in Ringer's solution was employed. The tissue extract was an insulin-free pancreatic extract supplied by Sharp and Dohme, marketed under the name of "Tissue Extract No. 568".

The results of 12 experiments are shown in Table I.

Discussion. Saturated solutions of chloroform in Ringer's solution produced only a slight constricting effect. After the vessels failed to respond any longer to the effect of trichlorethylene, epinephrine solution produced a marked constriction. After tissue extract, pitressin produced no significant constriction of the vessels; but the subsequent administration of trichlorethylene produced marked constriction.

Summary. It is of interest that these therapeutic agents used in the treatment of angio-spastic disease should antagonize the action of each other on the blood vessels of the frog.

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Reaction of Trichloracetic Acid and of Chloral Hydrate with Carotene.*

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Trichloracetic acetic acid (9 parts by weight of the crystallized acid and one part of water) gives immediately with carotene in chloroform solution an intense blue color. The reaction develops without the aid of external heat. Three drops of trichloracetic acid solution are mixed with 0.1 cc. of chloroform solution of carotene in order to develop the reaction. The color fades on the addition of water or of alcohol, but not on heating. Spectroscopic examination reveals absorption beginning at 640mμ and continuing to the end of the visible spectrum.

Carotene in solid form on exposure to air and sunlight changes from copper-colored crystals to a light brown powder. When kept in chloroform solution in a glass bottle exposed to sunlight the deep golden yellow color gradually changes to a light yellow brown. Solid

*The carotene used was obtained from the S.M.A. Corporation, Cleveland. It contains β-carotene and a small quantity of α-carotene. Cerevisterol and ergosterol-free cholesterol were obtained from Dr. Charles E. Bills, Director of Researeh, Mead Johnson and Company, Evansville, Indiana.

carotene or carotene in solution oxidized by exposure to air and sunlight gives a dirty greenish coloration, turning to dirty grayish on heating. When a sample of carotene in solid form or in solution capable of yielding the above reaction is exposed for a further period, it yields no color reaction with the trichloracetic acid reagent.

Rosenheim[1] reported a specific reaction for ergosterol based upon the procedure we have employed for carotene. Ergosterol gives an immediate red solution showing an absorption band at 500mμ. The red solution gradually changes to a clear blue solution yielding one absorption band at 570-580mμ and another at 650-680mμ. Cholesterol and other sterols give no reaction in the cold. On heating cholesterol in the water bath, however, a red solution develops showing an absorption band at 500 mμ. Rosenheim was able to detect by the use of trichloracetic acid at room temperature the presence of ergosterol in cholesterol. McDonald and Bills[2] demonstrated a series of color changes for iso-ergosterol with the Rosenheim reagent. Honeywell and Bills[3] reported that cerevisterol reacts with the Rosenheim reagent yielding with minute amounts of this sterol an initial red color, which soon fades, and with higher amounts a red color which gives way to a dirty green or even black coloration. With the Rosenheim reagent we observed with cerevisterol a pink coloration changing to dark brown. When the test with cerevisterol is made by heating the reaction mixture on a water bath, an evanescent light red color is formed, giving way to brown and finally to dark purple.

It is evident that the Rosenheim procedure for testing ergosterol applies equally well to carotene. The carotenoid pigment, however, is more sensitive to the trichloracetic acid reagent. Rosenheim reported the limit of sensitivity for ergosterol to be 0.005 mg., using 3 drops of trichloracetic acid and 0.1 cc. chloroform solution of the sterol. Under the same quantities of reagent and chloroform solution we found the limit of sensitivity to be 0.001 mg. for ergosterol and 0.0002 mg. for carotene. The tests given by the 2 compounds show marked differences. With ergosterol an initial red color is obtained and a final blue color; with carotene no preliminary red color appears and the blue color develops immediately. Examination of the reaction mixtures with the spectroscope reveals differences in the absorption bands in the visible spectrum.

Chloral hydrate also reacts with carotene. About 0.5 gm. of chloral hydrate is liquefied by placing in a small evaporating dish

[1] Rosenheim, O., *Biochem. J.*, 1929, **23**, 47.

[2] McDonald, F. G., and Bills, C. E., *J. Biol. Chem.*, 1930, **88**, 601.

[3] Honeywell, E. M., and Bills, C. E., *J. Biol. Chem.*, 1932, **99**, 71.

and heating on the water bath. One drop of concentrated hydrochloric acid and finally 0.1 cc. of a solution of carotene in chloroform are added. An intense blue color forms immediately. The color fades on the addition of water or alcohol. The limit of sensitivity following the above procedure is 0.001 mg. of carotene in 0.1 cc. of chloroform. The concentration of acid in the reaction mixture modifies the test. A chloroform solution of carotene mixed with an equal volume of concentrated hydrochloric acid yields at first a yellow brown color, changing to olive green and finally to light green. Solid carotene dissolves in liquefied chloral hydrate with the formation of a blue solution.

Rosenheim applied chloral hydrate as a reagent for ergosterol. He reported in 1929 that when solid ergosterol (1 mg. or less) is added to liquefied chloral hydrate (0.5 gm.), there develops a carmine red solution, changing within a minute to an evanescent green and finally to a deep blue, which persists for a considerable time. The test he reported to be specific for ergosterol, since carefully purified samples of cholesterol, sisterol, dihydrosisterol, γ-sitosterol, stigmasterol, zymosterol, fungisterol, isocholesterol, amyrol and coprosterol gave rise to colorless solutions with the chloral hydrate reagent. We have found, however, that ergosterol-free cholesterol gave when heated with chloral hydrate on the water bath a pink or reddish fluid.

An acid reaction, according to Rosenheim, is essential to the reaction. A saturated solution of chloral hydrate (80%) in water yielded a positive test with ergosterol when a drop of concentrated hydrochloric acid was added. Freshly distilled anhydrous chloral gave a positive test with ergosterol. The addition of a drop of water activated the mixture. Chloral or chloroform under laboratory conditions may undergo slight decomposition with the formation of traces of hydrochloric acid.

Conclusion. Trichloracetic acid and chloral hydrate are reagents that serve in the detection of ergosterol and of carotene. Ergosterol with the 2 reagents yields an initial red and a final blue color, while carotene gives only the blue color.