We wish to acknowledge here the stimulation accorded us by Dr. Ralph R. Mellen, now of the Western Pennsylvania Hospital, Pittsburgh, Pa., through discussion, correspondence and his own admirable papers treating the subject of microbic heredity, and also by Dr. F. E. Lutz, of the American Museum of Natural History, for his interest in general.

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COD-LIVER OIL AND THE ANTIMONY TRICHLORIDE REACTION FOR VITAMIN A

DURING the months of January, February and March, 1928, I was in the Lofoten Islands, Norway, making a rather comprehensive survey of matters of importance in connection with cod livers and the oil produced from these livers. Many samples of oil were exported by me to the United States for animal assay and other lines of investigation. In addition to work of this character I was desirous of securing definite information as to the vitamin values of absolutely fresh Norwegian cod-liver oils produced by different methods from livers treated in a variety of ways. The use of biological methods of assay being out of the question, I equipped myself with a Lovibond tintometer and proceeded to assay my oil samples for vitamin A by the antimony trichloride reaction which was being widely used in England for the quantitative estimation of this vitamin in cod-liver oil.

In the course of my tests I made an observation which appeared to cast grave doubt upon the validity of the claim that the blue color produced in cod-liver oil by the addition of antimony trichloride is due to vitamin A. Since my return from Europe a very comprehensive and critical study has been made in our laboratories of the antimony trichloride reaction, with the result that we believe we have demonstrated most conclusively that this reaction does not afford a reliable means of determining the vitamin A content of cod-liver oil. We are now preparing full details for early publication. Our first Norwegian observation which cast doubt upon the validity of the claims made for the color reaction follows.

It is pretty generally accepted that cod-liver oil deteriorates when left open to light and air and that this deterioration is indicated, among other things, by a decrease in the vitamin A potency. In order to learn just how rapidly the vitamin A of fresh Norwegian cod-liver oil is destroyed when free access of light and air is permitted, I took two small tin pans of the same size and introduced the same volume of fresh oil into each and took a third sample for immediate colorimetric examination. I then placed one pan of oil in a clean, dry, dark closet and left the other pan of oil outdoors during the day uncovered, thus affording free access to rain, snow, sunlight and such particles of cinders and other dirt as might be blown into it. At night the pan was brought into the laboratory until morning. In the event of a driving rain or a particularly heavy fall of snow during the day, the pan was rescued and brought into the laboratory, but the oil was actually exposed to the elements for a total of seventy-nine hours during a period of two weeks.

It is doubtful if any sample of cod-liver oil was ever treated in a more shameful manner, during the time that its companion sample was supposed to be conserving its vitamin A in the seclusion of the dark closet.

At the end of two weeks these two oils were examined by me in the tintometer, using the antimony trichloride procedure. When I found that the cod-liver oil which had been standing open to air and light gave *a deeper blue color* than the original oil or the oil kept in the dark, I concluded that I had made some error in the technique and discarded all solutions and reagents and did not bother to finish the test. However, after making up a new oil solution with a different sample of chloroform and using a different bottle of antimony trichloride, I again found that the cod-liver oil which had been standing outdoors for seventy-nine hours possessed a higher value than either the original oil or the oil I had carefully protected in the dark closet. I then began to wonder if my eyesight had been injured through the monotony incident to looking at nothing but codfish, cod livers and cod-liver oil for a rather long interval. The idea that the antimony trichloride reaction was inaccurate did not at that moment suggest itself to me. However, when I examined the three samples of oil again on the following day and verified my previous findings, my confidence in the accuracy of the reaction was materially lessened.

Starting from the above initial observation, we believe that we have collected a mass of experimental evidence which very definitely indicates that the antimony trichloride reaction is not an accurate measure of the vitamin A potency of cod-liver oil. If this is true we must still consider the animal assay as the only accurate method by which cod-liver oil may be assayed for vitamin A.

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