PRELIMINARY STUDIES OF THE ROSENHEIM, DRUMMOND COLOR TESTS OF VITAMINE A IN COD-LIVER OIL.*

BY E. C. TOWLE AND E. C. MERRILL.

In the *Lancet* of January 31, 1928, Messrs. Rosenheim and Drummond published the results of a fairly comprehensive study of the relation between their colorimetric test for vitamine A in cod-liver oil as compared with actual biological studies made on the same samples of oil. We believe this pioneer work of Messrs. Rosenheim and Drummond constitutes a valuable line of thought which eventually will lead to the perfecting of a chemically controlled test that may be depended upon as a quick means of estimating vitamine A in cod-liver oil.

Whatever follows, therefore, should be understood as constructive suggestion devoid of criticism of the originators of the test and written with the object of bringing about a concentration of study on this test which will relegate to the background the biological test which is obviously handicapped by the factor of time alone as to be insufficient for commercial needs. In the following the authors have made a special study of the Antimony Chloride method of colorimetrically estimated vitamine A in cod-liver oil described in the *Lancet* January 21, 1928—page 148, as follows:

Carr and Price, *Biochemical Journal*, 20 (1926), 497.

"*Antimony Chloride Method.*—By means of a standard pipette 2 c.cm. of the oil are measured into a measuring flask of 10 c.cm. capacity. The pipette is rinsed out three times with chloroform, and the solution made up to volume with the same solvent; 0.2 c.cm. (= 40 c.mm. oil) of the solution are measured into a test-tube (10 mm. diameter) and mixed with 2 c.cm. of a 30 per cent antimony chloride solution in chloroform delivered from a standard burette. Readings are taken as above described. Limit of error as above."

1. It has been noted that in following technique of the above-mentioned test that the development of blue color and its transitional changes to an ultimate red or muddy-brown color is very positively influenced by the temperature of the reaction mixture in the test-tube. Illustrative of this reaction we may state that in conducting the test at ordinary room temperature (approximately 25° C.) the blue color develops in a few seconds time, and progressively goes through changes from a blue color developing red rapidly and fading to the ultimate red or reddish brown within a few minutes. On the other hand, if the reaction mixture in the test-tube is cooled to a temperature of 10° C. or below, the rate of change of color proceeds at a lesser rate of speed and lasts nearly 20 times longer in duration than at room temperature. With this thought in mind, it is suggested that the ultimate control of the technique of this test may be perfected by specifying a definite working temperature which will enable one to operate under sufficient margin of time to make accurate reading of the color by Lovibond Tintometer or other means.

2. The color reaction in the Antimony Trichloride Colorimetric test appears to consist of two stages:

a. The development of an intense blue color.
b. The fading of the blue and the development of a red color.

In this study we wish to mention the three following experiments:

* Scientific Section, A. PH. A., Portland meeting, 1928.

Experiment No. 1.—This test was conducted in the regular way by the Antimony Trichloride method on some cod-liver oil of known vitamin content that had been subjected to biological assay. The characteristic blue appeared at once, and faded in the usual manner in a few minutes at room temperature.

Experiment No. 2.—Another portion of the original biologically assayed cod-liver oil was subjected to a temperature of approximately 100° C., at the same time being treated by a continuous stream of air passing through the oil. A sample of this oil subjected to the Antimony Trichloride test showed an immediate deep red color.

Experiment No. 3.—A sample of the original biologically assayed cod-liver oil of definite vitamin A content was tested by the Antimony Trichloride method and a few seconds after the reaction had commenced a small quantity of zinc dust was added. The speed of this test as compared with No. 1 test, which was conducted under identical conditions of temperature, was greatly retarded in rate of development of red color.

From the above experiments we would lean to the opinion that the development of red coloration is associated with an oxidation process.

3. Further study of the influence of alcohol to the reaction mixture, using the Antimony Trichloride method, result in the observation that the addition of minute quantities of alcohol tend to speed up the rate of the reaction and speed of change from blue to red color. This being the case, would it not be desirable to use a chloroform that is entirely free from alcohol, since the American market medicinal or U. S. P. chloroform permits the presence of between 99 and 99½% of chloroform and the remainder consisting of alcohol.

4. From "Lewkowitsch's Chemical Technology and Analysis of Oils, Fats and Waxes"—page 270—we quote as follows:

"A very delicate and characteristic reaction, described by Hager, was modified by Salkowski, as follows: A few centigrams of cholesterol are dissolved in 2 cc. of chloroform, an equal volume of concentrated sulphuric acid is added, and the mixture shaken. The chloroform solution is immediately colored blood-red, afterwards cherry-red and purple; the last tint remains for several days. (The sulphuric acid layer under the chloroform shows sometimes a strong green fluorescence.[1]) On pouring a few drops of the purple chloroform layer into a porcelain basin, the red colour changes rapidly to blue, green, and finally to yellow. On diluting the purple solution with more chloroform, it becomes nearly colourless or acquires an intense blue colour; if it now be shaken again with the sulphuric acid, the former colouration reappears. These changes of colour are said to be due to traces of water in the chloroform.[2]

"If a chloroform solution of cholesterol prepared from fats be shaken with concentrated sulphuric acid, the blue colouration is noticed at once; this indicates the presence of so-called "lipochromes," which have been shown to occur in cod-liver oil, the fat of the yolk of eggs, palm oil, and in small quantities in cow butter. But even in these cases the red colouration soon appears."

We believe there is a close analogy between the test of Hager as modified by Salkowski with the Carr and Price Antimony Trichloride reaction especially in

[1] In the case of wool fat cholesterol this green fluorescence is, in the author's opinion, due to the presence of isocholesterol.

[2] According to Herbig (*Dinglers polytech. J.*, 303 (1897), 191), cholesteryl palmitate and cholesteryl cerotate give the same colour reaction.

consideration of the so-called lipochromes as described by these investigators many years prior to the recognition of the various types of vitamins. Of special interest is that portion of the above quotation describing the color test on cholesterol: "On diluting the purple solution with more chloroform, it becomes nearly colourless *or* acquires an intense blue colour." We are wondering if the record of this test on cholesterol did not actually vary with the source of the cholesterol obtained, since the writer in a foot-note states as follows:

"Wool fat cholesterol does not show the violet-pink colouration given by gallstone cholesterol, but becomes red at once."

Reverting again to the Antimony Trichloride test, we note that the behavior of this reaction is influenced in a very uncertain manner by the additions of varying traces of water from which we infer that the balance of moisture content in this reaction mixture would seem to be a desirable subject for further study.

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A LITERATURE REVIEW ON THE PRODUCTION OF ANTIRACHITIC SUBSTANCES BY THE IRRADIATION PROCESS.*

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The development of our knowledge of the antirachitic vitamin within the past five years has been remarkable. No earlier than 1922 was differentiation made between the antiophthalmic vitamin A and the antirachitic vitamin D, occurring together in butter and cod liver oil and prior to that time and even in much literature published after that time, classed together as the fat-soluble A vitamin. Now, although we do not know the exact chemical structure of the vitamin D, we know it contains only carbon, hydrogen and oxygen and a method of synthesis has been arrived at. Simultaneously, our knowledge of the etiology of its deficiency disease, rickets, has shown advance.

Prior to the discovery that certain foods could be given antirachitic potency by irradiation with ultraviolet light, those rays had been used in the therapy of rickets. This work has been reviewed by Park (172). In some cases, eosin was administered before light treatment on the assumption that its ability to absorb ultraviolet light increased its action in the body (72). Rats fed on diets deficient in fat-soluble vitamins did not develop rickets when irradiated with light from a mercury vapor quartz lamp (178) although ophthalmia due to deficiency of vitamin A was not delayed (224). More recently, the effect of sunlight (11) and of ultraviolet light (63,156) in preventing leg weakness and rickets in poultry has been studied. The biologically active wave-lengths in sunlight were found by Luce to be below 296 $\mu\mu$ (153); by Huldschinsky to be from 289 to 320 $\mu\mu$ (128); and by Hess and Weinstock using Corning glass filters to be below 303 $\mu\mu$ or possibly 313 $\mu\mu$ (198). Similar protection of rats from rickets by much more prolonged irradiation (18 times) with Wood's light (about 365 $\mu\mu$) has also been reported (166).

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* Scientific Section, A. PH. A., Portland meeting, 1928.