pure sample of 4-acetamido-4'-methylsulfonylbiphenyl, recrystallized from glacial AcOH, melted at 267–268°. *Anal.* ($C_{15}H_{15}NO_3S$) C, H, N, S.

The 4-acetamido group of the biphenyl derivative described above was hydrolyzed by refluxing 1.89 g (0.01 mole) of 4-acetamido-4'-methylsulfonylbiphenyl in 20% HCl (100 ml) for *ca.* 20 hr. The reaction mixture was then filtered hot and the clear filtrate was made strongly basic with 10 *N* NaOH solution. The crude, hydrolysis product was collected by filtration and recrystallized (MeOH); 1.4 g (82%), mp 203–205°. *Anal.* ($C_{13}H_{13}NO_2S$) C, H, N, S.

Diazotization of the hydrolysis product was accomplished by adding an aqueous solution of $NaNO_2$ (1.6 g in 4 ml of H_2O) dropwise (20 min) to a vigorously stirred, cooled (0–5°) suspension of 4-amino-4'-methylsulfonylbiphenyl (2.3 g, 0.01 mole) in glacial AcOH (15 ml) and concentrated H_2SO_4 (15 ml). When diazotization was complete, the excess HNO_2 was decomposed by the cautious addition (30 min) of urea at 0–5°. The cold solution of the diazonium sulfate was then added slowly (20 min) to a refluxing H_2SO_4 solution (40 wt %) and, after addition was complete, the reation mixture was allowed to reflux an additional 15 min. Dilution of the acidic solution with H_2O afforded a crude solid which was collected by filtration, dissolved in 1 *N* NaOH (20 ml) and refiltered. Acidification of the clear filtrate using an excess of 1 *N* H_2SO_4 furnished a precipitate which was washed with two 75-ml portions of H_2O and then air dried. The yield of crude 4-hydroxy-4'-methylsulfonylbiphenyl obtained following the procedures outlined above melted at 189–190°; 1.7 g (75%). The ir absorption bands were as expected and the intermediate was used in the final step without further purification.

Following method A, 1.9 g (0.01 mole) of the sodio derivative of 4-hydroxy-4'-methylsulfonylbiphenyl was allowed to react with 1.3 g (0.01 mole) of 2-pyrrolidinylethyl chloride in refluxing DMF. After a 60-hr reflux period, the reaction mixture was cooled, filtered, and concentrated to a semisolid residue. Trituration of the crude yield with two 50-ml portions of H_2O yielded an insoluble product which was taken up in C_6H_6 (75 ml). The C_6H_6 solution was decolorized (charcoal), dried (Na_2SO_4), and then treated with excess HCl gas. The HCl salt which precipitated was dissolved in H_2O (100 ml), decolorized (charcoal), and filtered, and the clear, acidic filtrate was made basic (excess 1.0 *N* NaOH). The desired product (**32**) which separated from the basic solution was collected and recrystallized (C_6H_6), 1.0 g (41%), mp 153–154°.

1-{2-[*p*-(*p*-Bromophenyl)anilino]ethyl}pyrrolidine (15).—To a suspension consisting of 6.2 g (0.025 mole) of the lithio derivative of 4-amino-4'-bromobiphenyl in dry toluene (100 ml) was added 3.4 g (0.025 mole) of 2-pyrrolidinylethyl chloride and the reaction mixture was refluxed 22 hr. The LiCl was removed by filtration and the clear filtrate was concentrated to a semisolid residue which was dissolved in an Et_2O (50 ml) and C_6H_6 (50 ml) solution. The solution was then treated with an excess of dry HCl gas and the crude precipitated HCl salt was collected and dissolved in a minimum amount of H_2O. A gray-white solid separated from the acid solution after addition of an excess of aqueous KOH solution (10 *N*). The solid isolated in this manner was recrystallized once (heptane); 3.8 g (44%), mp 141–142°.

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1-{2-[4'-(Trifluoromethyl)-4-biphenylyloxy]ethyl}pyrrolidine. A Potent Hyposterolemic Agent

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The oral hypocholesteremic compound, 1-{2-[4'-(trifluoromethyl)-4-biphenylyloxy]ethyl}pyrrolidine (boxidine),[1] was studied in rats, mice, monkeys, and dogs and found to be active in all species. In the rat, the species studied most intensively, it was active at a dose of 0.0003% in the diet, equivalent to the ingestion of approximately 0.3 mg/kg of body weight. Triglycerides and phospholipids were reduced as well. Boxidine was ten times as active as *trans*-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride,[2] 1000 times as active as clofibrate,[3] and 50 times as active as triparanol.[4] It inhibited the biosynthesis of cholesterol at the 7-dehydrocholesterol stage but its primary mechanism of action may be the inhibition of sterol absorption.

Sustained interest in hypocholesteremic agents has resulted in a plethora of reports on compounds which lower blood cholesterol level by inhibiting its synthesis at various stages in the biosynthetic pathway. Most of these compounds exert their inhibitory action at the desmosterol stage,[4-6] whereas *trans*-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride inhibits at 7-dehydrocholesterol.[7] In the course of our search for means of lowering serum and tissue cholesterol, a class of compounds was discovered whose hyposterolemic activity may be due indirectly to the formation of 7-dehydrocholesterol and directly to the inhibition of sterol absorption. A preliminary study of these compounds has been reported[8] and the details of synthesis have been described.[9]

We wish to report on some biological studies done on a representative member of this class, 1-{2-[4'-(trifluoromethyl)-4-biphenylyloxy]ethyl}pyrrolidine (boxidine, **35**) in the series described by Bach, *et al.*[9]

(1) The name of this compound was approved by the U. S. Adopted Names Council; *J. Am. Med. Assoc.*, **203**, 143 (1968).

(2) AY9944, Ayerst Research Laboratories, Montreal, Canada.

(3) Atromid-S®, ethyl α-(p-chlorophenoxy)isobutyrate, Ayerst Research Laboratories, Montreal, Canada.

(4) T. R. Blohm and R. D. MacKenzie, *Arch. Biochem. Biophys.*, **85**, 245 (1959).

(5) E. W. Cantrall, R. Littel, S. M. Stolar, W. P. Cekleniak, H. J. Albers, S. Gordon, and S. Bernstein, *Steroids*, **1**, 173 (1963).

(6) R. E. Cousell, P. D. Klimstra, and R. E. Ranney, *J. Med. Pharm. Chem.*, **5**, 720 (1962).

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(9) F. L. Bach, J. C. Barclay, F. Kende, and E. Cohen, *J. Med. Chem.*, **11**, 987 (1968).

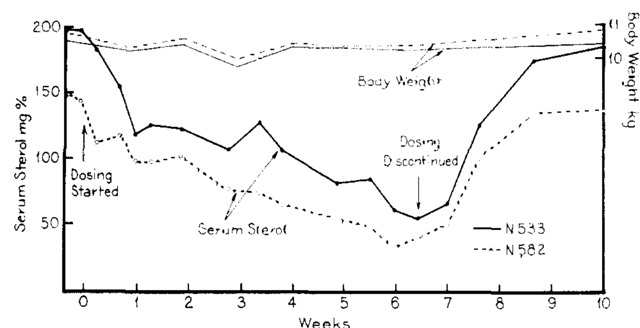

Figure 1.—The effect of boxidine on the serum sterol and body weight of dogs after a daily oral dose of 10 mg/kg.

Experimental Section

Boxidine was subjected to the following investigations: (a) efficacy in various species, *e.g.*, rats, mice, monkeys, and dogs; (b) time and dose response in rats at 1, 2, and 4 weeks; (c) 3 months feeding study in rats; (d) relative potency as compared to known hypocholesteremic agents; and (e) mechanism of action studies.

Animals and Diets.—Rats were male CFE strain obtained from Carworth Farms, New City, N. Y. Mice were male C3H strain obtained from our own breeding colony. They were housed two per wire bottom cage and fed the diets and water *ad libitum*. Diets were prepared by dissolving the compound in a few milliliters of acetone, pouring the solution over ground Purina Laboratory Chow, and allowing the solvent to evaporate while the diet was being thoroughly mixed. African green monkeys obtained from a commercial supplier and young adult male beagle dogs from our own breeding colony were used in the efficacy studies.

Sterol and Lipid Determination.—Serum sterols were determined on 0.1 ml of serum by Trinder[10] saponification and extraction procedure followed by the colorimetric analysis of Zlatkis, *et al.*[11] Quantification of 7-dehydrocholesterol was obtained by measuring the optical density at 281 mμ and determining the concentration from a standard curve. Corrections for 7-dehydrocholesterol in total sterols were calculated using a factor determined experimentally which indicated that 7-dehydrocholesterol yielded only half the optical density of cholesterol when treated with the $FeCl_3$-H_2SO_4 colorimetric reagent and measured at 570 mμ. Thus

$$\text{total sterol} = \text{sterol at 570 m}\mu + {}^1/_2\,(\text{sterol at 281 m}\mu)$$

Liver lipids were determined by homogenizing approximately 1 g of liver in 25 ml of $EtOH$–Et_2O (3:1) and following the method of Shipley, *et al.*[12] Total liver sterol was determined on an aliquot of the petroleum ether (bp 30–60°) extract obtained for total lipid determination. This sample was saponified and extracted by the Trinder method[10] followed by the colorimetric method described by Zlatkis, *et al.*[11]

Adrenal sterols were determined by saponifying both adrenal glands in 2 ml of 25% alcoholic KOH and treating as described for serum. Triglycerides were determined using the lipid extraction procedure of Nicolaysen and Nygaard[13] followed by the triglyceride determination of Van Handel.[14] Phospholipids were determined on the lipid extract above using the method of Bartlett.[15] Various combined serum and liver samples from various animal studies were saponified and sterols were extracted into petroleum ether. Aliquots of these extracts were applied to a gas–liquid partition chromatographic (glpc) column at the following conditions: Barber-Colman Model 10, 1% Epon Resin 1001 on Anakrom 60–70 mesh, 1.82 m × 4 mm id, column temperature 235°, cell temperature 260°, flash heater 280°, argon gas flow 20 cc/min at 30 psi, ^{90}Sr detector.

(10) P. Trinder, *Analyst*, **77**, 321 (1952).
(11) A. Zlatkis, B. Zak, and A. J. Boyle, *J. Lab. Clin. Med.*, **41**, 486 (1953).
(12) R. A. Shipley, E. B. Chudzek, and P. Gyorgy, *Arch. Biochem. Biophys.*, **16**, 301 (1948).
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Results and Discussion

Efficacy in Various Species. Rats. The results of a 6-day dose response to boxidine are shown in Table I.

TABLE I

EFFECT OF BOXIDINE ON SERUM STEROL OF RATS[a] AFTER 6 DAYS OF ADMINISTRATION

Dose, % in diet	Food intake, g/rat/day	Serum sterol, mg %	Serum sterol, % of control
0	18	81 ± 3.2[b]	
0.0005	17	42 ± 3.4	52
0.001	17	29 ± 4.6	36
0.003	18	21 ± 1.5	26
0.01	16	23 ± 3.6	28

[a] Male CFE rats, 125 ± 5 g initial weight, four per group except six controls. [b] Mean ± standard error.

A level of 0.0005% in the diet, which is equivalent to a daily dose of 0.5 mg/kg, caused approximately a 50% reduction in serum sterol.

Mice.—The results of a 6-day study are shown in Table II. As shown by the data, boxidine was highly

TABLE II

EFFECT OF BOXIDINE IN MICE[a] AFTER 6 DAYS OF ADMINISTRATION

Dose, % in diet	Food intake, g/mouse/day	Serum sterol, mg %	Serum sterol, % of control
0	4.2	132 ± 4.2[b]	
0.001	3.6	52 ± 5.6	39
0.003	3.4	30 ± 3.1	23
0.01	2.6	27 ± 3.5	20
0.03	1.8	30 ± 2.6	23

[a] Male C3H, 20 ± 2 g initial weight, six per group. [b] Mean ± standard error.

active at all levels tested. Reduced food intake was noted at the higher doses. Glpc analyses of the sera indicated small amounts of 7-dehydrocholesterol.

Monkeys.—The results of 1 week of oral administration at a dose of 5-mg/kg are shown in Table III.

TABLE III

EFFECT OF BOXIDINE ON SERUM STEROL OF MONKEYS[a] AFTER 7 DAYS OF ADMINISTRATION

Test time	Serum sterol, mg %
Pretreatment	148 (137–163)[b]
1 week (5 mg/kg)	140 (121–161)[c]
1 week off compound	100 (75–125)[c]
2 weeks off compound	98 (80–115)[c]
6 weeks off compound	180 (116–190)

[a] Three male African green monkeys, 2.88-kg average weight, were given a daily oral dose of 5 mg/kg in a no. 5 gelatin capsule. [b] Mean (range). [c] Small amounts of 7-dehydrocholesterol were found in the serum by glpc.

There appeared to be a delayed response to boxidine which became apparent after drug administration was terminated for 1 week. Complete return to pretreatment levels was observed at 6 weeks posttreatment.

Dogs.—The results of treating two dogs orally with boxidine are shown in Figure 1. Within 1 week of dosing at a level of 10 mg/kg there was a progressive reduction in serum sterol. Small quantities of 7-dehydrocholesterol appeared in the serum during the study. However, within 3 weeks after discontinuance of treatment, the sterol level returned to normal and

TABLE IV

EFFECT OF BOXIDINE ON SERUM LIPIDS OF RATS[a] AT VARIOUS TIME INTERVALS

Dose, % of diet	1 week Cholesterol mg %[b]	1 week Cholesterol % of control	2 weeks Cholesterol mg %[b]	2 weeks Cholesterol % of control	4 weeks Cholesterol, mg %	4 weeks 7-Dehydro-cholesterol, mg %	4 weeks Total % of control	4 weeks Phospholipids mg %	4 weeks Phospholipids % of control	4 weeks Triglycerides mg %	4 weeks Triglycerides % of control
0	89 ± 12[c]		89 ± 3.8		83 ± 7.8	0		95 ± 2.7		169 ± 13.4	
0.0001	85 ± 3.4	95	72 ± 3.3	81	78 ± 2.5	2 ± 0.2	96	91 ± 5.3	96	160 ± 15.1	95
0.0003	60 ± 8.2	67	44 ± 6.7	49	50 ± 4.3	11 ± 1.2	73	79 ± 5.0	83	109 ± 12.2	65
0.001	25 ± 2.8	28	21 ± 1.9	24	23 ± 1.9	18 ± 1.0	49	57 ± 6.4	60	113 ± 6.4	67
0.01	12 ± 1.0	13	12 ± 1.9	13	3 ± 1.5	12 ± 1.0	18	26 ± 3.1	27	61 ± 8.2	36
0.03	18 ± 1.9	20	7 ± 1.4	8	5 ± 1.4	17 ± 0.9	23	27 ± 4.4	28	86 ± 8.7	51

[a] Male, CFE strain, 125 ± 5 g initial weight. [b] Uncorrected for 7-dehydrocholesterol. [c] Mean ± standard error, six per group.

TABLE V

EFFECTS OF VARIOUS LEVELS OF BOXIDINE IN RATS[a] AFTER 4 WEEKS OF ADMINISTRATION

Dose, % of diet	Food intake, g/rat/day	Final body wt, g	Adrenal wt, mg	Adrenal wt/body wt, mg/100 g	Adrenal sterol,[b] mg/g	Liver wt, g	Liver wt/body wt, g/100 g	Liver sterol,[b] mg/g	Liver lipids, mg/g	Testes wt, g	Testes wt/body wt, g/100 g
0	18.2	285[c] ± 7.2	43.3 ± 2.4	15.1 ± 0.64	28.5 ± 1.8	12.8 ± 0.64	4.4 ± 0.11	2.4 ± 0.11	80.0 ± 0.11	3.1 ± 0.06	1.0 ± 0.03
0.0001	18.2	283 ± 5.3	46.6 ± 1.9	16.5 ± 0.61	22.9 ± 1.5	12.8 ± 0.33	4.5 ± 0.04	2.3 ± 0.08	81.4 ± 1.6	3.1 ± 0.09	1.0 ± 0.02
0.0003	16.2	264 ± 6.4	55.8 ± 2.1	21.1 ± 0.82	21.0 ± 1.7	12.4 ± 0.36	4.7 ± 0.06	2.1 ± 0.08	73.4 ± 1.8	3.0 ± 0.04	1.1 ± 0.04
0.001	16.8	270 ± 4.8	74.1 ± 3.0	27.3 ± 0.98	10.1 ± 1.2	13.7 ± 0.44	5.0 ± 0.08	1.8 ± 0.03	79.3 ± 2.0	3.1 ± 0.03	1.1 ± 0.04
0.01	9.1	156 ± 4.6	88.0 ± 5.3	56.1 ± 2.6	4.4 ± 1.0	8.2 ± 0.37	5.2 ± 0.16	1.4 ± 0.08	76.3 ± 2.9	2.6 ± 0.08	1.6 ± 0.03
0.03	11.0	176 ± 8.0	94.1 ± 4.2	53.5 ± 1.0	3.2 ± 0.43	9.0 ± 0.43	5.1 ± 0.08	1.4 ± 0.03	74.0 ± 3.0	2.7 ± 0.06	1.5 ± 0.04

[a] Male CFE strain, 125 ± 5 g initial weight. These are the same animals used in Table IV. [b] Uncorrected for 7-dehydrocholesterol. [c] Mean ± standard error, six per group.

7-dehydrocholesterol could not be detected. No effect on body weight was observed.

Time and Dose Response in Rats.—The hyposterolemic dose response in the rat at intervals of 1, 2, and 4 weeks and the effects of serum phospholipids and triglycerides are shown in Table IV. As can be seen from the data, the compound at 0.0003% in the diet was active in reducing lipid levels.

The actual quantities of 7-dehydrocholesterol and cholesterol were determined in the serum samples obtained at 4 weeks (Table IV). The results indicate that relatively small amounts of the former appear, whereas relatively large amounts of cholesterol disappear so that one cannot say that the 7-dehydrocholesterol replaces cholesterol. There is, in fact, an absolute reduction of total sterol with compound administration.

The effects of different levels of boxidine on various organs and their lipid content are shown in Table V. Adrenal sterol was markedly reduced and adrenal hypertrophy was noted. Liver lipids and perhaps sterols were unchanged. Examination of liver sterols by glpc revealed increasing quantities of 7-dehydrocholesterol with increasing dose levels. Hence, if the sterol analyses had been corrected upwards, they would have been approximately the same as controls. Food intake was reduced at the high dose levels which was subsequently reflected in reduced body, liver, and testes weights as compared with controls. It is of interest, however, that the ratio of testes or liver to body weight was increased, probably due to the marked decrease in body weight.

Three Months Feeding Study in Rats.—To protect the animals from the pharmacologic effects of boxidine resulting in marked sterol depletion, they were fed ground Purina Laboratory Chow supplemented with 1% cholesterol + 0.5% cholic acid. This group served as the control. A corresponding group of animals was fed the same supplemented diet with the addition of boxidine at a level of 0.03%. After 3 months, tail blood samples for hematological examination were obtained under light ether anesthesia and the animals were sacrificed by decapitation. Various organs were removed, weighed, and examined grossly.

Organ weights are shown in Table VI and the hematology study in Table VII. There was no significant difference between treated and control animals. The large livers in both treated and control animals were

TABLE VI

EFFECTS OF BOXIDINE ON BODY AND ORGAN WEIGHTS[a] OF RATS AFTER 3 MONTHS OF ADMINISTRATION

	Controls	0.03% boxidine[b]
Final body weight, g[c]	365.0 ± 5.8[d]	343.0 ± 6.5
Liver, g	22.5 ± 1.0	21.5 ± 1.2
Adrenal glands, mg	59.2 ± 3.2	57.2 ± 2.5
Kidneys, g	2.96 ± 0.10	2.78 ± 0.13
Heart, g	1.13 ± 0.07	1.09 ± 0.05
Testes, g	2.84 ± 0.08	2.70 ± 0.05
Seminal vesicles, g	1.10 ± 0.05	1.00 ± 0.07

[a] All organs were wet weight. [b] One death in this group. [c] Male CFE rats, 50 ± 5 g initial weight, six per group. All animals were on a 1% cholesterol + 0.5% cholic acid supplemented diet for 13 weeks. [d] Mean ± standard error.

TABLE VII

EFFECT OF BOXIDINE ON THE HEMATOLOGY OF THE RAT[a] AFTER 3 MONTHS OF ADMINISTRATION

	Control	0.03% boxidine
Serum sterol, mg %	219 ± 38[b]	211 ± 16
Hemoglobin, g/100 ml	12.4 ± 0.29	12.3 ± 0.50
Hematocrit, %	34.5 ± 1.3	35.2 ± 1.2
White cells, $10^3/mm^3$	7.8 ± 0.4	8.8 ± 1.1
Red cells, $10^6/mm^3$	6.06 ± 0.20	5.75 ± 0.21
Differential count, %		
Seg neutrophil	11.8 ± 2.1	11.6 ± 1.3
Lymphocytes	81.5 ± 2.4	84.0 ± 1.4
Band cells	0	0
Eosinophils	0.2 ± .2	1 ± 0.4
Monocytes	6.3 ± 1.5	3.4 ± 0.8
Myelocytes	0	0

[a] Same animals and conditions as in Table VI. [b] Mean ± standard error.

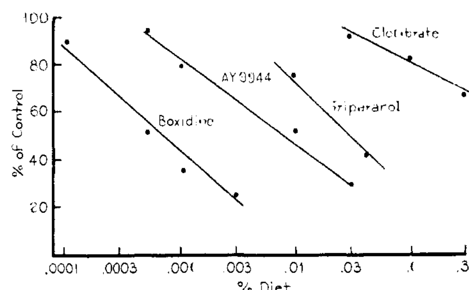

Figure 2.—Comparative levels of serum sterol of rats after receiving drug in the diet for 6 days.

probably due to the marked increase in liver sterol and lipids resulting from the cholesterol-supplemented diet. This type of diet is necessary to prevent lethal tissue cholesterol depletion observed with long-term high-dose regimen. Of particular interest is the observation that adrenal gland enlargement was not found in this instance although these animals received very high doses of compound. This would suggest that adrenal gland hyperplasia observed with boxidine (Table V) was associated with marked adrenal sterol depletion. When this was prevented (Table VI) adrenal size was normal. Similar findings have been reported with triparanol in which marked adrenal hypofunction was corrected by the addition of cholesterol to the diet.[16]

Relative Potency as Compared to Known Hypocholesteremic Agents.—A comparison of hypocholesteremic potency in rats was made with *trans*-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride (**1**), triparanol (**2**), and clofibrate (**3**) at various doses in the rat. As shown in Figure 2, boxidine was approximately ten times as active as **1**, 50 times as active as **2**, and 1000 times as active as **3**.

Mechanism of Action.—In some preliminary experiments on the absorption of sterols in the rat, it was observed that when boxidine was added to sterol-supplemented diets there were indications of inhibition of absorption. It has been our experience that the most consistent measurement indicative of absorption of dietary sterol was elevated liver sterol. Using this as our criterion, our initial studies indicated essentially no difference between the absorption of 7-dehydrocholesterol and cholesterol in the rat (Table VIII). Moreover the 7-dehydrocholesterol which was absorbed was converted in its entirety to cholesterol. When

(16) S. Gordon, S. Mauer, W. P. Cekleniak, and R. Partridge, *Endocrinology*, **72**, 643 (1963).

TABLE VIII

ABSORPTION OF STEROL IN THE RAT[a]

Treatment	Serum cholesterol, mg %	Liver cholesterol, mg/g
0.5% cholic acid	138 ± 6.3[b]	5.9 ± 0.6
0.5% cholic acid + 0.3% cholesterol	261 ± 24	25.8 ± 1.8
0.5% cholic acid + 0.3% 7-dehydrocholesterol	264 ± 19[c]	20.0 ± 1.2[c]

[a] Male, CFE rats 70 ± 5 g initial weight, eight per group, treated for 16 days. [b] Mean ± standard error. [c] Cholesterol was the sole sterol detected by glpc.

boxidine was added to the sterol-supplemented diet, there was a dramatic decrease in the absorption of 7-dehydrocholesterol (Table IX).

TABLE IX

EFFECT OF BOXIDINE ON ABSORPTION OF 7-DEHYDROCHOLESTEROL IN THE RAT[a]

Treatment	Sterol content: Serum, mg %	Liver, mg/g	Intestine,[b] mg/g
0.5% cholic acid + 0.3% 7-dehydrocholesterol	177 ± 25.7[c,d]	13.1 ± 2.3[d]	6.17[e]
0.5% cholic acid + 0.3% 7-dehydrocholesterol + 0.001% boxidine	26 ± 2.6[e]	2.8 ± 0.18[e]	4.54[e]
0.5% cholic acid + 0.001% boxidine	17 ± 5.4[e]	2.7 ± 0.21[e]	3.93[e]

[a] Male CFE weanling rats 45 ± 5 g initial weight, six per group, 4 weeks on treatment. [b] Pooled samples. [c] Mean ± standard error. [d] Cholesterol was the sole sterol as determined by glpc. [e] 7-Dehydrocholesterol was the predominant sterol by glpc.

These results suggest that a major factor responsible for the reduction of total sterol in the serum and tissues is the reduced ability of the boxidine-treated animal to reabsorb the cycling sterol in the biliary system. At each cycle some of the endogenously synthesized sterol (7-dehydrocholesterol) which was excreted with bile into the intestinal tract is prevented from being reabsorbed and is lost. Thus, there is a net loss of sterol at each cycle resulting in an over-all decrease in the total body sterol pool.

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