Inhibitors of sterol synthesis. Effects of a new fluorinated analog of 3β-hydroxy-5α-cholest-8(14)-en-15-one in rats

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Abstract 3β-Hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VII**), an analog of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) in which conversion to 26- and 25-oxygenated metabolites is blocked by the F_7-substitution, was administered to male Sprague-Dawley rats at levels of from 0.025 to 0.15% by weight in a ground chow diet. Administration of **VII** resulted in lowering of the levels of serum cholesterol at dosages as low as 0.025% by weight in diet. In marked contrast to **I, VII** had little or no effect on food consumption. Whereas administration of **I** at a level of 0.1% by weight in diet resulted in a cessation of growth, **VII,** at approximately the same molar concentration in diet, had only slight or no effect on changes in total body weight. Significant levels of 25,26,26,26,27,27,27-heptafluorocholesterol (**VIII**) were observed in serum and liver, indicating the conversion of **VII** to **VIII.** Characterization of **VIII** in liver was based upon the results of gas chromatography, low and high resolution mass spectral studies, infrared spectroscopy, and ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. The levels of **VIII** in serum appeared to be related to dosage and duration of administration of **VII.**—**Gerst, N., F. D. Pinkerton, A. Kisic, W. K. Wilson, S. Swaminathan, and G. J. Schroepfer, Jr.** Inhibitors of sterol synthesis. Effects of a new fluorinated analog of 3β-hydroxy-5α-cholest-8(14)-en-15-one in rats. *J. Lipid Res.* 1994. **35:** 1040–1056.

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3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) is a potent inhibitor of sterol biosynthesis and is highly active in lowering the levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity in cultured mammalian cells (1–5). This 15-ketosterol is convertible to cholesterol (Chol) in intact animals (6–12) and in cell-free preparations from rat liver (13, 14), and a scheme has been presented to account for the overall metabolism of **I** to Chol (14) (**Fig. 1**). Chol and its fatty acid esters have been shown to constitute the major metabolites of **I** found in blood and tissues at 48 h after its intravenous administration to bile duct-cannulated rats (9, 11). However, the major fate of **I** under these conditions is very rapid conversion to polar metabolites which are excreted in bile (9, 11) and of which a substantial fraction undergoes enterohepatic circulation (9). Studies (15–17) of the metabolism of **I** by rat liver mitochondria in the presence of NADPH have shown that under these conditions the major metabolites of **I** are (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (25R-**II**), the 25S-isomer of **II,** 3β-hydroxy-15-keto-5α-cholest-8(14)-en-26-oic acid (**III**), and 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one (**IV**) (**Fig. 2**). The results of further studies demonstrated that synthetic (25R)-**II** and **IV** are highly active, equivalent to **I**, in lowering the levels of HMG-CoA reductase activity in cultured mammalian cells (15, 18–20). In contrast to (25R)-**II** and **IV,** synthetic **III** had relatively low potency in lowering reductase activity (21). Other studies in HepG2 cells and in bile duct-cannulated rats have indicated very substantial metabolism to polar metabolites which appears to be initiated by hydroxylation at C-26 (J. S. Pyrek, S. Numazawa, N. Gerst, G. T. Emmons, F. D. Pinkerton, and G. J. Schroepfer, Jr., unpublished data). Two other potential in vivo metabolites of **I,** 3β-hydroxy-15-keto-5α-chol-8(14)-en-24-oic acid (**VI**) (22, 23) and (25R)-5α-cholest-8(14)-ene-3β,15β-26-triol (**V**) (24) have been shown to be moderately active and highly active, respectively, in the suppression of the levels of HMG-CoA reductase activity in cultured mammalian cells.

Oral administration of the parent 15-ketosterol (**I**) to rats results in an inhibition of Chol absorption (7, 25), a lowering of levels of acyl coenzyme A:cholesterol

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; Chol, cholesterol; ACAT, acyl coenzyme A:cholesterol acyltransferase; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography; GC, gas chromatography; TMS, trimethylsilyl; TMSOH, trimethylsilanol; SC, alkyl side chain; UV, ultraviolet; IR, infrared; MS, mass spectrometry or mass spectrum; NMR, nuclear magnetic resonance.

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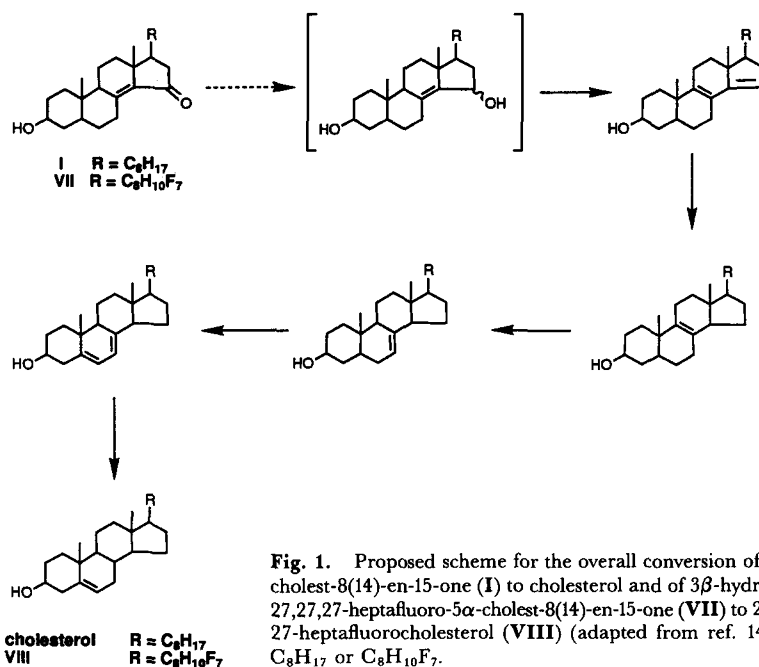

Fig. 1. Proposed scheme for the overall conversion of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) to cholesterol and of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VII**) to 25,26,26,26,27,27,27-heptafluorocholesterol (**VIII**) (adapted from ref. 14) wherein R = C_8H_{17} or $C_8H_{10}F_7$.

acyltransferase (ACAT) activity in jejunal microsomes (26), and a lowering of serum Chol levels (27–29). Oral administration of **I** is also associated with a reduction in food consumption and a suppression of the growth in rats (27–29) but not in nonhuman primates (30, 31). The lowering of serum Chol levels in nonhuman primates (30, 31) has been shown to be associated with a reduction in the levels of low density lipoprotein Chol and an elevation of the levels of high density lipoprotein Chol.

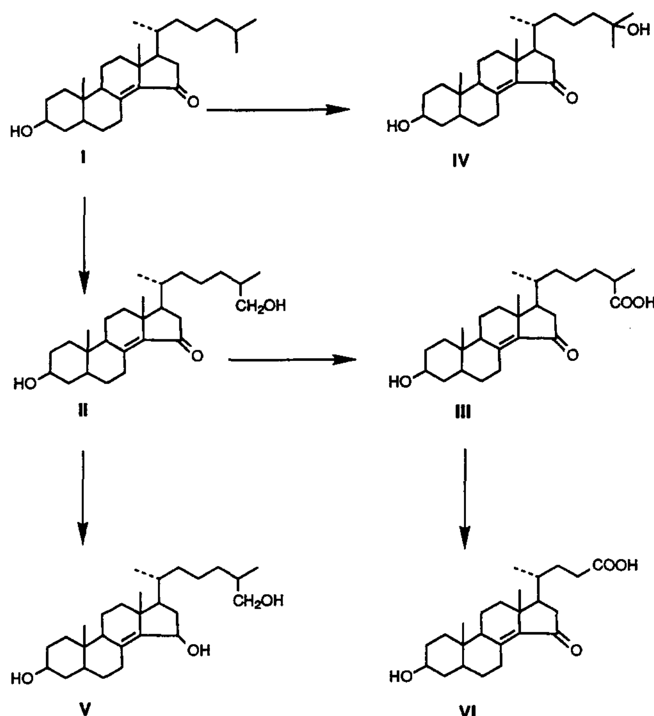

Fig. 2. Conversion of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) to side-chain oxygenated metabolites.

The significant formation of polar metabolites from **I** in animals and in HepG2 cells and the demonstration of the high activity of a number of these metabolites in decreasing the levels of HMG-CoA reductase activity raised the possibility that the effects of **I** might be partially or totally caused by metabolites of **I.** The ability of the parent 15-ketosterol **I** itself to lower HMG-CoA reductase activity is strongly indicated by the demonstration of its high potency in Chinese hamster ovary (CHO-K1) cells (2, 3, 5), a cell line in which little or no metabolism of **I** has been detected, apart from the formation of its fatty acid esters (5). Moreover, synthetic 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VII**), in which enzymatic oxidation at C-26 (and at C-25) is precluded, has a potency equivalent to **I** in lowering the levels of HMG-CoA reductase activity in both CHO-K1 cells and HepG2 cells (32).

While the results of these studies demonstrated the inherent activity of **I** in the lowering of HMG-CoA reductase activity, the significant formation of multiple polar metabolites in animals, the high potency of a number of these metabolites in lowering HMG-CoA reductase activity, and the demonstration of synergy between **I** and another oxysterol in lowering reductase activity (33) necessitates consideration of the polar metabolites of **I** for understanding the actions of **I** upon its administration to animals. Since most of the polar metabolites of **I** in intact animals appear to arise by initial oxidation of C-26, we have also explored the effects of oral administration of

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VII, in which oxidation at C-26 (and C-25) is blocked by the F_7 substitution. The results presented herein demonstrate the hypocholesterolemic effect of **VII** in rats. In contrast to **I,** oral administration of **VII** had little or no effect on food consumption.

EXPERIMENTAL PROCEDURES AND RESULTS

Materials and methods

3β-Hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VII;** designated F_7-15-ketosterol) and 25,26,26,26,27,27,27-heptafluorocholesterol (**VIII;** designated F_7-cholesterol) were prepared and characterized as described previously (32). Samples of **VII** used in the experiments described herein showed a purity of over 99% by ^1H nuclear magnetic resonance (NMR) and high performance liquid chromatography (HPLC). 3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) was prepared as described previously (34) and showed a single component on thin-layer chromatography (TLC). [7(n)-^3H]Chol (15 Ci per mmol) and [1,2,6,7(n)-^3H]Chol oleate (65.8 Ci per mmol) were obtained from Amersham (Arlington Heights, IL). [2,4-^3H]**I** (13.5 mCi per mmol) was prepared by a minor modification of a procedure described previously (35). Chol was purified by way of its dibromide derivative (36).

TLC was carried out on silica gel G plates (Analtech; Newark, DE). Components on the plates were visualized after spraying with 5% ammonium molybdate(VI) in 10% sulfuric acid followed by heating. Unless stated otherwise, solvent systems were: SS-1, 35% ether in hexane; SS-2, 15% ethyl acetate in hexane; SS-3, 20% ethyl acetate in hexane. HPLC was carried out using a Waters 600 Multisolvent Delivery System and a Shimadzu SPD 6A UV detector. Analytical HPLC was done using a 5 μm Customsil ODS C_{18} reversed phase column (250 mm × 4.6 mm) at a flow rate of 1 ml per min. Semi-preparative HPLC employed a 5 μm Spherisorb ODS-II C_{18} reversed phase column (250 mm × 9.4 mm). The HPLC columns were purchased from Custom LC (Houston, TX). Solvents for analytical studies were HPLC grade. Capillary gas chromatography (GC) was carried out on a Shimadzu GC-9A unit using splitless injection with nitrogen (1.3 kg per cm^2) as the carrier gas. The injector and flame-ionization detector were maintained at 290°C and the column temperature was programmed as follows: 100°C for 3 min; then 100°C to 250°C at 20°C per min; then 250°C for 10 min. The column used was Rt_x 1701 (15 m × 0.25 mm ID; 14% cyanopropylphenyl, 86% methyl polysiloxane; 0.1 μm film thickness; Restek Corporation; Bellefonte, PA). Trimethylsilyl (TMS) ether derivatives of the sterols were prepared by treatment with a 1:1 mixture (200 μl) of bis(trimethylsilyl)trifluoroacetamide and pyridine for 1 h under nitrogen at room temperature. The mixture was evaporated to dryness under nitrogen to a residue that was dissolved in hexane (500 μl) for GC analysis (1 μl aliquot). Colorimetric assays of sterols were carried out using the color reagent described by Abell et al. (37). Infrared (IR) spectra were obtained on a Mattson Galaxy 6020 Fourier-transform IR spectrometer with KBr pellets. Low resolution mass spectra (MS) were recorded on a Shimadzu QP-1000 quadrupole spectrometer with an electron energy of 70 eV and direct inlet sample introduction. GC-MS analyses were carried out as described previously (25, 26,) using a DB-5 capillary column (15 m × 0.25 mm; 0.1 μm film thickness; J & W Scientific, Inc.; Folsom, CA) with direct introduction of the effluent into the ion source of the mass spectrometer (Extrel ELQ-400). High resolution MS were recorded on a Kratos MS-50DA spectrometer at the Midwest Center for Mass Spectrometry (Lincoln, NE). Radioactivity was measured in a Packard Tri-Carb 1500 liquid scintillation spectrometer using Scintisol (Isolab; Akron, OH) as the scintillation fluid. NMR spectra were measured on an IBM AF300 (300.1 MHz for ^1H, 75.5 MHz for ^{13}C), a Bruker AMX500 (500.1 MHz for ^1H), or a Bruker AC250 (235.4 MHz for ^{19}F) spectrometer in $CDCl_3$ solution as described previously (32).

Effects of dietary administration of the F_7-15-ketosterol VII to rats

Male rats (100–140 g) of the Sprague-Dawley strain were purchased from Harlan Sprague-Dawley (Houston, TX) and housed in pairs for 6 days on a light (6:00 AM–6:00 PM)-dark cycle and fed a basal diet (Purina Formulab 5008) and water ad libitum. The animals were then divided into groups of eight animals each, such that the mean values of serum Chol and body weight were approximately the same. The animals were then housed individually and provided with the indicated diets. The body weight and food consumption of the individual rats were determined daily. Blood for serum sterol concentrations was obtained at ~8:00 AM from the tail vein on days 5 and 9 (days 5, 9, 15, and 19 in Experiment 5) and "neck blood" was obtained at the time of death on the morning of day 10 (day 20 in Experiment 5). The experiments were terminated with ketamine anesthesia (0.2 ml; 100 mg/ml) followed by decapitation. The following organs were removed, cleaned, and weighed: liver, heart, small intestine, kidneys, adrenal glands, testes, and spleen.

Five experiments involving the dietary administration of **VII** to rats were performed. Compound **VII** was administered in basal diet as described previously for studies of the effects of **I** in rats (6–8). In each experiment, Group A (designated ad libitum) had free access to the basal diet containing no added sterol (control animals). In Experiment 1, **VII** was administered at levels of 0.025% (Group

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B), 0.05% (Group C), 0.075% (Group D), and 0.10% (Group E) by weight in the basal diet. In Experiment 2, Group B received **I** (0.1% by weight) in the basal diet. Group C (designated as pair-fed to the 15-ketosterol) received basal diet with no added sterol but only in the amount consumed by its individual counterpart in Group B on the previous day. Group D received **VII** (0.125% by weight) in the basal diet. Group E (designated as pair-fed to the F_7-15-ketosterol) received basal diet with no added sterol but only in the amount consumed by its individual counterpart in Group D on the previous day. In Experiment 3, Group B received **VII** (0.1% by weight) in the basal diet. In Experiment 4, Groups B and C received **VII,** 0.125% and 0.150% by weight in the basal diet, respectively. Experiment 5 differed from the experiments noted above in its longer duration (20 days). Animals in Group B received **VII** (0.10% by weight) in the basal diet.

Serum Chol was measured by two methods. Day 0 values were determined using a commercial assay kit ("Single Vial"; Boehringer Mannheim Diagnostics; catalog number 236691). This methodology could not be applied to the determination of Chol in serum samples of rats treated with **VII** due to the presence of another sterol which also acted as a substrate for Chol oxidase (vide infra). Accordingly, Chol levels in serum were determined by GC. This approach also permitted simultaneous determination of the levels of **VII** and of a major metabolite of **VII** in serum. Routine capillary GC analyses of sterols in serum were made on a Rt_x 1701 column as described above.[2] Stigmasterol was used as an internal standard. In addition, recovery of sterol after saponification and extraction was monitored through the use of an internal standard of [7(n)-^{3}H]cholesteryl oleate. Routine saponification of samples (100 μl) involved treatment with 10% KOH in ethanol (500 μl) at 70°C followed by extraction with hexane (3 × 1.5 ml). After evaporation to dryness under nitrogen, the samples were silylated (vide supra) and subjected to GC analysis.

Statistical comparisons were made using a paired Student's *t* test. Unless indicated otherwise, variation is expressed as standard error of the mean.

Comparison of the responses of cholesterol and the F_7-cholesterol VIII in colorimetric and enzymatic assays

The F_7-Chol did not differ from Chol upon assay with Chol oxidase when absorbance was plotted as a function of the molar concentration of sterol. However, when assayed with the Liebermann-Burchard color reagent, the F_7-Chol gave considerably less color response per μmol of sterol than did Chol, approximately 63% that of Chol.

Isolation and characterization of the F_7-cholesterol from liver of animal treated with the F_7-15-ketosterol (VII)

Preliminary analyses of the sterols in serum from rats treated with the F_7-15-ketosterol indicated the presence of significant levels of a sterol with the chromatographic and GC-MS properties of **VIII** (vide infra). To obtain sufficient material to establish unequivocally the structure of this material, we pursued its isolation from liver. Accordingly, the liver (13.0 g) of one rat that received **VII** (0.10% in diet) for 20 days was homogenized with a 2:1 mixture (300 ml) of $CHCl_3$ and methanol. The resulting mixture was filtered and, after washing the filter twice with $CHCl_3$–methanol 2:1 (50 ml portions), the filtrate was washed twice with water (100 ml portions) and evaporated to dryness under reduced pressure. To remove traces of water, a mixture of benzene and methanol (7:3) was added and the solvent was removed under reduced pressure. The residue was dissolved in toluene (10 ml) and an aliquot (20 μl) was removed for GC analysis. This material was saponified with a mixture of 10% ethanolic KOH (500 μl) and water (100 μl) for 2 h at 70°C. The sterols were recovered by extraction with hexane (3 × 1.5 ml). After evaporation of the solvent, the TMS ethers were prepared and analyzed by GC on an Rt_x 1701 column. Two major components were observed that corresponded to the chromatographic behavior of the TMS derivatives of Chol (79%) and **VIII** (21%).[2]

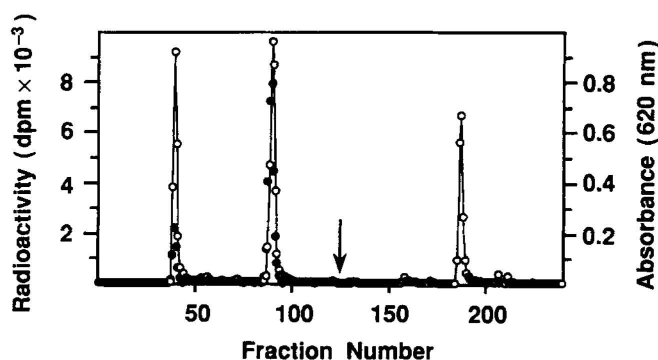

Fig. 3. Silicic acid-Super Cel column chromatographic analysis of total lipid extract of liver from a rat treated with 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (0.1% in diet for 20 days) along with internal standards of (in order of elution) [1,2,6,7(n)-^{3}H]cholesteryl oleate, [7(n)-^{3}H]cholesterol, and [2,4-^{3}H]3β-hydroxy-5α-cholest-8(14)-en-15-one; (○), radioactivity; (●), cholesterol or cholesteryl esters determined colorimetrically. The arrow at fraction 125 marks the change in the mobile phase from toluene to toluene–ether (92:8).

[2]Chromatographic properties of the F_7-15-ketosterol **VII** and F_7-cholesterol **VIII** on TLC, HPLC, and GC have been presented previously (32) along with comparable data for Chol and the 15-ketosterol **I**. Whereas the F_7 substitution had little effect on the chromatographic behavior of Chol or the 15-ketosterol **I** on TLC on silica gel, a marked shortening of retention time was observed for the free sterols on reversed phase HPLC and upon capillary GC of the free sterols and their acetate and TMS derivatives.

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The remainder of the total lipid extract of liver was applied to a 1:1 silicic acid-Super Cel column (100 cm × 2.2 cm) along with internal standards of [7(n)-^{3}H]Chol (10^6 dpm), [1,2,6,7(n)-^{3}H]Chol oleate (10^6 dpm), and [2,4-^{3}H]I (10^6 dpm). The column was eluted with toluene which, at fraction 125, was changed to toluene-ether 92:8. Aliquots were taken for assay of radioactivity and for colorimetric assay of Chol. The resulting chromatogram is shown in **Fig. 3.**

The contents of each of the fractions from 86 to 101, corresponding to the mobility of Chol, as determined colorimetrically, were analyzed by GC in the form of the TMS ether derivative. This analysis showed that fraction 86 consisted of Chol (>99%). Analyses of fractions 87-100 showed mixtures of two sterols with the chromatographic mobilities of the TMS ethers of Chol and F_7-Chol.[3]

The contents of fractions 86 through 100 from the silicic acid column were pooled, dissolved in a 9:1 mixture (1 ml) of methanol and ethyl acetate, and subjected to semipreparative HPLC on a reversed phase column using a mixture (99:1) of methanol and water as the eluting solvent at a flow rate of 3 ml per min. Two components were completely separated from each other. The major component (14.1 mg; retention time, 29.7 min) corresponded to the mobility of Chol. The less abundant component (4.7 mg; retention time of 12.3 min) represented **VIII** of high purity. It showed a single component by TLC (5% ethyl acetate in hexane, R_f 0.23) and, as its TMS ether derivative, a single component on capillary GC analyses on Rt_x 1701 and DB-5 columns. The MS of the TMS ether was essentially the same as that of the TMS derivative of an authentic sample of **VIII** (**Table 1**). In addition, the results of low resolution and high resolution MS studies of the free sterol (**Table 2**) were fully compatible with the assignment of structure as **VIII.** The suggested assignments for the fragment ions presented in Table 2 are in accord with high resolution MS measurements and previously reported fragmentations for Chol (41). The ^{1}H and ^{13}C NMR spectra of the isolated **VIII** were essentially the same as those of synthetic **VIII,** for which complete assignments have been presented (32). The ^{13}C NMR signals of isolated **VIII** matched those of synthetic **VIII** to ± 0.02 ppm and showed the same multiplicities and similar C-F couplings for C-23 and C-24. Although the signal to noise ratio (~20:1) of the ^{13}C NMR spectrum of the isolated sample was insufficient to observe the extremely weak multiplets for C-25, C-26, and C-27 (32), the ^{19}F NMR spectrum showed F-25, F-26, and F-27 with chemical shifts and coupling patterns essentially identical to those of synthetic **VIII.** The 500-MHz ^{1}H NMR spectrum of the isolated **VIII** indicated high purity as judged by the absence of extraneous signals in the methyl region (detection limit 0.5%) and in the δ_H 2.5-10 region (detection limit ~2%). Numerous ^{1}H signals were sufficiently resolved to estimate chemical shifts and coupling constants. These ^{1}H NMR data for metabolic **VIII** matched those of synthetic **VIII** to ± 0.01 ppm (chemical shifts) or ± 0.2 Hz (couplings). The IR spectrum of the isolated **VIII** was also fully compatible with the assigned structure. The major absorbances were at 3500, 3000-2840, 1696, 1611, 1470, 1381, 1362, 1314, 1294, 1242, 1225, 1159, 1088, 1049, 1003, 940, 719, and 665 cm^{-1}. These data are to be compared with those of Chol: 3440, 3000-2840, 1466, 1377, 1316, 1221, 1159, 1051, 940, and 719 cm^{-1}. Although the IR spectra of the isolated

[3]The TMS derivative of the material in fraction 101, representing a very minor portion of the material eluted from the silicic acid column in the C_{27} monohydroxysterol region, was analyzed by GC-MS. In addition to the TMS derivatives of **VIII** (22%, t_R 6.7 min) and Chol (71%, t_R 7.1 min), a third component (~7%) was observed at 6.8 min. Its MS was compatible with that of the TMS derivative of a F_7-C_{27} monohydroxysterol, i.e., molecular ion at *m/z* 584 (48%) and ions at *m/z* 494 (17%; M-TMSOH), 479 (41%; M-TMSOH-CH_3), 345 (13%; M-SC), and 255 (50%; M-SC-TMSOH), 107 (100%). The lack of a base peak at *m/z* 129, which is characteristic of TMS ethers of 3-hydroxy-Δ^5-sterols (38-40), and other considerations suggest that this component may represent F_7-5α-cholest-7-en-3β-ol or its Δ^8-isomer.

TABLE 1. Mass spectral data for samples of the TMS derivative of F_7-cholesterol detected in serum and in liver along with those for the TMS derivative of authentic F_7-cholesterol[a]

Ion	Tentative Assignment	Relative Abundance		
		Serum	Liver	Synthetic
m/z			%	
584	M^+	12	10	28
569	$M-CH_3$[b]	3	3	4
494	M-TMSOH[b]	31	30	45
479	M-TMSOH-CH_3[b]	16	14	15
455	M-TMSOH-C_3H_3[b]	30	25	50
401	M-TMSOH-C_7H_9	3	4	4
386		3	5	4
373	M-TMSOH-C_9H_{13}[c]	23	14	19
359	M-TMSOH-$C_{10}H_{15}$	2	4	4
345	M-SC or M-TMSOH-$C_{11}H_{17}$	3	3	4
333		3	4	5
331	M-TMSOH-$C_{12}H_{19}$	3	3	3
319		5	3	6
291		3	6	5
283		2	3	4
279		3	3	3
277		3	4	4
255	M-TMSOH-SC[b]	12	11	12
213	M-TMSOH-SC-C_3H_6[b]	9	7	8
159		11	14	17
145		24	30	26
129	$(CH_3)_3SiO^+=CHCH=CH_2$[b]	100	100	100

[a]Major ions above *m/z* 100 from GC-MS data acquired at 70 eV.
[b]Analagous losses for the TMS ether of cholesterol have been described (40).
[c]The TMS ether of cholesterol shows M-211 in comparable abundance (40).

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TABLE 2. Mass spectral data on F_7-cholesterol isolated from rat liver after dietary administration of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one[a]

Ion	Relative Abundance	Suggested Assignment	Elemental Composition	Observed Exact Mass[b]
m/z	%			
512	100[b]; 76[c]; 100[d]	M^+	$C_{27}7H_{39}OF_7$	512.2892 (+0.3)[f]
510	8; 2; 11	M-2H	$C_{27}H_{37}OF_7$	510.2731 (−0.2)
497	29; 30; 33	$M-CH_3$[e]	$C_{26}H_{36}OF_7$	497.2644 (+1.0)
494	77; 74; 98	$M-H_2O$[e]	$C_{27}H_{37}F_7$	494.2767 (−1.7)
479	54; 67; 87	$M-H_2O-CH_3$[e]	$C_{26}H_{34}F_7$	479.2536 (−1.3)
455	6; 8; 8	$M-C_3H_5O$	$C_{24}H_{34}F_7$	455.2517 (−3.2)
452	7; 18; 14	$M-C_3H_8O$	$C_{24}H_{31}F_7$	452.2302 (−1.2)
427	34; 68; 71	$M-C_5H_9O$[e]	$C_{22}H_{30}F_7$	427.2221 (−1.5)
401	70; 100; 95	$M-C_7H_{11}O$[e]	$C_{20}H_{28}F_7$	401.2073 (−0.6)
387	4; 9; 6	$M-C_8H_{12}O$	$C_{19}H_{26}F_7$	387.1929 (+0.6)
373	23; 33; 27	$M-C_9H_{15}O$[e]	$C_{18}H_{24}F_7$	373.1742 (−2.4)
332	6; 6; 6	$M-C_{12}H_{20}O$	$C_{15}H_{19}F_7$	332.1387 (+1.2)
331	4; 16; 11	$M-C_{12}H_{21}O$	$C_{15}H_{18}F_7$	331.1286 (−1.1)
319	3; 12; 10	$M-C_{13}H_{21}O$	$C_{14}H_{18}F_7$	319.1297 (+0.1)
291	3; 8; 8	$M-C_{15}H_{25}O$	$C_{12}H_{14}F_7$	291.0984 (+0.1)
273	4; 11; 11	M-SC[e]	$C_{19}H_{29}O$	273.2217 (−0.1)
255	8; 16; 24	$M-SC-H_2O$[e]	$C_{19}H_{27}$	255.2114 (+0.1)
231	5; 11; 15	$M-[SC + C_{15}..C_{17}] + H$[e]	$C_{16}H_{23}O$	231.1755 (+0.6)
213	14; 29; 42	$M-[SC + C_{15}..C_{17}] + H - H_2O$[e]	$C_{16}H_{21}$	213.1652 (+0.9)

[a] Major ions above *m/z* 200.
[b] From high resolution MS of isolated F_7-sterol.
[c] From low resolution MS of isolated **VIII**.
[d] From high resolution MS of synthetic **VIII**.
[e] These assignments are compatible with assignments given previously for analogous ions in the spectrum of cholesterol (41).
[f] Difference (in millimass units) of observed mass from calculated value.

VIII and Chol differed markedly, the results show that the heptafluoro functionality has little effect on the IR spectrum except for the anticipated series of strong peaks in the region from 940 to 1300 cm^{-1} which can be attributed to C–F stretching vibrations (42, 43). More importantly, the spectrum of **VIII** from liver was essentially identical to that obtained from an authentic sample prepared by chemical synthesis (32).

The contents of fractions 38 through 45, corresponding to the mobility of Chol esters, from the silicic acid column were saponified and the resulting free sterols were subjected to semi-preparative reversed phase HPLC as described above. The major component (3.8 mg) corresponded to the mobility of Chol. The less abundant component (0.95 mg) corresponded to the mobility of **VIII.**

A portion of the material in fraction 195, corresponding to the mobility of the **VII** was analyzed by GC–MS in the form of its TMS ether derivative. The MS was essentially the same as that of an authentic sample (**Table 3**).

The liver (10.94 g) of another rat treated for 10 days with **VII** (0.15% in diet; Experiment 4) was studied in a similar fashion. Capillary GC analysis of a small portion of the Folch extract (followed by routine saponification and silylation) showed two main components with the retention times for the TMS ether of Chol (87.5%) and of **VIII** (12.5%). The material in the remainder of the Folch extract was subjected to silicic acid column chromatography after the addition of labeled standards noted above. Material with the general mobilities of esters of **I** and free **I** were analyzed by capillary GC–MS as their TMS derivatives after mild alkaline hydrolysis of the esters. The levels of **VII** present in the free and ester fractions, approximately 0.8–0.9 and 0.1–0.2 μg per g of liver, respectively, were much lower than those of **VIII.** The materials with the general mobilities of Chol esters and Chol were, after standard hydrolysis of the esters, subjected to semi-preparative reversed phase HPLC (as described above) with gravimetric determinations of the amount of Chol and **VIII** (followed by characterization of the sterols by GC–MS of their TMS derivatives). The levels of **VIII** present in the free and ester fractions were approximately 310 and 65 μg per g of liver, respectively.

Nature of sterols in serum of rats treated with the F_7-15-ketosterol (VII)

A pooled sample of serum from animals treated with **VII** was prepared by combining aliquots (200 μl) of serum from each of the experimental rats at the termination of Experiment 5 (0.1% **VII** for 20 days). A pooled sample of serum obtained from the corresponding control animals at the termination of Experiment 5 was prepared

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TABLE 3. Mass spectral data for samples of the TMS derivative of F_7-15-ketosterol detected in serum and in liver along with those for the TMS derivative of authentic 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one[a]

Ion	Tentative Assignment[b]	Relative Abundance (%)		
		Serum	Liver	Synthetic
m/z				
598	M^+	100	25	52
583	M-CH_3	15	3	7
580	M-H_2O	7		2
508	M-TMSOH	29	12	13
493	M-TMSOH-CH_3	55	21	30
490	M-TMSOH-H_2O			6
479		10	4	4
475	M-TMSOH-CH_3-H_2O	9	6	6
465		6	2	2
413	Ion A	35	16	15
402	Ion B	25	12	15
387	Ion D or ion B-CH_3	32	26	20
373		8	3	3
369	Ion D-H_2O or ion B-CH_3-H_2O	9	3	2
359	M-SC	11	2	4
341	M-SC-H_2O	41	17	20
331	Ion C	6	4	4
299		10		
297	Ion D-TMSOH		3	3
284		15	14	10
269	M-SC-TMSOH	24	18	20
251	M-TMSOH-SC-H_2O	68	100	100

[a]Major ions above *m/z* 250 from GC-MS data acquired at 70 eV.
[b]The definitions of ions A, B, C, and D have been presented previously (16, 23) for electron ionization-induced fragmentations of $\Delta^{8(14)}$-15-ketosterols and their derivatives.

in an identical fashion. These pooled samples were extracted 3 times with $CHCl_3$-methanol 2:1 (5 ml portions). The residues obtained after evaporation of the solvent were subjected to mild alkaline hydrolysis (treatment with potassium carbonate (20 mg) in methanol (3 ml) for 3 h at 50°C) followed by extraction with methyl-*t*-butyl ether (3 × 5 ml). An aliquot was silylated and analyzed by capillary GC. The chromatogram of the pooled sample from the treated animals differed from that of the control animals in the presence of a significant peak at 10.88 min (corresponding to the retention time of the TMS ether of **VIII**) which was absent in the case of the control serum. The material with the retention time of **VIII** represented 32% of the combination of **VIII** and Chol (based upon integration of the two peaks).

The remainder of the material obtained after mild saponification of the pooled samples of serum was subjected to reversed phase HPLC as described above. The HPLC profiles for the samples from the treated and control animals differed in the presence of a significant component with the mobility of **VIII** in the treated animals that was absent in the control sample. This material showed, as its TMS derivative, a single component on capillary GC analysis on a DB-5 column with the same retention time as that of an authentic standard. The MS of the TMS derivative of the isolated **VIII** was fully compatible with this structure and essentially identical to that of an authentic sample (Table 1).

The material with the mobility of **VII** on HPLC was analyzed by GC-MS. Its MS was essentially identical to that of the TMS derivative of authentic **VII** (Table 3). The level of the total **VII** (free plus esterified) in the pooled sample was very low (~3 μM). Similar results were obtained from the analyses of pooled samples of serum from animals treated with **VII** (0.10% in diet for 10 days) (Experiment 1).

These results indicate the presence of significant levels of **VIII** in serum and only trace levels of material with the properties of **VII** in serum. Other F_7-sterols, which could be anticipated as intermediates in the overall conversion of **VII** to **VIII,** were not detected in serum. As **VIII** gives essentially the same response as Chol upon assay with Chol oxidase, determination of the effects of **VII** on the levels of Chol required the use of GC analysis to measure separately the levels of Chol and **VIII.**

The 15-ketosterol **I** has been shown to undergo very significant decomposition under standard saponification conditions (13). The experiments described above involved mild conditions of saponification (i.e., potassium carbonate in methanol) of the total lipid extract of serum so as to avoid base-catalyzed decomposition of **VII** present in serum. The demonstration of only trace amounts of **VII** in serum justified the use of standard saponification conditions (10% ethanolic KOH) in the routine processing of serum samples prior to capillary GC analyses.

Effects of F_7-15-ketosterol administration on levels of cholesterol, F_7-cholesterol, and total sterols in serum and on food consumption, changes in body weight, and weights of selected organs

Experiment 1. Experiment 1 involved administration of **VII** at levels of 0.025%, 0.050%, 0.075%, and 0.10% in the diet. **Table 4** presents the effects of increasing doses of **VII** on the levels of Chol and **VIII** in serum. Mean values of serum Chol of the treated rats were significantly lower ($P < 0.002$ at each dosage) than the corresponding day 0 values for the same groups of rats prior to treatment. When compared with the mean value for the untreated animals on days 5 and 9, mean serum Chol levels were significantly lower ($P < 0.03$ in each case) for the treated animals at all dose levels. Significant levels of **VIII** were observed in each of the groups of animals treated with **VII.** On day 5, the mean levels of **VIII** were higher in animals treated at dosages of 0.05% ($P = 0.0013$), 0.075% ($P = 0.0001$), and 0.10% ($P = 0.0002$) than the animals treated at a dosage of 0.025%. On day 9, the mean levels of **VIII** were significantly higher in animals

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TABLE 4. Effects of dietary 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (F_7-15-ketosterol) on the levels of serum sterols (mean ± SEM, n = 8) in male Sprague-Dawley rats

Experiment	Day	F_7-15-Ketosterol (% in Diet)	Serum Sterols (mM)		
			Cholesterol	F_7-Cholesterol	Total Sterols
1	0	A. 0.000	2.85 ± 0.07		2.85 ± 0.07
		B. 0.025	2.86 ± 0.06		2.86 ± 0.06
		C. 0.050	2.86 ± 0.05		2.86 ± 0.05
		D. 0.075	2.87 ± 0.05		2.87 ± 0.05
		E. 0.100	2.92 ± 0.05		2.92 ± 0.05
	5	A. 0.000	2.60 ± 0.12	0.0 ± 0.0	2.60 ± 0.12
		B. 0.025	2.02 ± 0.12[e]	0.13 ± 0.02	2.15 ± 0.13[ns]
		C. 0.050	2.06 ± 0.07[d]	0.34 ± 0.03	2.40 ± 0.10[ns]
		D. 0.075	1.76 ± 0.05[b]	0.32 ± 0.02	2.08 ± 0.07[d]
		E. 0.100	1.97 ± 0.09[e]	0.34 ± 0.03	2.31 ± 0.11[ns]
	9	A. 0.000	2.62 ± 0.06	0.0 ± 0.0	2.62 ± 0.06
		B. 0.025	2.10 ± 0.10[a]	0.17 ± 0.02	2.27 ± 0.12[c]
		C. 0.050	1.83 ± 0.07[a]	0.32 ± 0.03	2.15 ± 0.10[c]
		D. 0.075	1.76 ± 0.12[b]	0.39 ± 0.05	2.15 ± 0.16[e]
		E. 0.100	1.90 ± 0.10[a]	0.44 ± 0.05	2.34 ± 0.14[ns]
3	0	A. 0.000	2.66 ± 0.03		2.66 ± 0.03
		B. 0.100	2.66 ± 0.03		2.66 ± 0.03
	9	A. 0.000	2.59 ± 0.09	0.0 ± 0.0	2.59 ± 0.09
		B. 0.100	1.76 ± 0.04[a]	0.17 ± 0.01	1.93 ± 0.03[b]
4	0	A. 0.000	2.84 ± 0.06		2.84 ± 0.06
		B. 0.125	2.85 ± 0.05		2.85 ± 0.05
		C. 0.150	2.82 ± 0.05		2.82 ± 0.05
	5	A. 0.000	2.56 ± 0.12	0.0 ± 0.0	2.56 ± 0.12
		B. 0.125	1.78 ± 0.09[c]	0.33 ± 0.04	2.11 ± 0.12[e]
		C. 0.150	1.68 ± 0.11[c]	0.33 ± 0.03	2.01 ± 0.14[e]
	9	A. 0.000	2.61 ± 0.12	0.0 ± 0.0	2.61 ± 0.12
		B. 0.125	1.77 ± 0.08[a]	0.44 ± 0.04	2.21 ± 0.11[c]
		C. 0.150	1.79 ± 0.07[b]	0.47 ± 0.04	2.26 ± 0.09[ns]
5	0	A. 0.000	2.84 ± 0.05		2.84 ± 0.05
		B. 0.100	2.85 ± 0.05		2.85 ± 0.05
	5	A. 0.000	2.62 ± 0.11	0.0 ± 0.0	2.62 ± 0.11
		B. 0.100	1.99 ± 0.06[c]	0.42 ± 0.02	2.41 ± 0.07[ns]
	9	A. 0.000	2.64 ± 0.11	0.0 ± 0.0	2.64 ± 0.11
		B. 0.100	1.71 ± 0.11[c]	0.42 ± 0.06	2.13 ± 0.17[ns]
	15	A. 0.000	2.49 ± 0.07	0.0 ± 0.0	2.49 ± 0.07
		B. 0.100	1.70 ± 0.03[b]	0.60 ± 0.04	2.30 ± 0.06[ns]
	19	A. 0.000	2.59 ± 0.08	0.0 ± 0.0	2.59 ± 0.08
		B. 0.100	1.65 ± 0.07[b]	0.70 ± 0.08	2.35 ± 0.11[ns]

Comparisons with ad libitum control group on same day: [a] $P = 0.0001$; [b] $0.001 > P > 0.0001$; [c] $0.005 > P > 0.001$; [d] $0.01 > P > 0.005$; [e] $0.05 > P > 0.01$; [ns] $P > 0.05$.

treated with 0.05% ($P = 0.005$), 0.075% ($P = 0.0087$), and 0.10% ($P = 0.0038$) than the animals treated at a dosage of 0.025%.

The mean values of total sterols in serum on days 5 and 9 in each of the treated groups were significantly lower ($P \leq 0.006$) than those for the same animals prior to treatment (day 0 values). However, the mean values of total sterols in serum in each of the treatment groups were not significantly lower than those of the untreated control group on day 5, with the exception of the animals treated at a level of 0.075%. On day 9, total sterols were reduced modestly except for the animals treated at a level of 0.10%.

No effect of administration of **VII** on the mean values of food consumption was detected at any of the dose levels tested (data not presented). Similarly, little or no effect of **VII** on the mean values of body weight was observed. However, slightly lower mean values of body weight were observed in the animals fed **VII** at a level of 0.10% on day 4 (−3.2%, $P = 0.043$), day 6 (−3.2%; $P = 0.011$), day 9 (−4.1%; $P = 0.0012$), and day 10 (−3.8%; $P = 0.014$). As the various groups of animals were not perfectly matched with respect to initial mean values of body weight, the effect of administration of **VII** was also evaluated with respect to percentage changes in body weight from initial values (**Table 5**). This analysis indicated that administration of **VII** at levels of 0.075% and 0.10% was associated with moderately decreased values of growth. However, when the percent change in weight was calculated on a daily basis for each animal rather than as a percentage of day 0 values, there were only two significant differences in

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TABLE 5. Effects of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (F_7-15-ketosterol; at levels of 0.025, 0.050, 0.075, and 0.10% in diet for 10 days) on mean percentage changes in body weight (± SEM, n = 8) in male Sprague-Dawley rats (Experiment 1)

	Percentage Change in Body Weight (g)				
Day	Group A % in Diet 0.000	Group B % in Diet 0.025	Group C % in Diet 0.050	Group D % in Diet 0.075	Group E % in Diet 0.100
1	1.6 ± 1.5	0.7 ± 2.1^{ns}	0.7 ± 1.7^{ns}	0.0 ± 1.7^{ns}	−2.2 ± 1.6^{b}
2	5.6 ± 1.3	4.7 ± 2.1^{ns}	5.3 ± 1.9^{ns}	4.6 ± 1.8^{ns}	2.0 ± 1.7^{b}
3	9.4 ± 1.3	8.3 ± 2.2^{ns}	8.3 ± 1.8^{ns}	7.6 ± 2.0^{ns}	4.5 ± 1.6^{b}
4	12.9 ± 1.2	11.5 ± 2.3^{ns}	11.5 ± 1.9^{ns}	10.5 ± 2.2^{ns}	5.2 ± 1.3^{a}
5	18.7 ± 1.1	16.6 ± 2.2^{ns}	16.9 ± 1.9^{ns}	15.2 ± 2.2^{ns}	11.5 ± 1.3^{b}
6	19.7 ± 1.0	17.9 ± 2.1^{ns}	18.2 ± 1.6^{ns}	16.0 ± 2.5^{c}	11.5 ± 1.3^{a}
7	22.9 ± 1.1	20.2 ± 2.6^{ns}	20.7 ± 1.7^{ns}	18.4 ± 2.6^{c}	14.3 ± 1.0^{b}
8	26.8 ± 1.3	23.2 ± 2.6^{ns}	24.1 ± 1.6^{ns}	21.4 ± 2.8^{c}	16.9 ± 1.1^{b}
9	31.6 ± 1.3	31.9 ± 2.7^{ns}	27.8 ± 1.8^{ns}	24.5 ± 3.0^{c}	21.5 ± 1.2^{b}
10	31.5 ± 1.3	27.8 ± 2.9^{ns}	25.5 ± 1.8^{c}	25.5 ± 3.3^{c}	21.8 ± 0.9^{b}

Differences in mean values from the control animals (group A) on the individual days: $^{ns}P > 0.05$; $^{a}P < 0.001$; $^{b}0.001 < P < 0.01$; $^{c}0.01 < P < 0.05$.

the mean values of daily weight change between the ad libitum group and any of the groups receiving **VII.** On day 9, the weight of the ad libitum group increased by 3.8 ± 0.3% while the weight of the 0.075% group increased by 2.5 ± 0.3% ($P = 0.0067$). On day 10, the ad libitum group lost weight (−0.1 ± 0.3%) while the weight of the 0.05% group increased by 1.3 ± 0.5% ($P = 0.0078$).

Table 6 presents the effects of **VII** on organ weights. The major finding was increased weight of small intestine for the animals treated with **VII** at levels of 0.05%, 0.075%, and 0.10%, an increase that appeared to be dose-related. The mean weight of liver from rats treated at a level of 0.05% in diet was higher than that of the control animals. However, no effect of administration of **VII** was observed at higher doses (0.075% and 0.10% in diet). The mean weight of kidneys in rats treated at a level of 0.10% was less than that of control animals. This difference was not observed for the kidney weight expressed as a percentage of total body weight. The mean weight of testes of animals treated at a level of 0.10% was slightly higher than that of control animals.

Experiment 2. Experiment 2 involved administration of **VII** (0.125%) and the 15-ketosterol (0.10%) in diet. Also included were ad libitum control animals and pair-fed control animals for each of the treatment groups. **Table 7** presents the effects of approximately the same molar concentrations of **I** and its F_7 analog **VII** in the diet on the levels of Chol and **VIII** in serum. Administration of **I** (0.10% by weight) significantly reduced mean serum

TABLE 6. Effects of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (F_7-15-ketosterol; at levels of 0.025, 0.050, 0.075, and 0.10% in diet for 10 days) on mean weights (g ± SEM, n = 8) of selected organs (Experiment 1)

	Weight (g ± SEM)				
Organ	Group A % in Diet 0.000	Group B % in Diet 0.025	Group C % in Diet 0.050	Group D % in Diet 0.075	Group E % in Diet 0.100
Liver[a]	10.05 ± 0.20	10.44 ± 0.28	11.04 ± 0.36	10.55 ± 0.45	10.00 ± 0.24
Small intestine[b]	6.59 ± 0.19	6.67 ± 0.21	7.32 ± 0.24	8.59 ± 0.38	9.70 ± 0.41
Spleen	0.70 ± 0.02	0.69 ± 0.03	0.72 ± 0.02	0.67 ± 0.03	0.67 ± 0.02
Kidneys[c]	1.92 ± 0.05	1.88 ± 0.05	1.88 ± 0.05	1.85 ± 0.04	1.76 ± 0.04
Adrenals	0.042 ± 0.001	0.045 ± 0.002	0.042 ± 0.003	0.042 ± 0.002	0.042 ± 0.002
Heart	0.86 ± 0.04	0.82 ± 0.02	0.85 ± 0.02	0.84 ± 0.03	0.79 ± 0.04
Testes[d]	2.82 ± 0.03	2.96 ± 0.07	2.80 ± 0.13	2.95 ± 0.06	2.97 ± 0.08

[a]The mean weight of the livers was higher in group C ($P = 0.013$) than that of the control group A.
[b]The mean weights of small intestine were significantly higher in group C ($P = 0.02$), group D ($P = 0.0009$), and group E ($P = 0.0001$) than that of the control group A. The mean weights of groups A and B did not differ significantly.
[c]The mean weight of kidneys in group E was less ($P = 0.011$) than that of the control group A.
[d]The mean weight of testes in group D was slightly higher ($P = 0.028$) than that of the control group A.

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TABLE 7. Effects of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (0.125% in diet for 10 days) and 3β-hydroxy-5α-cholest-8(14)-en-15-one (0.10% in diet for 10 days) on the levels of serum sterols (mean ± SEM, n = 8) in male Sprague-Dawley rats (Experiment 2)

		Serum Sterols		
Day	Diet	Cholesterol	F_7-Cholesterol	Total Sterols
			mM	
0	A. Ad libitum	3.09 ± 0.12		3.09 ± 0.12
	B. 15-Ketosterol (0.1%)	3.08 ± 0.09		3.08 ± 0.09
	C. Pair-fed to B	3.08 ± 0.09		3.08 ± 0.09
	D. F_7-15-Ketosterol (0.125%)	3.09 ± 0.11		3.09 ± 0.11
	E. Pair-fed to D	3.08 ± 0.11		3.08 ± 0.11
5	A. Ad libitum	2.68 ± 0.08	0.0 ± 0.0	2.68 ± 0.08
	B. 15-Ketosterol (0.1%)[a]	1.34 ± 0.08	0.0 ± 0.0	1.34 ± 0.08
	C. Pair-fed to B	2.22 ± 0.16	0.0 ± 0.0	2.22 ± 0.16
	D. F_7-15-Ketosterol (0.125%)	1.86 ± 0.06	0.30 ± 0.03	2.16 ± 0.08
	E. Pair-fed to D	2.37 ± 0.08	0.0 ± 0.0	2.37 ± 0.08
9	A. Ad libitum	2.63 ± 0.11	0.0 ± 0.0	2.63 ± 0.11
	B. 15-Ketosterol (0.1%)[a]	0.79 ± 0.09	0.0 ± 0.0	0.79 ± 0.09
	C. Pair-fed to B	2.22 ± 0.09	0.0 ± 0.0	2.22 ± 0.09
	D. F_7-15-Ketosterol (0.125%)	1.82 ± 0.04	0.39 ± 0.03	2.21 ± 0.05
	E. Pair-fed to D	2.39 ± 0.09	0.0 ± 0.0	2.39 ± 0.09

[a]n = 7.

Chol levels relative to mean day 0 values for the same group of animals prior to treatment ($P = 0.0001$), to mean values for ad libitum controls on the same day ($P < 0.0003$), and to its pair-fed control group on the same day ($P < 0.005$). **VII** (0.125% by weight in the diet) also reduced serum Chol levels. Mean levels of serum Chol on days 5 and 9 were significantly reduced relative to mean day 0 values for the same group of animals prior to treatment ($P < 0.0011$), to mean values for ad libitum controls on the same day ($P < 0.0004$), and to its pair-fed control group on the same day ($P < 0.003$). **VIII** was detected only in the serum of animals treated with **VII.** Mean values on day 9 were significantly ($P = 0.04$) higher than on day 5. The mean values of total sterols in serum on days 5 and 9 were significantly lower ($P \leq 0.0001$) than those of the same animals prior to treatment (day 0 values) and they were lower ($P \leq 0.0001$) than those of the untreated control group on the same days.

Increasing the dosage of **VII** to a level of 0.125% in diet (approximately the molar equivalent of administration of **I** at a level of 0.10%) had no significant effect on food consumption (**Table 8**). In contrast, administration of **I** markedly suppressed food consumption which was associated with an arrest of growth of the animals (**Fig. 4**). **VII,** at a level of 0.125% in diet, had no effect on growth.

The striking enlargement of the small intestine of rats fed **I** (0.10% in diet) is shown in **Table 9.** Feeding **VII** (0.125% in diet) was also associated with a significant in-

TABLE 8. Effects of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (F_7-15-ketosterol; 0.125% in diet for 10 days) and 3β-hydroxy-5α-cholest-8(14)-en-15-one (15-ketosterol; 0.10% in diet for 10 days) on mean values of daily food consumption (g ± SEM, n = 8) in male Sprague-Dawley rats (Experiment 2)

	Food Consumption (g)				
Day	A. Ad Libitum	B. 15-Ketosterol (0.1%)[a]	C. Pair-Fed to Group B	D. F_7-15-Ketosterol (0.125%)	E. Pair-Fed to Group D
1	19.2 ± 0.9	12.2 ± 1.9	19.2 ± 1.3	16.7 ± 2.0	17.7 ± 2.3
2	17.9 ± 1.0	7.7 ± 1.7	12.2 ± 1.9	17.1 ± 1.3	15.8 ± 1.9
3	18.7 ± 0.6	8.5 ± 0.9	7.7 ± 1.7	18.0 ± 0.5	16.8 ± 1.3
4	19.5 ± 0.6	9.0 ± 0.8	8.5 ± 0.9	19.0 ± 0.3	17.8 ± 0.4
5	19.0 ± 0.5	9.0 ± 0.8	9.0 ± 0.8	17.6 ± 0.7	18.7 ± 0.2
6	18.5 ± 0.5	9.9 ± 0.7	9.0 ± 0.8	17.9 ± 0.4	17.4 ± 0.6
7	19.5 ± 0.5	10.9 ± 0.9	9.9 ± 0.7	19.6 ± 0.5	17.7 ± 0.4
8	20.4 ± 0.6	12.1 ± 1.0	10.9 ± 0.9	19.6 ± 0.5	19.4 ± 0.5
9	20.3 ± 0.4	12.1 ± 1.0	12.1 ± 1.0	19.5 ± 0.5	19.5 ± 0.5
10	19.0 ± 0.6	11.3 ± 1.6	12.1 ± 1.0	20.6 ± 0.9	19.5 ± 0.5

[a]n = 7.

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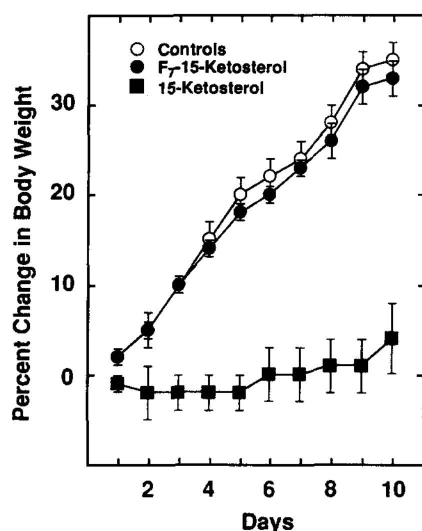

Fig. 4. Effects of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) (0.1% in diet) and 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VII**) (0.125% in diet) on percentage changes in body weight (± SEM, n = 8) in (○), ad libitum controls; (●) F_7-15-ketosterol; (■) 15-ketosterol.

crease in the weight of small intestine. However, this increase was considerably less than that observed with **I.**

Experiment 3. Experiment 3 involved administration of **VII** at a level of 0.10% in the diet. Table 4 presents the effects of **VII** on the levels of Chol and **VIII** in serum. **VII** caused a significant reduction of serum Chol levels relative to those of the same group prior to treatment (P = 0.0001) and to controls on the same day (P = 0.0001). The levels of **VIII** in serum of the animals treated with **VII** (0.10% in diet) were lower than those observed in Experiment 1 (see above).

As in the cases of Experiments 1 and 2, no effect of **VII** on food consumption was observed (data not shown). In contrast to the case of Experiment 1, **VII,** at a level of 0.10% in diet, had no effect on body weight (data not presented). In this experiment, no differences in mean values of organ weights were detected except in the cases of small intestine (experimental, 7.82 ± 0.32 g; control, 5.85 ± 0.25 g; P = 0.0018) and liver (experimental, 10.74 ± 0.32 g; control, 9.79 ± 0.27 g; P = 0.018).

Experiment 4. This experiment involved administration of **VII** at levels of 0.125% and 0.15% in the diet. Table 4 presents the effects of **VII** on the levels of Chol, **VIII,** and total sterols in serum. At a dosage of 0.125% in diet, serum Chol levels were significantly reduced on day 5 (P = 0.026) and day 9 (P = 0.0001) relative to the same animals prior to treatment. At a dosage of 0.15%, serum Chol levels were significantly lower (P = 0.0001 on days 5 and 9) relative to the same animals prior to treatment. Serum Chol levels were also significantly lower in both treatment groups on days 5 and 9 ($P < 0.002$ in each case) relative to ad libitum control animals on the same days. Significant amounts of **VIII** were observed in the serum of animals treated with **VII.** The levels of **VIII** in these animals were significantly higher (P = 0.0001) on day 9 than on day 5.

Administration of **VII** at levels of 0.125% and 0.15% in diet had little or no effect on food consumption by the rats (data not presented). At these dosages, no significant differences were observed between mean values of body weight in the treated rats and those of control rats on any of the days of the study. However, a moderate suppression of growth in this experiment was indicated when the changes in body weight were expressed as percentage changes from day 0 values. Mean values for the animals treated at the 0.125% dosage level were lower ($P < 0.025$) than those of control animals on days 6–10). At the higher dosage level (0.15%), the mean values in treated rats were lower ($P < 0.02$) than control values on days 8–10. At the termination of the experiment (day 10), the mean value for the percentage change in body weight of the controls was

TABLE 9. Effects of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (F_7-15-ketosterol; 0.125% in diet for 10 days) and 3β-hydroxy-5α-cholest-8(14)-en-15-one (15-ketosterol; 0.10% in diet for 10 days) on the mean weights (g ± SEM, n = 8) of selected organs in male Sprague-Dawley rats (Experiment 2)

	Weight (g ± SEM)				
Organ	A. Ad Libitum	B. 15-Ketosterol (0.1%)[a]	C. Pair-Fed to Group B	D. F_7-15-Ketosterol (0.125%)	E. Pair-Fed to Group D
Liver	9.91 ± 0.37	5.94 ± 0.53	6.66 ± 0.37	10.07 ± 0.46	9.30 ± 0.45
Small intestine[b]	6.31 ± 0.28	12.27 ± 0.60	4.96 ± 0.22	7.59 ± 0.61	6.08 ± 0.18
Spleen	0.65 ± 0.02	0.43 ± 0.04	0.46 ± 0.02	0.62 ± 0.02	0.65 ± 0.03
Kidneys	1.92 ± 0.07	1.31 ± 0.05	1.47 ± 0.05	1.76 ± 0.08	1.91 ± 0.07
Adrenals	0.045 ± 0.004	0.035 ± 0.001	0.038 ± 0.002	0.042 ± 0.002	0.044 ± 0.001
Heart	0.89 ± 0.03	0.59 ± 0.03	0.68 ± 0.02	0.82 ± 0.02	0.88 ± 0.02
Testes	3.17 ± 0.08	2.96 ± 0.12	3.07 ± 0.09	3.02 ± 0.04	3.08 ± 0.15

[a]n = 7.
[b]The mean value of the weights of the small intestines from the F_7-15-ketosterol pair-fed group (E) animals was less (P = 0.0018) than that from the control group (A). The mean value of the weights of the small intestines from the F_7-15-ketosterol fed animals (D) was greater (P = 0.036) than that from the F_7-15-ketosterol pair-fed group (E).

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+34.2 ± 2.2%, whereas those for rats treated at dosages of 0.125% and 0.15% were +22.1 ± 3.7% and +25.5 ± 2.2%, respectively. These results differ from those of Experiment 3 in which no effect of **VII** (0.125% in diet) on growth was noted. Nonetheless, the moderate suppression of growth by **VII** at 0.125% on diet in this experiment was not increased at the higher dosage (0.15%) and was clearly different from the marked effect of **I** on growth. Administration of **VII** (0.125% and 0.15%) was associated with increased weight of small intestine (10.45 ± 0.71 g and 10.13 ± 0.40 g, respectively, vs. control, 6.86 ± 0.13 g; P = 0.0001). The mean weights of spleen and heart in the two experimental groups were less than those of the control groups; however, no significant differences in the weights of spleen and heart were observed when expressed as percentages of total body weight (data not presented). The mean value of testicular weight (expressed as a percentage of total body weight) was higher in animals receiving **VII** at 0.125% in diet than in control animals (1.34 ± 0.05% vs. 1.13 ± 0.04%; P = 0.022); however, no effect on testicular weight was observed at a dosage level of 0.15% in diet.

Experiment 5. Experiment 5 involved administration of **VII** at a dosage of 0.10% in diet over a 20-day period. Table 4 presents the effects of extended treatment with **VII** on the levels of Chol and **VIII** in serum. Serum Chol levels were significantly reduced ($P < 0.0001$ on each day) relative to the same animals prior to treatment and also to ad libitum control animals on each of the days ($P < 0.006$ in each case). The levels of **VIII** were significantly higher ($P < 0.03$ in each case) on days 15 and 19, relative to the mean value on day 5 and they were significantly higher ($P < 0.05$ in each case) on days 15 and 19, relative to the mean value on day 9. The mean levels of total sterols on day 5 (P = 0.0006), day 9 (P = 0.0031), day 15 (P = 0.0007), and day 19 (P = 0.0255) in the treated group were lower than those for the same groups of animals prior to treatment (day 0 values). However, the mean levels of total sterols in serum did not differ significantly from the untreated control values on each of the days.

In contrast to the cases of Experiment 1 and Experiment 3, administration of **VII,** at a level of 0.1% in the diet, was associated with slightly lower food consumption (average of 11.9%) on days 2 through 9. Thereafter, with the exception of day 13 (−7.8% lower than that of control animals), there were no significant differences in food consumption in the treated and control animals.

Extended treatment with the **VII** at a level of 0.10% in diet had little effect on growth as measured by percentage change in body weight from initial values. At the termination of the experiment, values for control and treated animals did not differ significantly (day 20: control, +55.0 ± 3.1% vs. experimental, +49.6 ± 2.0%). The values for percentage change in body weight differed significantly only on days 9 and 11–16. The average values in the treated animals on days 11–16 were ~16% lower than that of control animals. Administration of **VII** was associated with an increased weight of small intestine (+32.6%; P = 0.0005). The weights of small intestine (expressed as a percentage of total body weight) in the steroid-treated and control animals were 3.31 ± 0.07% and 2.29 ± 0.06%, respectively (P = 0.0001). The mean values for the weight of kidneys were less (1.91 ± 0.01 g vs. 2.25 ± 0.07 g, P = 0.002; as a percentage of total body weight, 0.69 ± 0.01% vs. 0.75 ± 0.01%, P = 0.0046) in the animals receiving **VII.** Whereas the mean weights of liver did not differ in the experimental and control animals, the livers of treated rats were slightly larger (+11.5%) when expressed as a percentage of total body weight (4.45 ± 0.06% vs. 3.99 ± 0.11%, P = 0.0028). The mean weight of testes of treated animals was slightly less (−9.0%; P = 0.042); however, when expressed as a percentage of total body weight, there was no difference in the weight of testes (treated, 1.15 ± 0.02%; control, 1.14 ± 0.01%).

DISCUSSION

3β-Hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VII**) was synthesized (32) to evaluate the effects of the F_7 substitution on the actions of the parent 15-ketosterol **I. VII** has been shown to be highly active in lowering the levels of HMG-CoA reductase activity in CHO-K1 cells and in HepG2 cells, with a potency equivalent to that of **I** (32). The possibility that the effects of **VII** on the levels of reductase activity in the cultured cells might be due to the F_7 substitution was excluded by the demonstration that synthetic **VIII,** containing the same F_7 substitution, had little or no effect on HMG-CoA reductase activity in CHO-K1 cells (32). In the present study we have explored the effects of dietary administration of **VII** in rats.

Dietary administration of **I** to rats in a ground chow diet (Purina Formulab 5008) or in a "cholesterol-free test diet" results in a marked suppression of food consumption (7, 27–29). The results presented in Table 8 illustrate this effect, which has been observed at concentrations as low as 0.05% in diet (D. H. Needleman and G. J. Schroepfer, Jr., unpublished data). This decreased food consumption induced by the dietary administration of **I** to rats is associated with a marked suppression of the growth of the animals as measured by determination of total body weight (7, 27–29) (Fig. 4). These actions of **I** required the inclusion of experimental protocols involving pair-fed control animals to interpret the effects of its oral administration. Dietary administration of **I** to rats is also associated with a significant enlargement of the small intestine, for which the morphology (relative to ad libitum and

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pair-fed control animals) has been described (29). It is important to note that oral administration of **I** to nonhuman primates resulted in a significant reduction of serum Chol levels which was not associated with a suppression of food consumption, effects on body weight (30, 31) or on the morphology of small intestine (29).

Administration of **VII** to rats had little or no effect on food consumption at all of the doses studied (i.e., from 0.025% to 0.15% by weight in diet). These observations are in marked contrast to those made with **I.** The mechanism(s) involved in the marked lowering of food consumption by **I** in rats has not been determined. The very substantial metabolism of **I** to polar metabolites (most of which appear to arise by initial oxidation at C-26) which are rapidly excreted in bile, raises the possibility that these polar metabolites may be involved in the suppression of food consumption. This suggestion is greatly strengthened by the demonstration that dietary administration of **VII** (in which oxidation at C-26 and C-27 is blocked by the F_7 substitution) to rats had little or no effect on food consumption, even at very high dosage levels. These findings suggest that one or more of the polar metabolites of **I** may be the active species involved in the suppression of food intake observed in this species.

Dietary administration of **VII** to rats had only slight or no effects on body weight. These observations are in contrast to the marked effects of dietary administration of **I** to rats on body weight. Fig. 4 provides a striking illustration of the differences between the effects of **VII** and **I.** In this experiment, dietary administration of **I** at a level of 0.10% resulted in clear arrest of growth of the animals. In contrast, dietary administration of **VII,** at an approximately equimolar level in diet, had no effect on growth as measured by change in total body weight.

Administration of **VII** to rats resulted in the accumulation of a sterol in liver and blood with the properties of **VIII.** This sterol was found in both free and esterified states and represented ~21% of total sterol in liver after 20 days of administration of the **VII** at a dosage level of 0.10%. In the same experiment, **VIII** represented ~30% of total sterol in serum on day 19. **VIII,** isolated from liver, showed the same chromatographic behavior as the synthetic **VIII.** The IR spectrum of **VIII** from liver was essentially identical to that of the synthetic **VIII.** Whereas the IR spectrum of **VIII** differed markedly from that of Chol, the differences were those attributable to known C-F stretching vibrations (42, 43). The mass spectrum of the TMS derivative of **VIII** present in liver (and in serum) was, allowing for minor differences in operating conditions, essentially the same as that of the TMS ether of authentic **VIII** (Table 1). In addition, the spectra of the TMS derivative of **VIII** samples were fully compatible with those of TMS ethers of 3-hydroxy-Δ^5-steroids (38-40). The mass spectrum of the underivatized **VIII** was also essentially the same as that of the synthetic **VIII.** Further, the results of high resolution MS measurements were in close accord with established or suggested (41) fragmentations of Chol (Table 2).[4] The ^{1}H ^{13}C, and ^{19}F NMR spectra of **VIII** isolated from liver were essentially the same as those of synthetic **VIII.**

The occurrence of **VIII** in liver and blood indicates its formation from **VII,** presumably by the same sequence of reactions involved in the overall conversion of **I** to Chol (Fig. 1). The substantial amounts of **VIII** observed in liver and serum suggest no adverse effect of the F_7 substitution in the side chain on the enzymatic reactions involved in the overall conversion of the $\Delta^{8(14)}$-15-one to the Δ^5-sterol. Detailed studies of this matter will await the availability of **VII** in labeled form. No intermediates in the conversion of **VII** to **VIII** were detected in serum. However, trace amounts of an F_7-monounsaturated sterol (possibly the Δ^7- or Δ^8-analog of **VIII**) were detected in liver. Very low levels of the administered **VII** were detected in liver and in serum.

The presence of significant levels of **VIII** in the serum of animals receiving **VII** complicated the determination of the levels of Chol. Since **VIII** gave an essentially identical response as Chol in routine Chol oxidase-based assay of Chol, use of enzymatic assay would only provide information on the levels of total sterols (i.e., Chol plus **VIII**) in serum. **VIII** gave significantly less color response than Chol with the Liebermann-Burchard reagent,[5] a finding

[4]Interestingly, the MS of samples of **VIII** did not show ions corresponding to loss of HF. The MS of 25-fluoro-6β-methoxy-3α,5-cyclo-5α-cholestane has been reported (44) to show ions of high abundance due to loss of HF (i.e., M-HF, M-CH_3-HF, and M-CH_3OH-HF). Similarly, the MS of 3β-acetoxy-25-fluorocholest-5-ene (44) and of 25-fluorocholesterol (45) were reported to show ions of high abundance corresponding to M-CH_3COOH-HF and M-HF, respectively. The results of recent detailed studies (46) of the spectral properties of 25-fluorocholesterol showed the presence of significant ions resulting from loss of HF including M-HF, M-CH_3-HF, M-H_2O-HF, M-H_2O-CH_3-HF, and M-H_2O-C_5H_7-HF. The absence of significant ions from loss of HF in the MS of **VIII** most probably arises from a suppression of the loss of fluorine from C-25 by the fluorine substitution at C-26 and C-27.

[5]The finding that **VIII** gave only ~63% of the color response with the Liebermann-Burchard color reagent as that observed with Chol is worthy of note. Desmosterol (cholesta-5,24-dien-3β-ol) has also been found to give a lower color response than Chol with this reagent (47, 48), with reported color yields which were 52% (47) and 60% (48) that of Chol. To our knowledge, the precise chemical basis for this observation has not been established. In the present study we have not established the origin of the reduced color yield of the F_7-Chol with the Liebermann-Burchard reagent. Napoli et al. (44) have reported that heating of 25-fluoro-6β-methoxy-3α,5-cyclo-5α-cholestane in glacial acetic acid at 70°C for 24 h gives a mixture of Δ^{24}- and Δ^{25}-olefins and 25-acetoxy compounds. It should be noted that acetic acid is a component (along with acetic anhydride and concentrated sulfuric acid) of the Liebermann-Burchard color reagent. Upon exposure of 25-acetoxy sterols to the color reagent, formation of Δ^{24} and Δ^{25} unsaturated sterols could be anticipated. If the reactions described by Napoli et al. (44) for the 25-fluorosterol carry over to the case of **VIII,** its treatment with the Liebermann-Burchard color reagent might yield the $\Delta^{5,24}$-sterol exclusively. The formation of this $\Delta^{5,24}$-sterol may account for the reduced color yield given by **VIII** with the color reagent.

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that precluded the use of this color reagent for assay not only of the levels of Chol in serum but also of the levels of the combination of Chol and **VIII.** Therefore, the levels of Chol in serum were determined by capillary GC under conditions in which very substantial separation of Chol and its F_7-analog is readily achieved. In addition, the same analyses provided critical data on the levels of **VIII** in serum.

Table 4 presents a summary of the effects of administration of **VII** on the levels of **VIII** in serum. In general, these results indicate that the levels of **VIII** in serum are related to dosage and duration of administration of **VII.** The effect of dosage on the levels of **VIII** is well illustrated in Experiment 1, in which a clear positive relationship can be noted between the levels of **VIII** in serum on day 9 and the dosage of **VII** over the range from 0.025% to 0.10% in the diet. Combining these data with those of other experiments indicated that further increases in dosages resulted in only modest additional increases in the levels of **VIII** in serum on day 9, with maximum levels of **VIII** of 24 mg/dl at 0.15% **VII** in the diet. The relationship between **VIII** levels in serum and dosage of **VII** appeared to be consistent from one experiment to another except for Experiment 3. In this case, a lower level of **VIII** (8.6 mg/dl) in serum was observed on day 9 at a dosage of 0.10% than those observed in Experiments 1 and 5 (22.5 mg/dl and 21.3 mg/dl, respectively) at the same dose.

The levels of **VIII** in serum appeared to increase with the duration of administration of **VII** when it was fed at levels of 0.1% or higher in the diet. Thus, in Experiments 1, 2, and 4 (involving administration of **VII** at doses of 0.10%, 0.125%, and 0.15% in the diet), the levels of **VIII** were higher on day 9 than on day 5. In Experiment 5, in which **VII** was fed at 0.1% in the diet for a longer period of time, the levels of **VIII** on days 15 and 19 were higher than those on days 5 and 9, with a maximum value of 36 mg/dl on day 19.

Dietary administration of **VII** resulted in a significant lowering of the levels of Chol in serum in each experiment at each of the doses studied (Table 4). The reduction of serum Chol relative to values in the same animals prior to treatment on day 0 ranged from 28% to 42% in the individual experiments, while the lowering of serum Chol relative to control animals on the same day ranged from 20% to 36% (Table 4).

It is important to note that the lowering of serum Chol levels in rats upon administration of **VII** occurred under conditions in which no effect upon food consumption was observed, a finding not made previously with the parent 15-ketosterol **I** in rats. In addition, while metabolites of **I** formed by initial oxidation in the side chain at C-26 or C-25 may contribute to the lowering of serum Chol levels in animals after its administration, the results presented herein indicate that substantial lowering of serum Chol levels can be achieved in the absence of the formation of these metabolites. Moreover, **VII** lowered serum Chol significantly at very low levels in the diet, i.e., at as low a concentration as 0.025%. This concentration of **VII** corresponds to a level of **I** in the diet of 0.019% after adjustment for the substantial difference in molecular weight between **VII** and **I.** Significant hypocholesterolemic action of **I** in rats has only been observed at a level as low as 0.05% in a Chol-free test diet, and the hypocholesterolemic effect seen under these conditions at this dosage was not sustained upon extended administration. In addition, the reduction of serum Chol levels by **VII** at 0.025% in the diet was not associated with any enlargement of small intestine (vide infra).

When the results of the administration of **VII** were examined with respect to effects on the levels of total serum sterols, i.e., Chol plus **VIII,** total sterols were significantly reduced relative to pretreatment (day 0) values in each experiment and at each dosage level (from 18% to 29%) at the termination of the various experiments. When the same data were analyzed relative to levels in control animals on the same days, only slight or moderate lowering of total sterols was observed. In addition, in Experiment 5 no significant lowering of total sterols was observed and, in Experiments 1 and 4, no significant lowering was observed on day 9 at dosage levels of **VII** of 0.10% and 0.15% in diet.

The effects of dietary administration of **VII** on the weights of liver, small intestine, spleen, kidneys, adrenals, heart, and testes were also evaluated. No effect of administration of **VII** on the weights of adrenals was detected in any of the experiments. No clear effects of **VII** on the weights of heart and spleen were detected. In Experiment 4 (at doses of 0.125% and 0.15% by weight in diet), the mean weights of heart and spleen were less than those of controls; however, when organ weight was expressed as a percentage of total body weight, no differences were observed between treated and control animals. There was no consistent effect on the weight of kidneys. In Experiment 1, a slightly lower value of the weight of kidneys was observed at a dosage of 0.10%; however, no difference was observed when the organ weight was expressed as a percentage of total body weight. In Experiment 5, administration of **VII** at the same dosage level was associated with lower weights of kidneys. However, no effect on kidney weight was observed at higher dosage levels (0.125% and 0.15%). Administration of **VII** had no consistent effect on liver weight. In Experiment 1, a higher value for liver weight was observed at a dosage level of 0.05% in diet; however, no effect of **VII** at higher dosages (0.075% and 0.10%) was observed. In Experiments 3 and 5 administration of **VII** at a level of 0.10% was associated with slightly increased levels of liver weight; however, no effect was observed at higher dosages (0.125% and 0.15%) in two other experiments (2 and 4). The only consistent observation made with regard to the

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TABLE 10. Summary of effects of dietary administration of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (F_7-15-ketosterol) and 3β-hydroxy-5α-cholest-8(14)-en-15-one (15-ketosterol) on the weight of small intestine

Sterol	% in Diet	Experiment	Percentage Difference from Ad Libitum Control: From Weight in g	Percentage Difference from Ad Libitum Control: From % of Total Body Weight
F_7-15-Ketosterol	0.025	1	+ 1.2[a]	+ 1.1[a]
	0.050	1	+ 11.1	+ 10.7
	0.075	1	+ 30.3	+ 28.9
	0.100	1	+ 47.2	+ 52.9
	0.100	3	+ 33.7	+ 31.3
	0.100	5	+ 44.5	+ 32.7
	0.125	2	+ 20.3	+ 26.7
	0.125	4	+ 52.3	+ 56.8
	0.150	4	+ 47.7	+ 51.5
15-Ketosterol	0.100	2	+ 84.9	+ 157.8

[a]Not significant ($P > 0.05$).

effect of **VII** on organ weights was an increased weight of small intestine, an effect that was observed in each experiment and at each dosage level except that of 0.025%. These results are summarized in **Table 10.** The magnitude of this effect was considerably less than that caused by **I.**

It is not known whether the accumulation of **VIII** in blood and tissues confers beneficial or adverse effects. The availability of synthetic **VIII** (32) provides opportunities for exploring the effects of the F_7 substitution in Chol in cultured cells and intact animals, as well as in synthetic and natural membrane preparations. An alternative approach to the enrichment of cells with **VIII** is the in vivo modification of sterol composition by the administration of **VII** to animals as described herein.

In summary, administration of **VII** in basal diet to rats results in a lowering of serum Chol levels which, in marked contrast to **I,** was not associated with a lowering of food consumption. The hypocholesterolemic action of **VII** was observed at much lower concentrations in the diet than for **I. VII** was converted to **VIII** which was shown to be present at significant levels in liver and serum. These results, coupled with our previously established knowledge of the reactions involved in the overall conversion of the **I** to Chol, provide the basis for the design of analogs of **VII** in which its metabolism to **VIII** is blocked and for which enhanced hypocholesterolemic action can be anticipated.

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