simultaneous salt solution administration in dogs with high intestinal obstruction. Since burns are chemically characterized by hypochloremia and hyperazotemia we are experimenting with Cortin and salt solution as a detoxifying agent. The results will be reported elsewhere.

8444 C

Differentiation of Ergosterol from Cholesterol.

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Ergosterol and cholesterol are sterols each containing in its molecule 3 benzene rings with the configuration found in phenanthrene, one 5-membered ring with a side chain and one secondary alcohol grouping. Ergosterol, however, has one more carbon atom than cholesterol, one hydrogen in the side chain being replaced by a methyl group, and it also has 3 double bonds instead of one double bond.

The greater degree of unsaturation of ergosterol argues for its greater reactivity. Heilbron and Spring[1] report that ergosterol yields a red color with antimony trichloride, but no reaction takes place with α- and β-dihydroergosterol, with α- and β-ergostenol (tetrahydroergosterol) with allo-α-ergostenol (hexahydroergosterol), or with cholesterol. They also report that the trichloracetic acid reaction with ergosterol, which is negative with cholesterol, is not given by partially or completely saturated derivatives of ergosterol.

Several other tests reported for ergosterol fail to give responses with cholesterol. Among these we may mention the reaction (1) with chloral hydrate,[2] (2) with a solution of bromine in chloroform (Tortelli-Jaffé reaction)[1] (3) with concentrated nitric acid or with a solution of mercuric acetate in nitric acid,[3] and (4) with a solution of selenium dioxide.[4] Montignie[4] and also Callow and

[1] Heilbron, I. M., and Spring, F. S., *Biochem. J.*, 1930, **24**, 133.

[2] Rosenheim, O., *Biochem. J.*, 1929, **23**, 49.

[3] Rosenheim, O., and Callow, R. K., *Biochem. J.*, 1931, **25**, 24.

[4] Montignie, E., *Bull. Soc. Chem.*, 1932, **51**, 144.

[5] Callow, R. K., and Rosenheim, O., *J. Chemical Society of London*, 1933, 387.

Rosenheim[5] state that selenium dioxide is reduced to free selenium not only by ergosterol but also by dihydroergosterol. A test to differentiate ergosterol from cholesterol may be carried out by use of a reagent containing concentrated sulphuric acid with sodium selenite, the sterol being dissolved in chloroform.[6] The chloroform layer remains free from color in case of ergosterol, while the acid layer changes to dark red brown to light brown with no green fluorescence. In case of cholesterol a deep bluish purple develops in the chloroform layer, while the lower or acid layer is red brown without a display of green fluorescence. The test cannot sharply differentiate ergosterol from cholesterol in mixtures of these 2 sterols, and is therefore not serviceable in detection of small traces of ergosterol in cholesterol.

We now report a method for differentiating ergosterol from cholesterol in chloroform solution by means of a reagent containing 3 volumes of 2% aqueous solution of sodium selenite and 1 volume of concentrated hydrochloric acid. A reagent containing equal volumes of 2% sodium selenite solution and orthophosphoric acid (85%) or one containing 3 volumes of the selenite solution and one volume of concentrated sulphuric acid is also effective. The best results, however, are obtained with the selenite reagent acidified with hydrochloric acid. In alkaline medium the sodium selenite solution does not react.

The chloroform for dissolving the sterol must be free from substances that reduce the selenite to elemental selenium. Non-reactive chloroform may be obtained by washing U.S.P. chloroform 3 times with water in the proportion of one volume of organic solvent to 3 volumes of distilled water.

The reaction is carried out as follows: 2 cc. of a chloroform solution of ergosterol together with 2 cc. of the selenite reagent acidified with hydrochloric acid placed in a test tube are heated on the water bath until the chloroform layer is evaporated. The evaporation takes about a minute. Upon replacing the 2 cc. of chloroform lost, a deep orange to barely discernible yellow color develops in the chloroform layer, the intensity of the color depending upon the concentration of the sterol. The aqueous layer remains colorless. Up to and including 0.2 mg. of ergosterol the orange color predominates, while below that amount the light yellow color develops. The yellow color is well recognizable with 0.05 mg. of ergosterol and is still perceptible with 0.01 mg. of ergosterol.

[6] Levine, V. E., and Richman, E., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 832, 833, 1010.

The colors developed in the reaction are partly due to reduced selenium and partly due to the oxidation of the sterol by the selenite. Potassium cyanide reacts with free selenium in neutral or slightly alkaline to form a colorless solution of potassium selenocyanide (KCNSe). We have treated the reaction product obtained from selenite and ergosterol with a solution of potassium cyanide, and have found but slight diminution in the characteristic color. The failure to decolorize may be due partly to the presence of colored product derived from the oxidation of the sterol and partly to the fact that the free selenium may be adsorbed upon the sterol or some of its reaction products so that it may not be free to react with potassium cyanide.

Ergosterol irradiated with the Hanovia mercury lamp at a distance of 30 inches for one hour also reacts. Non-irradiated ergosterol shows a more gradual change of color from deep orange to yellow, whereas the irradiated ergosterol gives a sharp gradation from a definite orange with 1 mg. of the sterol to a slight orange yellow with 0.5 mg. to a definite yellow with quantities ranging from 0.2 mg. to 0.01 mg.

Cholesterol, non-irradiated as well as irradiated under the same conditions as ergosterol, yields no color reaction. Cholic acid, which is related to the sterols in chemical composition, does not react. Carotene is also not reactive. Cerevisterol, which, according to Honeywell and Bills,[6] has two double bonds, gives a positive reaction, although Callow and Rosenheim find that apocholic acid reduces selenium dioxide. Cerevisterol, which according to Honeywell and Bills,[7] has two double bonds, gives a positive response. Carotene does not react.

Calciferol, one product isolated from irradiated ergosterol, and possessed of vitamin D potency, responds to the test but not as intensely as irradiated or non-irradiated ergosterol. One milligram of calciferol dissolved in chloroform gives a test comparable to that given by 0.2 mg. of ergosterol. The limit of sensitivity for calciferol is 0.25 mg. Callow and Rosenheim[5] report that lumisterol reacts with selenium dioxide to liberate selenium.

Ergosterol in chloroform solution also reacts with 5% solution of selenious acid, and with a reagent containing 3 volumes of 2% sodium selenate solution and one volume of concentrated hydrochloric acid. Cholesterol does not react with the above reagents.

The selenite reagent acidified with hydrochloric acid is very useful in the detection of minute quantities of ergosterol in choles-

[7] Honeywell, E. A., and Bills, C. E., *J. Biol. Chem.*, 1933, **103**, 515.

terol. Ordinary cholesterol, according to Bills,[8] contains less than 5 parts of ergosterol in 1000 parts or less than 0.5%. We dissolved one gram of cholesterol in 5 cc. of washed chloroform and added 2 cc. of the selenite-hydrochloric acid reagent. A reaction was obtained, which when compared to a series of standards, indicated the presence of 0.25 mg. of ergosterol in the one gram of cholesterol (0.025%). We made up a mixture of 100 mg. of ergosterol-free cholesterol and 0.025 mg. of ergosterol (0.025%) in 5 cc. of chloroform, applied the test and obtained a positive reaction.

Conclusion. A mixture made up in the proportion of three volumes of 2% aqueous solution of sodium selenite and one volume of concentrated hydrochloric acid is useful for the detection of irradiated or non-irradiated ergosterol and calciferol. The reagent is useful in distinguishing ergosterol from cholesterol, since the latter does not react. It is also very serviceable in the detection of small amounts of ergosterol in samples of cholesterol.

8445 C

Further Studies of Sex Skin Reactions in Macaca Mulatta.

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It has previously been reported[1] that the sex skin reactions of rhesus macaques, which can be called forth by injection of oestriol, eventually spread over a wide extra-genital area if the injections of this hormone are continued for longer than a month. Minor degrees of this extra-genital oedema can be observed in many untreated cyclic females at certain periods of the year, but the full manifestations possible after prolonged oestriol treatment have not been observed to date in a considerable experience with untreated macaques. In the further study of these phenomena, it was of interest to see whether the results obtained with oestriol could be duplicated with oestrone. Also, in view of the symmetrical character of the sex skin reactions, it was felt desirable to investigate a few relationships of the segmental nerve supply of the affected areas to the reactions occurring therein.

[8] Bills, C. E., *Physiol. Rev.*, 1935, **15**, 1.

[1] Bachman, C., Collip, J. B., and Selye, H., *Proc. Roy. Soc. Lond.*, 1935, **117**, 16.