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A Color Reaction for Carotene.

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Carotene in chloroform solution reacts with formaldehyde in sulphuric acid to form a characteristic deep violet zone between the chloroform and acid layer. The violet zone on shaking disseminates in the acid giving rise to a violet colored lower layer, while the chloroform layer above remains colorless. The addition of 2 to 3 drops of acetic anhydride causes a transitory blue to appear in the acid layer, provided a high concentration of carotene is present in the chloroform solution.

The test, which is considered positive on the appearance of a characteristic deep violet zone, is very sensitive, serving to detect 0.01 mg. of carotene in 2.5 cc. of chloroform. To carry out the test equal volumes of chloroform and formaldehyde-sulphuric acid mixture should be employed. For convenience we have used 2.5 cc. of the chloroform solution with 2.5 cc. of the aldehyde-acid mixture. This mixture is made up in the proportion of 1 volume of 37 to 40% formaldehyde to 50 volumes of concentrated sulphuric acid. Both the formaldehyde and the sulphuric acid should be chemically pure, and the mixture freshly made before using.

Cholesterol, according to Whitby,[1] gives a characteristic reaction with the formaldehyde-sulphuric acid mixture described above. With 1 to 2 mg. of the sterol dissolved in 2 cc. of chloroform he reported a cherry red color in the upper chloroform layer and a brownish red color in the lower sulphuric acid layer, which also showed an intense green fluorescence. When 2 to 3 drops of acetic anhydride were added to the chloroform layer previously transferred to a dry test tube, a blue color formed. With 0.1 mg. of cholesterol in 2 cc. of chloroform he obtained a deep reddish brown color and a very marked fluorescence in the acid layer, but no color in the chloroform layer. With 0.01 mg. of the sterol he could develop only a faint fluorescence in the acid layer and no coloration in the chloroform layer.

With 0.1 mg. of cholesterol in 2.5 cc. of chloroform we observed with the Whitby procedure a brown color and green fluorescence in

[1] Whitby, G. S., *Biochem. J.*, 1923, **17**, 5.

the acid layer, but no coloration in the chloroform layer. With 0.2 mg. cholesterol in 2.5 cc. chloroform we were able to observe a faint pink in the chloroform layer. From the standpoint of color formation in the upper chloroform layers, we may state that cholesterol is not as sensitive as carotene to the formaldehyde-sulphuric acid reaction.

Using Salkowski's procedure for the qualitative detection of cholesterol, we obtained an indigo blue color with a chloroform solution of carotene to which concentrated sulphuric acid had been added. This indigo blue also develops when crystals of carotene are dissolved in concentrated sulphuric acid. We are therefore led to the belief that the violet zone produced with a chloroform solution of carotene on the addition of formaldehyde-sulphuric acid mixture represents a blending of the blue color arising in the presence of the acid with a red shade probably formed by the intervention of the formaldehyde.

The copper-colored crystals of carotene turn pale yellowish brown on exposure to air and sunlight. The oxidized carotene does not respond to the formaldehyde-sulphuric acid reagent in the characteristic way, developing a brown zone instead of one which is deep violet.

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Liebermann-Burchard Reaction with Compounds Containing Five-Membered Monoheterocyclic Rings.

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Sterols,[1] terpenes,[2] carotene,[3] vitamin A[4] and 5-membered monoheterocyclic ring compounds[5] have the characteristic in common that they react with the Carr-Price reagent, antimony trichloride in

[1] Wokes, A. F., *Biochem. J.*, 1928, **22**, 830; Heilbron, M., and Spring, F. S., *Biochem. J.*, 1930, **24**, 133; Seel, H., *Arch. ex. Path. u. Pharmakol.*, 1931, **159**, 92.

[2] Levine, V. E., and Richman, E., *Biochem. J.*, 1933, **27**, No. 6, in press.

[3] Moore, T., *Lancet*, 1929, **1**, 499; von Euler, B., von Euler, H., and Hellström, H., *Biochem. Z.*, 1928, **220**, 370; Karrer, P., *Bull. Soc. Chim.*, 1928, **43**, 1041.

[4] Carr, F., and Price, T. A., *Lancet*, 1929, **2**, 806; Willimott, B. G., and Wokes, F., *Lancet*, 1927, **2**, 8.

[5] Levine, V. E., and Richman, E., *J. Biol. Chem.*, 1933, **101**, 373.