A COMPANION OF CHOLESTEROL

Sir:

Evidence of low-order carcinogenicity of a variety of cholesterol-rich lipid fractions[1] and of high carcinogenic potency[2] of Spielman and Meyer's[3] crude progesterone preparation derived from cholesterol by bromination, oxidation, and debromination suggests the existence of a possibly endogenous non-aromatic steroid carcinogen related to or derived from cholesterol. The hypothesis that the substance is an abnormal ester of cholesterol will be discussed in a paper with Dr. W. P. Schneider. Consideration of the alternate possibility that the carcinogen is a product of oxidation of cholesterol, perhaps a variant of one of the hormone structures, prompted a restudy of oxidation with hexavalent chromium and with selenium dioxide. In the former instance experimentation was greatly facilitated by use of a solution of sodium dichromate dihydrate in glacial acetic acid; with addition of 1 volume of benzene per 2 volumes of acetic acid, oxidations can be conducted in homogeneous solution at 0°. The four known oxidation products were all isolated;[4] Δ^5-cholestenone was found to be the precursor of the chief product, Δ^4-cholestene-3,6-dione; a new product is a cholestenedione, m.p. 189°, dec., $[\alpha]^{23}D$ +27° Chf, +31° Di; $\lambda_{max.}^{Chf}$ 5.85, 6.23 μ; $\lambda_{max.}^{EtOH}$ 236 mμ (log ϵ 4.16); found: C, 81.46; H, 10.82.

The observation that in both the dichromate and selenium dioxide oxidations of commercial cholesterol (Wilson), chromatography led to isolation of small amounts of substances that did not appear to be derivable from cholesterol prompted investigation of the homogeneity of the starting material. Repeated crystallization of the acetic acid complex effected slow distribution of companion steroids into the mother-liquor fractions that eventually sufficed for isolation of cholestanol as acetate, m.p. and mixed m.p. 110–111°, found: C, 80.75; H, 11.65, following bromination, and, by chromatography of the acetate mixture, of an isomer of cholesterol, m.p. 125–126°, $[\alpha]^{27}D$ + 5.7° Chf, found: C, 83.70; H, 12.15; acetate, m.p. 118–119°, $[\alpha]^{22}D$ + 1.5° Chf, found: C, 81.07; H, 11.50. This substance and its acetate showed no depression when mixed with Δ^7-cholesterol, m.p. 125–126°, $[\alpha]^{23}D$ + 3.9° Chf, +10.0° Di, and its acetate, m.p. 118–119°, $[\alpha]^{23}D$ + 2.4° Chf, +9.4° Di; it is thus 5,6-dihydroprovitamin D_3.[5]

Δ^7-Cholestenol[5] is characterized by high sensitivity to oxidation. A solution at 25° of 1 mg. of material in 0.5 cc. of benzene plus 0.5 cc. of 0.1 *M* selenious acid in aqueous acetic acid turns yellow in 2–3 minutes and deposits red selenium in 10–15 minutes; the reaction is rapid even at 0°. The test appears to be positive only for steroids of the A/B-*trans* series having a double bond or a dienic system adjacent to a 14α-hydrogen atom. Kogi Nakanishi has developed a microanalytical procedure based on this reaction applicable to a 5–15 mg. sample; some results for % Δ^7-stenol in cholesterol samples processed by Dr. Bidyut Bhattacharyya are: spinal-cord (Wilson): 0.62; beef adrenal, 0.65; liver, 0.35; normal plasma, 0.42–1.35; gall stone, 2.19–3.11; wool fat, 2.97; egg yolk, 4.34; cholesterol purified through the dibromide or by 22 crystallizations from acetic acid, 0.0.

Treatment of Δ^7-cholestenyl acetate with 2 moles of NBS in ether–methanol gave, in 27% yield, $\Delta^{7,9(11)}$-cholestadienyl acetate, m.p. and mixed m.p. 118–119°, $\lambda_{max.}^{EtOH}$ 236, 243, 250 mμ (log ϵ 4.10, 4.15, 3.96), found: C, 81.47; H, 10.69.[6] Since NBS in methanol is equivalent to bromine, it can be inferred that in the Spielman–Meyer process the Δ^7-cholestenol present was converted initially into $\Delta^{7,9(11)}$-cholestadienol and hence that the carcinogen may be an oxidation pronuct of this diene alcohol. A number of products of oxidation of $\Delta^{7,9(11)}$-dienes have been reported,[7] among them a monoepoxide.[7b] In view of the demonstrated carcinogenicity of the diepoxide of vinylcyclohexene,[8] it seems possible that the substance may be 7,8,9,11-diepoxycholestanol. This conceivably could be formed in lard-injected cholesterol[1] by the action of peroxides of lard on lathosterol; cholesterol administered in sesame oil, which contains a natural antioxidant preventing peroxidation, has given no tumors.[2]

(1) I. Hieger, *Brit. J. Cancer*, **3**, 123 (1949).

(2) F. Bischoff and J. J. Rupp, *Cancer Research*, **6**, 403 (1946).

(3) M. A. Spielman and R. K. Meyer, THIS JOURNAL, **61**, 893 (1939).

(4) The formation of "α-oxycholestenol," J. Mauthner and W. Suida, *Monatsh.*, **17**, 579 (1896), has not previously been confirmed. My material had the constants: m.p. 187.5–188°, $[\alpha]D$ −6° Di, −10° Chf, $\lambda_{max.}^{EtOH}$ 237 mμ (log ϵ 3.83).

(5) The term lathosterol (Gr. *latho-*, undetected) for this substance is in use in our laboratory to describe material of biological origin.

(6) J. E. Herz has established that Δ^7-cholestenyl benzoate can be substituted for the $\Delta^{7,9(11)}$-diene in our previously described process for C_{11}-oxygenation with NBS, Ref. 7d.

(7) (a) L. F. Fieser and co-workers, THIS JOURNAL, **73**, 2397 (1951); (b) E. M. Chamberlain, *et al.*, *ibid.*, **73**, 2396 (1951); (c) G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 3546 (1951); (d) L. F. Fieser and co-workers, *ibid.*, **73**, 4053 (1951).

(8) J. A. Hendry, R. F. Homer, F. L. Rose and A. L. Walpole, *Brit. J. Pharmacol. and Chemotherap.*, **6**, 235–255 (1951).

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THE SYNTHESIS OF 5-HYDROXYTRYPTAMINE

Sir:

The isolation[1] of the substance believed to be responsible for the vasoconstrictor activity of serum and the proposal[2] that the active principle is 5-hydroxytryptamine prompted this investigation.

5-Benzyloxyindole[3] on treatment with formaldehyde and dimethylamine gave 5-benzyloxygramine, m.p. 138° (*Anal.* Calcd. for $C_{18}H_{20}N_2$: C, 77.09; H, 7.19; N, 9.99. Found: C, 77.38; H, 7.02; N, 10.01). Using the procedure of Snyder,[4] this Mannich base was heated with sodium cyanide in aqueous ethanol to yield 5-benzyloxyindole-3-acetamide, m.p. 158° (*Anal.* Calcd. for $C_{17}H_{16}N_2O_2$: C, 72.83; H, 5.75; N, 10.00. Found: C, 72.93; H, 5.78; N, 10.27). The acetamide on reduction

(1) M. M. Rapport, A. A. Green and I. H. Page, *J. Biol. Chem.*, **176**, 1243 (1948).

(2) M. M. Rapport, *ibid.*, **180**, 961 (1949).

(3) H. Burton and J. L. Stoves, *J. Chem. Soc.*, 1726 (1937).

(4) H. R. Snyder and F. J. Pilgrim, THIS JOURNAL, **70**, 3770 (1948).