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.

chloroform solution, and with the Shear reagent, aniline-hydrochloric acid.[6] Reasoning on the probability of the existence of some common factor in the chemistry of sterols, terpenes, carotene and vitamin A, we determined to find out whether the Liebermann-Burchard reagent, used qualitatively in the detection of cholesterol and quantitatively in its estimation, was also given by the other compounds in the group we have mentioned.

We accordingly studied the Liebermann-Burchard test with respect to certain 5-membered monoheterocyclic compounds. Unless otherwise stated, we adopted the procedure of dissolving 1 to 2 drops of a given liquid compound to be tested or 10 to 20 mg. of a solid compound in 2 cc. of chloroform, adding 10 drops of acetic anhydride and 1 to 3 drops of concentrated sulphuric acid. The color reaction undergoes many variations depending upon the concentration in chloroform of the compound tested and the quantity of concentrated sulphuric acid added.

Pyrrol at first gave a yellow turbidity, which gradually turned to very deep orange. On standing a deep blood-orange precipitate separated out, leaving a colorless liquid above. On the addition of sulphuric acid alone slightly heavy droplets separate at the bottom. On standing these droplets solidified and developed an orange tinge, which gradually darkened. Thiophene on the addition of 5 drops to 2 cc. of chloroform gave yellow oily droplets at the bottom of the tube. On standing over night the droplets disappeared and the liquid contents of the tube turned green. The reaction was very slow. The green color, however, once formed became deeper and lasted more than 2 weeks. Black granules finally precipitated out in the colored mixture. With sulphuric acid alone yellow droplets formed at the bottom. These gradually turned deep reddish brown, and at the end of a week the colorless liquid assumed a very slight green tinge.

With furfurane a green viscid precipitate formed, which on standing dissolved to form a deep grass-green solution, turning violet and subsequently deep blue, and finally returning to deep grass-green. The reaction with furfurane is very characteristic and very intense. The final color is very stable and remained for more than a week. With sulphuric acid alone, a red-brown gummy precipitate developed, the liquid reaction mixture remaining colorless. With dimethyl furfurane yellowish droplets were formed, clinging to the sides of

[6] Levine, V. E., and Seaman, C. L., *Biochem. J.*, 1933, **27**, No. 6, in press; Levine, V. E., and Shaughnessy, E. J., *Biochem. J.*, 1933, **27**, No. 6, in press.

the tube in a colorless liquid reaction mixture. The droplets changed to deep orange, and finally the colorless liquid assumed a very deep orange, which changed to deep cherry-red. With sulphuric acid alone yellow, oily droplets formed, which finally turned brown.

The Liebermann-Burchard reagent also reacted with other derivatives of furfurane. Furfuryl alcohol gave heavy black precipitate and an intense black liquid. On dilution with 1 to 2 volumes of chloroform, the reaction mixture showed up a green color. Tetrahydrofurfuryl alcohol in chloroform solution after the addition of acetic anhydride developed a green color with 1 drop of sulphuric acid.

Furfural developed a succession of colors ranging from yellow to green to cherry-red, to brown, to purple and finally to deep green. Furfuraldoxime gave a green-yellow color when acetic anhydride alone was added. On the addition of 1 drop of sulphuric acid, the color changed to reddish yellow and finally became reddish brown. Furfural acetone yielded a yellowish brown color which, on the addition of an extra drop of sulphuric acid turned green. Furfural acetophenone produced a greenish yellow, changing to red and finally to brown; furfural diacetate, a cherry-red. Furylacrolein after the addition of 1 drop of sulphuric acid formed a yellow color which changed to green. On the addition of another drop of acid, the color became intense blue. Furylacrolein oxime gave a deep chlorophyll green coloration.

Hydrofuramide turned brown with a greenish tinge. On dilution of the reaction mixture with an equal volume of chloroform the color turned decidedly green with an olive tinge. Both furil and furoin gave the characteristic green color reaction, which is similar to that obtained with cholesterol.

Furoic acid and furoamide displayed no reactivity. The latter is insoluble in chloroform. Furonitrile, dissolving very slowly, yielded a colorless mixture at first, later changing to olive-green. Furylacrylic acid gave a bright red solution which changed to purple and finally to green. Furylacrolyl chloride yielded droplets which eventually turned green. Furylacrylamide produced a yellowish coloration which gradually became green.

We are led to the conclusion that the Liebermann-Burchard reagent not only reacts with cholesterol, but also with thiophene and with furfurane and many of its derivatives.