, a comparison of the results obtained by two such methods on oils which had been assayed biologically.

The first of these tests—the Carr and Price antimony chloride test—is well known and widely used. During the years of its use in our several laboratories the minute details of the test, upon which depends the value of the result, have been subjected to careful study and control. Much of that work was done in our Biological Research Laboratories.

The second test is a physical one, and depends upon the intensity of the absorption band at 3280 Å. in the ultraviolet.

These chemical and physical tests are of great value in both manufacturing and research. They are rapid and inexpensive, and may therefore be used at every intermediate step in both laboratory and plant, supplying quickly and cheaply approximate data which make possible quick decisions and rapid progress, with a reasonable expectancy that the biological assay will confirm the correctness of the action taken. It is necessary, of course, always to keep in mind the limits of accuracy of these tests.

Various workers,[2-8] using the spectrometer, or physical method, have shown that there exists a definite and consistent relationship between the intensity of the absorption band at 3280 Å. in the ultraviolet and the biological assay for Vitamin A.

---

* Section on Practical Pharmacy and Dispensing, A. PH. A., Portland meeting, 1935.

[1] Research Department of the Chemical and Pharmaceutical Laboratories, E. R. Squibb and Sons, Brooklyn, N. Y.

[2] Morton and Heilbron, *Biochem. J.*, 22, 993 (1928).

[3] Drummond and Morton, *Ibid.*, 23, 785 (1929).

[4] Coward, Dyer, Morton and Gaddum, *Ibid.*, 25, 1102 (1931).

[5] Evers, Norman and Smith, Brit. Pharm. Conference (July 24–27, 1933); through *Pharm. J.*, 131, 128 (1933).

[6] Lathbury, *Biochem. J.*, 28, 2254 (1934).

[7] Chevallier and Chabre, *Bull. soc. chim. biol.*, 16, 1461 (1934).

[8] Emment, *et al.*, Symposium, Am. Chem. Soc. meeting, New York, April 1935.

It was highly desirable, therefore, to compare the newer, physical method with the antimony trichloride color test.

Our preliminary findings show that the spectrometric method gives no better estimation of Vitamin A potency than does the colorimetric antimony trichloride test as used by us.

Eleven oils which had been assayed biologically were tested by the antimony trichloride method and were then sent to laboratories having equipment for the spectrometric assay. Nine oils were sent to Laboratory "A;" four of these plus two additional oils were sent to Laboratory "B." In Table I are listed the results of all three assay methods.

TABLE I.—ASSAY IN TERMS OF U. S. P. 1934 REVISION UNITS OF VITAMIN A PER GM.

Oil No.	Biological Method.	Antimony Tricbloride Method.	% Deviation.	Spectrometric Method. Laboratory A.	% Deviation.	Laboratory B.	% Deviation.
1	85,750	75,600	−11.8	76,440	−10.9	...	..
2	107,450	78,400	−27.0	94,080	−12.4	...	..
3	2,192	2,240	+ 2.2	2,784	+27.0	3,000	+36.8
4	2,156	2,054	− 4.7	2,523	+17.0	...	..
5	3,080	3,156	+ 2.5	2,850	− 7.4	3,760	+21.7
6	2,156	2,279	+ 5.7	2,497	+15.8	...	..
7	2,478	2,409	− 2.8	2,497	+ 0.8	2,730	+10.2
8	3,780	3,347	−11.5	45,225*	Very large	4,100	+ 8.5
9	60,900	64,440	+ 7.3	60,750	− 0.2	...	..
10	236,000	226,000	− 4.2	...	.....	249,000	+ 5.5
11	171,000	156,800	− 9.6	...	.....	110,000	−35.6

* Laboratory A rechecked this figure and found it to be correct.

In Table II the results have been grouped according to the extent to which they differ from the biological assay.

TABLE II.

Deviation from Biological Assay.	Antimony Trichloride Method.	Spectrometric Method. Laboratory A.	Laboratory B.
± 5%	45.4%	22.2%	..
± 5-10%	27.3	11.1	33.3%
±10-20%	18.2	44.4	16.7
±20-30%	9.1	11.1	16.7
±30-40%	..	..	33.3
>± 40%	..	11.1	..

DISCUSSION.

With one exception (Sample No. 11) Laboratory "B" has consistently and with wide variation, obtained higher values for Vitamin A potency than is shown by biological assay.

Excluding Sample No. 8 (we believe the value given for this oil by Laboratory "A" is erroneous and should probably be 4522.5) Laboratory "A" has obtained on oils of high potency (Samples Nos. 1, 2, 9) Vitamin A values in reasonable agreement with the results of biological assay. Greater variations occur, however, in the oils of low potency (Samples Nos. 3, 4, 5, 6, 7).

Except in the case of Sample No. 2 the antimony trichloride test, as used by us, has given Vitamin A values which are in fair agreement with those obtained through biological assay.

We gratefully acknowledge the assistance of the Biological Laboratories of E. R. Squibb and Sons in conducting the biological assays reported herein.

ENTERIC COATINGS. II. EXCRETION STUDIES WITH SODIUM SALICYLATE TABLETS.*

BY MILTON WRUBLE.

In an earlier paper (1) the use of calcium sulphide-methylene blue tablets was found of value in checking the effectiveness of enteric coatings qualitatively. As a further step in this direction it was thought desirable to make a quantitative evaluation. Since salicylates are excreted more or less quantitatively, are widely used in medicine and in many cases produce irritation in the stomach when unprotected by a suitable coating, they were of particular interest in this connection.

Salicylates are excreted quite rapidly but incompletely in the urine, mainly as such, and to a small extent as salicyluric acid (2) and a number of other products. Stockman (3) states: "Salicylic acid and salicylates are conjugated and excreted as salicyluric acid. Persons taking up to 180 grains of sodium salicylate per day eliminate no free salicylic acid." Holmes has found (4) that for doses of sodium salicylate of from 2 to 5 Gm. the salicylic: salicyluric ratio is constant at the value of 40:60.

The absorption of salicylates is quite rapid and for this reason exceedingly small amounts are at times found in the feces, more often none at all. With full therapeutic doses of about 15 Gm., Hanzlik, Scott and Thoburn (5) and Hanzlik and Wetzel (6) were able to recover in the urine about 75 to 80 per cent of the total administered. They concluded that about 20 per cent of the salicylate was destroyed in its passage through the body.

The rate and duration of excretion of salicylates varies with the dosage, the individual and the individual's state of health. In general, it has been found by Blanchier (7) that with doses of 1 to 2 Gm., excretion is completed in 22 hours; Ehrmann (8) found that excretion lasted from 36 to 48 hours in normal individuals; Geissler (9) noted that complete elimination takes place in 12 hours. However, Sée (10) states that it ordinarily lasts from 24 to 48 hours.

The quantitative recovery of salicylates from tissues and body fluids involves difficulties and complexities not present in foods and simple aqueous solutions. This can be readily appreciated since in passing through the body the salicyl group is conjugated with glycocoll forming a salicyluric acid, whose properties differ from salicylic acid. Moreover, the presence of colloidal and other interfering substances prevent a smooth and quantitative recovery of salicyclic acid.

A number of quantitative methods for the determination of salicylates in urine have been developed. None appears entirely satisfactory. A critical survey of these methods has been adequately made by Thoburn and Hanzlik (11). Recently Merz (12) and Blume and Breuning (13) have outlined extraction methods for the determination of salicylates in urine.

* The Research Laboratories, The Upjohn Company, Kalamazoo, Michigan.