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A Test for Cholesterol Based Upon the Use of Sulphuric Acid Containing Selenious Acid.

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Cholesterol dissolved in chloroform gives a characteristic color test with concentrated sulphuric acid containing in solution 125 mg. of sodium selenite for every 25 cc. of acid. The test is made by adding to a chloroform solution of cholesterol an equal volume of the selenious-sulphuric acid reagent. The reaction is positive when the top layer assumes a deep beautiful purple, while the lower layer assumes a red brown to a very dark brown color, but does not show any green fluorescence.

The test is more sensitive than the Salkowski test. The chloroform solution containing 0.0025% of cholesterol does not yield the typical color layers observed with the concentrated sulfuric acid in the Salkowski test, which is concentrated sulphuric acid. Such solution does not give the cherry red color in the top layer, and does not give in the bottom layer a red color by direct light and a green fluorescence in transmitted light. Instead it yields only a faint trace of pink in the top layer, and in the bottom layer a slight yellow color in direct light and a slight green fluorescence by transmitted light. On the other hand, the same strength solution of cholesterol with an equal volume of the selenious-sulphuric acid reagent still gives a deep purple in the top layer, and in the bottom layer a reddish brown without fluorescence.

With a 0.001% cholesterol solution the reaction is still positive with the new reagent, while with the sulphuric acid in the Salkowski test the reaction is atypical, no color being present in the top layer and but a very faint yellow color in the bottom layer, which by transmitted light gives a very slight green fluorescence. A 0.0005% cholesterol solution does not yield anything typical or atypical with both reagents.

A mixture of sulphuric acid with tellurous acid proved ineffective owing to the insolubility of the tellurium compound in the acid. A suspension of sodium tellurite was used containing 125 mg. per 25 cc. of acid. When mixed with an equal volume of cholesterol solution the upper layer was colored red to orange or orange brown, while the lower layer was of reddish brown to light orange with a

greenish fluorescence in transmitted light. The lower layer after a while became white, due to the settling of the insoluble tellurium compound. On the whole it may be stated that no advantage was gained by the addition of the tellurium compound to the concentrated sulphuric used as the reagent to test for cholesterol.

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A Test to Differentiate Ergosterol From Cholesterol.

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When sulphuric acid or a mixture of selenious and sulphuric acids is used as a reagent for cholesterol dissolved in chloroform, the two layers, that is the chloroform and the acid layer, assume different colorations. With sulphuric acid alone the chloroform layer is cherry red and the acid layer shows green fluorescence. With the selenious-sulphuric acid mixture, the chloroform layer assumes a deep beautiful purple, while the lower layer is red brown but does not display green fluorescence.

When, however, ergosterol is used with either reagent, the acid layer is colored *but the chloroform layer remains free from color.* With concentrated sulphuric acid and an equal volume of chloroform solution, the acid layer ranges in color from a brownish red in a 1%, to cherry red in a 0.5%, to light cherry red in a 0.1%, to orange in a 0.04%, and to yellowish orange in a 0.025% solution of ergosterol. In all these concentrations the typical green fluorescence is also observed in the acid layer.

With concentrated sulphuric acid containing 125 mg. of sodium selenite per 25 cc. of acid, the chloroform layer ranges in color from dark red brown to light red brown in the above concentrations of ergosterol while no green fluorescence is noted. *The chloroform layer remains uncolored.*

From the above results the conclusion may be drawn that cholesterol on treatment with acid forms a decomposition product or products giving rise to colored solutions in chloroform and in sulphuric acid or in both. Ergosterol, on the other hand, seems to give a decomposition product or products dissolving with color formation only in sulphuric acid.

Solutions of cholesterol in chloroform remain colorless, while