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Effect of Sulphur Dioxide on Vitamin A Activity of Cod Liver Oil, Butter, and Alfalfa.

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The work here presented was conceived by the observations of Bills[1] on the action of various reagents on the antirachitic vitamin, and was carried to completion in 1927. It occurred to us that an extension of Bills' work to vitamin A might be of value in throwing light on the chemical nature of this factor.

Of the reagents examined by Bills, sulphur dioxide was found to be without action on the antirachitic vitamin. Early in our investigation, however, we found the effect of this substance on vitamin A in cod liver oil to be of such a magnitude as to render desirable an examination of vitamin A from other sources.

In doing so we were guided by two principal considerations: (1) whether vitamin A activity is the property of a single chemical individual or that of a specific atomic grouping possessed in common by several different molecules; (2) assuming that vitamin A of the green plant would be as susceptible to destruction by sulphur dioxide as vitamin A of animal origin, it occurred to us that one important aspect of the damage to vegetation by smelter smoke would be the destruction of vitamin A. With these considerations before us we gave primary attention to experimentation with sulphur dioxide. Cod liver oil, butter, and extracts of alfalfa were used.

Cod liver oil was treated by bubbling with the purified gas at temperatures of 20° to 100° for periods of 15 minutes to 2 hours. The excess of sulphur dioxide remaining at the end of the experimental time was removed by exposure *in vacuo*.

Butter was melted at 60°, freed of casein by filtration, and treated with sulphur dioxide at 60° for 1 to 24 hours.

Alfalfa "leaves and blossoms" of high vitamin A activity were exhaustively extracted at 35° with many portions of alcohol for a total period of 35 hours. The combined extracts were concentrated on a steam bath and divided into 2 portions. One portion was reserved for the control diet, the other was treated with sulphur dioxide by bubbling at 60° for 1 hour. The cod liver oil, butter, and alfalfa extract, treated and untreated, were added to dextrin and

[1] Bills, C. E., *J. Biol. Chem.*, 1925, lxiv, 1.

[2] Steenbock, H., *Science*, 1923, lviii, 449.

incorporated in the diet in various known proportions. 119 animals were used in the experiments on cod liver oil, 54 on butter, and 103 on alfalfa and spinach. In testing for vitamin A the growth method of Drummond and Coward[3] and the curative method of Steenbock and Coward[4] were employed. The results may be summarized as follows:

In cod liver oil, treatment with sulphur dioxide for as short a time as 15 minutes at room temperature was sufficient to cause a complete loss of the growth-promoting, anti-xerophthalmic activity. The destruction was complete as indicated by feeding the treated oil over a wide range (0.25 to 5% of the whole ration). Under fairly similar conditions (1 hour at 60°) the destruction of vitamin A in alfalfa extracts was inappreciable. Butter occupied a somewhat similar position. Though treated for 2 hours at 60° and fed at a level of 5%, the extract continued to show marked vitamin A activity. Even after 22 hours of treatment much of the activity remained. When fed at the lower level of 2%, partial destruction of the vitamin became evident.

In conclusion, the vitamin A activity of cod liver oil is to be regarded as the property of a substance or substances not contained in significant quantities in butter and extracts of alfalfa, or else vitamin A of plant origin is associated with a protective substance not found in cod liver oil. These alternative explanations will be discussed in the final report with the assistance of additional experimental data on the inactivation of vitamin A.

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Effect of Adrenalin and Asphyxia on Blood Sugar of Amytalized Dogs.

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Recent work has thrown doubt on the presumption that use of amytal (isoamyl ethyl barbituric acid) as an anesthetic is without interference to blood sugar regulation.[1] In spite of the finding of

[3] Drummond, J. C., and Coward, K. H., *Biochem. J.*, 1920, xiv, 668.

[4] Steenbock, H., and Coward, K. H., *J. Biol. Chem.*, 1927, lxxii, 765.

[1] Olmsted and Giragossintz, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 103.