Effect of long-term administration of AY-9944, an inhibitor of 7-dehydrocholesterol Δ^7-reductase, on serum and tissue lipids in the rat

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ABSTRACT The effect of long-term administration of AY-9944, a specific inhibitor of cholesterol biosynthesis, was examined in rats maintained on diets with low and high cholesterol and fat content. Sterol and phospholipid levels were determined in the serum, liver, adrenals, lungs, and brain after 6 and 12 months of feeding AY-9944 at several dose levels.

In all the tissues examined, the cholesterol content was lowered and the cholesterol was partly replaced by 7-dehydrocholesterol biosynthesized instead of cholesterol in the presence of AY-9944. Cholesterol levels were particularly low in the serum and adrenals, while 7-dehydrocholesterol accumulated in the lungs. The fall in cholesterol and appearance of 7-dehydrocholesterol were reversible. Alterations of this type in the brain indicated that sterol metabolism is active in the adult rat brain. Addition of cholesterol to the diet reduced the effect of the inhibitor by eliminating the liver as a site of sterol synthesis.

KEY WORDS rat · cholesterol biosynthesis · inhibition · 7-dehydrocholesterol Δ^7-reductase · cholesterol lowering · 7-dehydrocholesterol accumulation · AY-9944, chronic administration · lung · phospholipid levels · brain sterol metabolism · atherogenic diet

Trans-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride (AY-9944) (1) interferes with the biosynthesis of cholesterol by specifically inhibiting the 7-dehydrocholesterol Δ^7-reductase system (2–6). In laboratory animals given AY-9944, the cholesterol content in the serum is greatly reduced, but only partly replaced by 7-dehydrocholesterol (7–12) biosynthesized instead of cholesterol in the presence of AY-9944. Because cholesterol is a precursor of steroid hormones and a structural constituent of cells, we were interested in the effect of chronic administration of this inhibitor on serum and tissue lipids. Rats were fed AY-9944 at several dose levels and the tissue sterol and phospholipid contents were determined after 6 and 12 months of treatment. The reversibility of the changes in lipid content produced by 6 months of AY-9944 treatment was assessed after a 6 month rest period. The effect of long-term administration of AY-9944 was also investigated in rats fed a diet supplemented with cholesterol, butter fat, and cholate.

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METHODS

Treatment of Animals

Albino rats (Charles River) were divided into four groups, each consisting of 60 males and 60 females. One half of each group was fed Purina chow (referred to as "standard diet"), while the other half received Purina chow supplemented with 1% cholesterol, 0.5% sodium cholate, and 7.5% butter fat (referred to as "atherogenic diet"). The first group was used as control and the three others were fed either 5, 10, or 36 μmoles/kg body weight (i.e., about 2.5, 5, or 18 mg/kg) of AY-9944, respectively. After 6 months of treatment, 10 male and 10 female rats from each group were killed and the sterol and phospholipid contents were determined in the serum, liver, lungs, adrenals, and brain.

The same procedure was repeated after 12 months. After 18 months of treatment, the sterol content was determined in the cerebrum and cerebellum of the remaining animals.

The reversibility of changes produced by long-term treatment with AY-9944 was investigated in two studies. In the first, rats were fed the standard diet containing AY-9944 (corresponding to a daily dose of about 5 mg/kg) over a period of 6 months; AY-9944 was withdrawn, the animals were allowed to recover for 6 months, and the tissue lipids were analyzed. In the second study, rats were fed the atherogenic diet containing AY-9944 (about 5 mg/kg per day) for 6 months; AY-9944 was withdrawn, and the animals were transferred to the standard diet and allowed to recover for 6 months before analysis of tissue lipids.

Analysis of Lipids

Phospholipids and sterols were determined in an ethanol–ether 3:1 extract (13) of the serum; the tissues were first homogenized in ethanol–ether and then extracted. Phospholipids contained in the ethanol–ether extracts were digested (14) and the phosphate was determined by the semiautomated procedure of Kraml (15). For sterol analysis, an aliquot of the extract was hydrolyzed with ethanolic KOH (1 ml of approximately 18 N aqueous KOH solution, 4 ml of ethanol, and 5 ml of water) in the presence of pyrogallol (16) and the neutral lipids were extracted with hexane. With one exception,[1] 7-dehydrocholesterol was calculated from its absorption at 281.5 mμ (16).[2] Total sterols were determined by the method of Zlatkis, Zak, and Boyle (21) as adapted for the AutoAnalyzer (method Np-24) and corrected for the 7-dehydrocholesterol content: since with the reagent of Zlatkis et al. 7-dehydrocholesterol produces 40% as much color as cholesterol, the amount of 7-dehydrocholesterol calculated from the UV spectrum was multiplied by 0.4 and subtracted from the "total sterol" value; the difference was considered to represent the cholesterol content (8). Total sterol levels shown in the tables represent the sum of cholesterol and 7-dehydrocholesterol values.

RESULTS

Standard Diet

The effect of chronic treatment with AY-9944 (2.5 and 5 mg/kg per day) on the sterol and phospholipid levels in rat serum and tissues is presented in Tables 1 and 2 and in Fig. 1.

7-Dehydrocholesterol

The concentrations of 7-dehydrocholesterol produced by both dose levels in the serum, liver (of male rats), and adrenals were similar in magnitude and were not significantly altered by prolonged treatment with AY-9944. In contrast, the levels in the lung increased with time and, in male rats, appeared to be dose-dependent. In the brain, the higher dose tended to produce higher levels; prolonged treatment resulted in higher 7-dehydrocholesterol content.

Total Sterols

The cholesterol content in all the tissues examined was lowered; the cholesterol was partially replaced by 7-dehydrocholesterol biosynthesized instead of cholesterol in the presence of AY-9944. The extent to which 7-dehydrocholesterol replaced cholesterol varied from tissue to tissue (Fig. 1). Serum sterol concentrations were significantly reduced, except after the low dose in male rats, where serum sterols were similar to those of untreated controls. Although the sterol content of the adrenals was considerably reduced, an accumulation of sterols, mainly 7-dehydrocholesterol, was found in the lungs. Liver total sterols tended to be elevated. Brain sterol levels were similar to those in untreated controls.

Phospholipids

In the serum, a consistent fall in phospholipids was detected (Table 2) with the higher dose of AY-9944; prolonged treatment caused no further reduction. Variations observed in the liver, adrenal, and brain phospholipid content were not consistent. In the lungs, the two doses caused similar accumulation of phospholipids, which increased as treatment was prolonged.

Atherogenic Diet

The effect of AY-9944 (5 and 18 mg/kg per day) on the sterol and phospholipid levels in rats fed the atherogenic diet is presented in Tables 3, 5, and 6. The atherogenic diet alone produced high cholesterol levels in all

[1] In one experiment, namely in the examination of the brain of rats fed AY-9944 for 18 months (see Table 5), the Liebermann–Burchard reagent was used to determine the "fast-acting" 7-dehydrocholesterol. The method is not specific, as many Δ^{7},$\Delta^{5,7}$-sterols, and even their peroxides (8), or sterols readily convertible into the former, react immediately with the reagent (17–19). In view of the site of inhibition of AY-9944, we assume that the "fast-acting" sterols consisted mainly of 7-dehydrocholesterol. The assumption is based on earlier reported findings, e.g. in the brain of rats treated with AY-9944, where the levels of "fast-acting" sterols were similar to those of 7-dehydrocholesterol measured by its absorption at 281.5 mμ (8); identical values for "fast-acting" sterols and 7-dehydrocholesterol as determined by gas–liquid chromatography were obtained with sera from rats treated chronically with AY-9944 (11).

[2] $\Delta^{5,7,24}$-Cholestatrien-3β-ol also absorbs at 281.5 mμ (4,20). Its presence in animals treated with AY-9944 was established when the 24-dehydrocholesterol Δ^{24}-reductase was independently inhibited (4) or when its activity was not increased, as in newborn rats (10); the triene was not detected in adult rats (10).

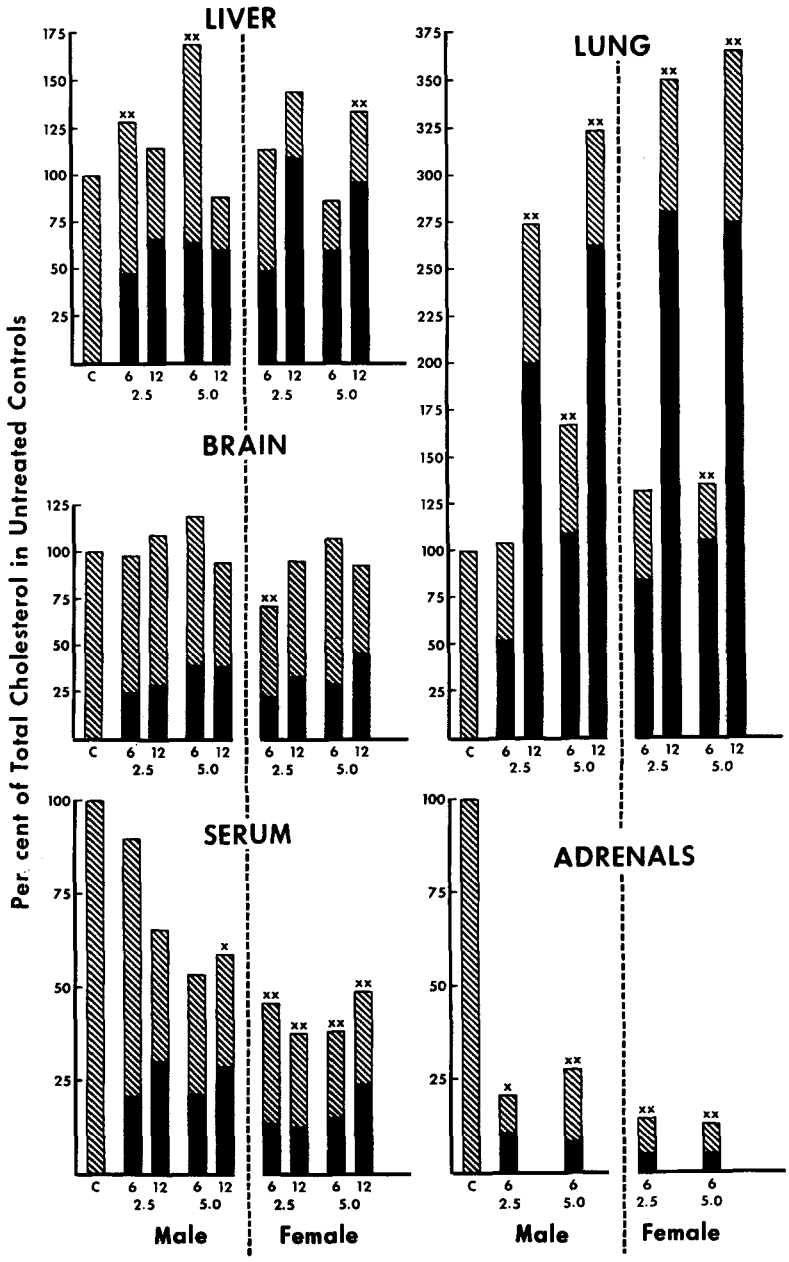

FIG. 1. Sterol composition in the serum and tissues of rats treated chronically with AY-9944. 7-Dehydrocholesterol (black bar) and cholesterol (shaded bar) in treated rats are expressed as a percentage of cholesterol found in untreated controls; values are based on mean levels determined after 6 and 12 months of feeding 2.5 and 5.0 mg/kg per day of AY-9944, respectively (see Table 1). Statistical significance of difference in total sterol levels between control and treated groups is indicated by *x*, $P < 0.05$ and *xx*, $P < 0.01$, respectively. C, values for controls.

tissues except the brain; the effect was greater in the serum and lungs of female rats. A similar, though less pronounced tendency was observed with the phospholipids.

7-Dehydrocholesterol

As shown in Table 3, all the tissues examined contained 7-dehydrocholesterol, whose concentrations tended to reflect the dose level of AY-9944. The absolute levels of 7-dehydrocholesterol in rats fed AY-9944 with the atherogenic diet differed from those found in rats fed AY-9944 with the standard diet: they were lower in the lungs and brain, and higher in the serum (of females), liver, and adrenals (Table 4). However, on a percentage basis, total sterols in rats fed the atherogenic diet and AY-9944 contained much less 7-dehydrocholesterol than those in rats fed the standard diet and AY-9944 (Table 4).

TABLE 1 EFFECT OF LONG-TERM TREATMENT WITH AY-9944 ON SERUM AND TISSUE STEROLS IN RATS FED THE STANDARD DIET

Tissue	Sex	Months of Treatment	Control	AY-9944-Treated				Recovery*
				7-Dehydrocholesterol		Total Sterols		
			Cholesterol (6)	2.5 mg/kg (10)	5 mg/kg (9)	2.5 mg/kg (10)	5 mg/kg (9)	Cholesterol† (5)
Serum	M	6	88 ± 7	18 ± 1.5	19 ± 1.5	79 ± 3	47 ± 2‡	—
		12	90 ± 12	27 ± 3	26 ± 4	59 ± 9	53 ± 4§	113 ± 12
	F	6	107 ± 6	14 ± 1	16 ± 3	49 ± 2‡	41 ± 4‡	—
		12	88 ± 8	11 ± 3	21 ± 1	33 ± 9‡	43 ± 5‡	92 ± 12
Liver	M	6	345 ± 18	162 ± 11	221 ± 10	443 ± 22‡	581 ± 76‡	—
		12	279 ± 28	184 ± 11	169 ± 11	320 ± 12	247 ± 14	288 ± 20
	F	6	413 ± 41	203 ± 8	230 ± 6	470 ± 23	355 ± 17	—
		12	228 ± 10	248 ± 8	218 ± 10	328 ± 14§	304 ± 16‡	265 ± 19
Adrenals	M	6	3750 ± 620	394 ± 57	312 ± 36	771 ± 66§	1040 ± 112‡	2970 ± 413
	F	6	4220 ± 315	206 ± 23	205 ± 41	607 ± 19‡	537 ± 78‡	2750 ± 168
Lungs	M	6	728 ± 102	381 ± 28	870 ± 86	757 ± 81	1220 ± 102‡	—
		12	541 ± 21	1080 ± 86 (8)	1420 ± 198	1480 ± 64†(8)	1750 ± 34‡	509 ± 27 (4)
	F	6	646 ± 33	556 ± 45	669 ± 57	855 ± 49	873 ± 63‡	—
		12	414 ± 28	1150 ± 164	1130 ± 76	1450 ± 188‡	1510 ± 154‡	559 ± 18
Brain	M	6	1730 ± 139	422 ± 25	672 ± 33	1694 ± 46	2060 ± 99	—
		12	2050 ± 122	589 ± 35	785 ± 36	2240 ± 44	1930 ± 43	1950 ± 36
	F	6	2340 ± 156	523 ± 21	681 ± 33	1660 ± 166‡	2500 ± 46	—
		12	1880 ± 79	632 ± 19	850 ± 95	1780 ± 39	1740 ± 139	1940 ± 31

Rats were fed approximately 2.5 mg/kg (5 μmole/kg) or 5 mg/kg of AY-9944 per day for 12 months and sampled after 6 and 12 months, respectively. Total sterols and 7-dehydrocholesterol were determined as described in the text. Results are expressed as mg/100 g of tissue or as mg/100 ml of serum and given as mean ± SEM. Number of rats per group is in parentheses.

* 6 months' treatment with AY-9944 (5 mg/kg per day) followed by 6 months' recovery.

† No 7-dehydrocholesterol detected.

‡ $P < 0.01$ for difference from control.

§ $P < 0.05$ for difference from control.

Total Sterols

Except in the adrenals, where elevated sterol levels were observed after the higher dose, no consistent effect of AY-9944 was detected in the rest of the examined tissues of rats fed the atherogenic diet and AY-9944 (Table 3). After 18 months of this treatment, the reduced cholesterol content in the cerebrum and cerebellum was compensated for by 7-dehydrocholesterol[1] (Table 5); the 7-dehydrocholesterol/cholesterol ratio in the cerebrum was approximately twice that in the cerebellum.

Phospholipids

Considerable accumulation of phospholipids in the lungs was noted with the higher dose of AY-9944; it increased with time and was more pronounced in female rats (Table 6). Similar results were obtained in rats treated with AY-9944 for 18 months and fed the atherogenic diet (22).

REVERSIBILITY OF CHANGES PRODUCED BY AY-9944

In rats fed 5 mg/kg per day of AY-9944 for 6 months and allowed to recover for 6 months, levels of serum and tissue sterol (Table 1) and of phospholipid (Table 2) returned to pretreatment values; no 7-dehydrocholesterol was detected in any serum or tissue samples. A similar recovery was found in rats fed AY-9944 with the atherogenic diet followed by a 6 month rest period on the standard diet (Tables 3 and 6).

BODY AND ORGAN WEIGHTS

As illustrated in Table 7, animals receiving AY-9944 weighed less than untreated rats used as controls, and had enlarged adrenals (23). A similar observation was made in rats fed AY-9944 for 3 months (11). In animals fed the atherogenic diet during treatment with AY-9944, body weights were lower than in corresponding controls and only an occasional increase in adrenal weight and decrease in liver weight was observed.

DISCUSSION

Long-term administration of AY-9944 to rats resulted in the accumulation of 7-dehydrocholesterol, the substrate of the enzyme system inhibited by AY-9944. In all the tissues examined, the cholesterol content was lowered and the cholesterol was partly replaced by 7-dehydrocholesterol. The extent to which these changes took place varied from tissue to tissue; the observed

TABLE 2 EFFECT OF LONG-TERM TREATMENT WITH AY-9944 ON SERUM AND TISSUE PHOSPHOLIPIDS IN RATS FED THE STANDARD DIET

Tissue	Sex	Months of Treatment	Control (6)	AY-9944-Treated 2.5 mg/kg (10)	AY-9944-Treated 5 mg/kg (9)	Recovery* (5)
Serum	M	6	138 ± 4	121 ± 21	93 ± 4† (8)	—
		12	216 ± 26	240 ± 31	99 ± 8†	239 ± 24
	F	6	196 ± 21	138 ± 42	91 ± 10†	—
		12	234 ± 19	100 ± 16†	91 ± 13†	280 ± 48
Liver	M	6	3610 ± 168	3634 ± 217	3626 ± 90	—
		12	3757 ± 234	4027 ± 121	3175 ± 112	4060 ± 80
	F	6	3341 ± 95	2979 ± 98‡	3494 ± 119	—
		12	3075 ± 122	3906 ± 168	3584 ± 101	3247 ± 92
Adrenals	M	6	6376 ± 1066	4873 ± 412	5821 ± 322	4876 ± 381
	F	6	4565 ± 539	4058 ± 341	4376 ± 122	4161 ± 387
Lungs	M	6	2493 ± 201	2656 ± 179	3438 ± 224†	—
		12	2263 ± 70	6324 ± 922†(8)	7397 ± 1860‡	2334 ± 54(4)
	F	6	2587 ± 130	3347 ± 323	3450 ± 258†	—
		12	2132 ± 136	7274 ± 1307†	8279 ± 2090‡	2285 ± 107
Brain	M	6	4818 ± 118	5188 ± 102	5620 ± 180†	—
		12	5556 ± 224	6215 ± 107	5502 ± 95	5511 ± 109
	F	6	4788 ± 219	4488 ± 111	5263 ± 138	—
		12	5463 ± 90	5442 ± 75	5518 ± 298	5036 ± 77

Rats were fed approximately 2.5 mg/kg (5 μmole/kg) or 5 mg/kg of AY-9944 per day for 12 months and sampled after 6 and 12 months, respectively. Phospholipids were determined as described in the text. Results are expressed as mg/100 g of tissue or mg/100 ml of serum and given as mean ± SEM. Number of rats is in parentheses.

* 6 months' treatment with AY-9944 (5 mg/kg per day) followed by 6 months' recovery.

† $P < 0.01$ for difference from controls.

‡ $P < 0.05$ for difference from controls.

variation may reflect tissue-specific differences in sterol metabolism (24–26). Our results are similar to those obtained by Horlick (11) in rats fed AY-9944 for 3 months. Horlick, who used thin-layer and gas-liquid chromatography to examine the sterol composition in three major cholesterogenic tissues (liver, adrenals, and skin) of AY-9944-treated rats, found no significant changes except a reduction in cholesterol and increase in 7-dehydrocholesterol (11). We assume that feeding AY-9944 to rats for periods longer than 3 months did not produce any additional changes in the sterol composition.

The 7-dehydrocholesterol found in rats fed AY-9944 and the standard diet was endogenous, i.e. biosynthesized instead of cholesterol in the presence of AY-9944. Because extrahepatic sites of cholesterol synthesis contain similar enzyme systems (e.g. 26–28), the synthesis of cholesterol was inhibited in any tissue with cholesterogenic activity to which AY-9944 was transported. In this respect, it is of interest that radioautographic studies in mice[3] indicated rapid transport of ^{14}C-labeled AY-9944 to organs with cholesterogenic activity, including intestine, adrenals, lungs, and brain (26). However, except in the brain (see below), it was impossible to distinguish the 7-dehydrocholesterol transported to a given tissue from that biosynthesized in situ.

It is likely that the 7-dehydrocholesterol found in the liver and the serum was synthesized mainly in the liver. In principle, chronic treatment with AY-9944 at a daily dose higher than that required for complete inhibition (29, 10) should finally result in a similar content of 7-dehydrocholesterol. Indeed, the two doses of AY-9944 produced similar levels of 7-dehydrocholesterol in the liver; the fact that the levels were not changed by prolonged treatment with AY-9944 suggested that a steady state had been attained. In contrast, the level of 7-dehydrocholesterol in the lungs was dose-dependent and was further increased by prolonged treatment. This accumulation of 7-dehydrocholesterol indicated a remarkable affinity between the lung and 7-dehydrocholesterol; the increase in 7-dehydrocholesterol was associated with elevated phospholipid levels.

Another characteristic of AY-9944 treatment is the greatly reduced cholesterol content in all the tissues examined. As the fall in cholesterol was partly compensated for by 7-dehydrocholesterol, total sterol levels in a given tissue depended on the amount of accumulated 7-dehydrocholesterol. Thus, in the serum, and to a greater extent in the adrenals, total sterols were much

[3] Unpublished experiments by Doctors L. Berlinguet, M. L. Givner, and D. Dvornik.

TABLE 3 EFFECT OF LONG-TERM TREATMENT WITH AY-9944 ON SERUM AND TISSUE STEROLS IN RATS FED ATHEROGENIC DIET

Tissue	Sex	Months of Treatment	Control (Atherogenic Diet)	Atherogenic Diet and AY-9944				Recovery†	Control (Standard Diet)
				7-Dehydrocholesterol		Total Sterols			
			Cholesterol* (6)	5 mg/kg (10)	18 mg/kg (10)	5 mg/kg (10)	18 mg/kg (10)	Cholesterol* (9)	Cholesterol (6)
Serum	M	6	307 ± 22	15 ± 2	27 ± 3	316 ± 33	353 ± 38	—	—
		12	667 ± 177	34 ± 15	22 ± 4	605 ± 240	264 ± 39‡	235 ± 32	90 ± 12
	F	6	654 ± 80	45 ± 6	83 ± 12	594 ± 109	953 ± 135	—	—
		12	1790 ± 531	141 ± 31	106 ± 17	822 ± 201	1400 ± 244	74 ± 12	88 ± 8
Liver	M	6	9800 ± 369	305 ± 16	631 ± 43	7230 ± 220	11098 ± 420	—	
		12	11300 ± 905	312 ± 21	783 ± 45 (9)	8914 ± 835	10500 ± 790 (9)	741 ± 105	279 ± 27
	F	6	11600 ± 1370	789 ± 41	861 ± 101	12700 ± 970	11200 ± 1240	—	—
		12	9890 ± 965	804 ± 47	853 ± 49	8770 ± 341	11605 ± 844	362 ± 31	228 ± 10
Adrenals	M	6	11000 ± 1500	1300 ± 252	2790 ± 132	15200 ± 2400	19400 ± 808§	—	—
		12	14170 ± 1870	1140 ± 189	1930 ± 109	9260 ± 116	12100 ± 548	2980 ± 345(10)	2920 ± 465(3)
	F	6	12000 ± 1350	2120 ± 187	4590 ± 502	11600 ± 669	19400 ± 1240‡		—
		12	11750 ± 343	2680 ± 178	2880 ± 262	14600 ± 1110	16000 ± 1370‡	3130 ± 281	2380 ± 262(3)
Lungs	M	6	873 ± 91	66 ± 4	135 ± 13	991 ± 57	1100 ± 67	—	—
		12	974 ± 131	74 ± 19	220 ± 59	680 ± 96	1150 ± 102	506 ± 32 (8)	541 ± 21
	F	6	1240 ± 128	158 ± 11	217 ± 33	1210 ± 157	1400 ± 162	—	—
		12	1170 ± 125	271 ± 61	302 ± 44	1330 ± 154	2070 ± 309‡	573 ± 64	414 ± 28
Brain	M	6	2410 ± 179	408 ± 33	620 ± 61	2570 ± 92	2440 ± 110	—	—
		12	1870 ± 121	354 ± 35	860 ± 32	1860 ± 49	2000 ± 67	1780 ± 42	2050 ± 122
	F	6	1740 ± 169	554 ± 42	819 ± 23	1380 ± 85	2030 ± 105	—	—
		12	1880 ± 121	849 ± 26	1040 ± 36	2120 ± 48	2150 ± 76	2070 ± 43	1880 ± 79

Rats were fed approximately 5 mg/kg (10 μmole/kg) or 18 mg/kg of AY-9944 per day for 12 months and sampled after 6 and 12 months, respectively. Total sterols and 7-dehydrocholesterol were determined as described in the text. Results are expressed as mg/100 g of tissue or as mg/100 ml of serum and given as mean ± SEM. Number of rats per group is in parentheses.

* No 7-dehydrocholesterol detected.

† 6 months' treatment with AY-9944 (5 mg/kg per day), rats fed atherogenic diet, followed by 6 months' recovery on standard diet.

‡ $P < 0.05$ for difference from controls.

§ $P < 0.01$ for difference from controls.

lower than in the corresponding untreated controls; they tended to be higher in the liver and were greatly elevated in the lung. In the brain, however, total sterol levels were not changed.

The effect of long-term administration of AY-9944 on rat phospholipids was less complex than the effect on sterols: we observed consistent changes only in the serum, where the higher dose produced a fall, and in the lungs. where both doses caused accumulation of phospholipids (22).

Rats survived the chronically low cholesterol levels produced by a daily dose of 2.5 mg/kg and about 50% survived 4 months of feeding 5 mg/kg per day of AY-9944. Both the fall in cholesterol and the appearance of 7-dehydrocholesterol were reversible. Earlier we have made similar observations in rats (29) and in pigs treated with AY-9944 (12).

In rats fed a diet low in cholesterol, the circulating cholesterol is mainly endogenous and synthesized in the liver; this is changed by increased intake of dietary cholesterol, which suppresses the rate of hepatic cholesterogenesis (30). Consequently, in rats fed cholesterol, the effect of an inhibitor of cholesterol biosynthesis should be small and restricted to those extrahepatic tissues whose cholesterogenic activity is not affected by the levels of ingested cholesterol (26). Interestingly, 7-dehydrocholesterol was found in all the examined tissues of rats fed the atherogenic diet together with AY-9944. Since the high level of ingested cholesterol should have virtually eliminated the liver as source of endogenous sterols, any 7-dehydrocholesterol formed endogenously should have been extrahepatic (31). On the other hand, as commercially available cholesterol usually contains traces of 7-dehydrocholesterol and small amounts of Δ^7-cholestenol (32), the 7-dehydrocholesterol found in these animals may have originated in the diet: Δ^7-cholestenol and 7-dehydrocholesterol are intermediates in cholesterol biosynthesis (33) and since once absorbed, they would be converted to cholesterol (34), they would not be detected in rats fed the atherogenic diet (Table 3). However, since in the presence of AY-9944 the enzymatic conversion of Δ^7-cholestenol to 7-dehydrocholesterol is not affected (5) but the conversion of the latter to cholesterol is blocked, any absorbed Δ^7-sterols of dietary origin would have accumulated as 7-dehydrocholesterol. In the present study Δ^7-sterols were not removed from

TABLE 4 7-DEHYDROCHOLESTEROL CONTENT IN SERUM AND TISSUE STEROLS OF RATS TREATED CHRONICALLY WITH AY-9944 AND FED STANDARD OR ATHEROGENIC DIETS

	Sex	Months of Treatment	% 7-Dehydrocholesterol in Total Sterols			
			Standard Diet and AY-9944		Atherogenic Diet and AY-9944	
			2.5 kg/mg	5 mg/kg	5 mg/kg	18 mg/kg
Serum	M	6	23	40	5	8
		12	46	49	6	8
	F	6	29	39	8	9
		12	33	49	17	7
Liver	M	6	37	38	4	6
		12	57.5	68	4	8
	F	6	43	69	6	8
		12	76	72	9	7
Adrenals	M	6	51	30	9	14
		12	—	44	12	16
	F	6	34	38	18	23
		12	—	58	18	18
Lungs	M	6	50	71.5	7	12
		12	73	81	11	19
	F	6	64	77	13	16
		12	80	75	20	15
Brain	M	6	25	33	16	25
		12	26	41	19	43
	F	6	31	27	40	40
		12	35	49	40	48

Rats were treated as described for Tables 1 and 3. 10 animals per group.

TABLE 5 EFFECT OF LONG-TERM TREATMENT WITH AY-9944 ON BRAIN STEROLS IN RATS FED ATHEROGENIC DIET

Tissue	Sex	Group	Cholesterol	"Fast-Acting" Sterols
Cerebrum	M	Control (3)	17.0 ± 1.0	0
		Treated (14)	8.8 ± 0.6	9.0 ± 0.4
	F	Control (2)*	14.5; 15.9	0
		Treated (11)	6.7 ± 0.7	10.6 ± 0.6
Cerebellum	M	Control (2)*	31.4; 41.7	0
		Treated (14)	18.3 ± 0.8	10.5 ± 0.5
	F	Control (2)*	28.9; 32.2	0
		Treated (10)	15.1 ± 1.1	10.1 ± 0.6

Rats were fed approximately 18 mg/kg of AY-9944 per day for 18 months. Cholesterol and "fast-acting" sterols were determined as described in the text and in footnote 1. Results are expressed as mg/g and given as mean ± SEM. Number of rats per group is in parentheses.

* Individual values.

the cholesterol added to the diet,[4] and their contribution of 7-dehydrocholesterol to the circulating sterol pool cannot be assessed in rats fed the atherogenic diet and AY-9944.

BRAIN STEROL METABOLISM AND AY-9944

The effect of AY-9944 on rat brain cholesterol synthesis and its age-dependent rate was examined earlier: after a single oral dose of the agent, Givner and Dvornik (8) observed greatly reduced incorporation of labeled acetate into cholesterol in both 9-day and 15-month old rats, and established that the "fast-acting" sterols in the brain of treated animals consisted mainly of 7-dehydrocholesterol. Fumagalli, Niemiro, and Paoletti (10) have also identified 7-dehydrocholesterol in the brain of

[4] A commercially available sample of cholesterol similar to that added to the atherogenic diet used in the present study contained 0.03% 7-dehydrocholesterol, calculated from the absorption at 281.5 mμ, and 2% "fast-acting" sterols expressed as Δ^7-cholestenol.

TABLE 6 EFFECTS OF LONG-TERM TREATMENT WITH AY-9944 ON SERUM AND TISSUE PHOSPHOLIPIDS IN RATS FED ATHEROGENIC DIET

Tissue	Sex	Months of Treatment	Control (Atherogenic Diet) (6)	Atherogenic Diet and AY-9944		Recovery* (9)	Control (Standard Diet) (6)
				5 mg/kg (10)	18 mg/kg (10)		
Serum	M	6	248 ± 15	256 ± 21	288 ± 20	—	—
		12	429 ± 102	418 ± 120	313 ± 91	403 ± 68 (10)	216 ± 26 (5)
	F	6	421 ± 14	556 ± 156	589 ± 48†	—	—
		12	1030 ± 234	801 ± 45	939 ± 95	300 ± 25 (10)	234 ± 19
Liver	M	6	3060 ± 131	2990 ± 95	3190 ± 119	—	—
		12	3030 ± 152	3280 ± 370	3120 ± 192	3390 ± 100 (10)	3760 ± 234
	F	6	2690 ± 193	2940 ± 83	3010 ± 151	—	—
		12	2830 ± 264	2040 ± 183	3150 ± 124	3090 ± 62	3070 ± 122
Adrenals	M	6	3610 ± 250	3520 ± 173	4010 ± 364	—	—
		12	3120 ± 450	4020 ± 435	4760 ± 386	4490 ± 359 (10)	4000 ± 155 (3)
	F	6	3710 ± 289	3800 ± 218	3710 ± 269	—	—
		12	3850 ± 415	4240 ± 154	4730 ± 343	4750 ± 590	4230 ± 721 (3)
Lungs	M	6	2130 ± 125	2180 ± 112	2860 ± 152‡	—	—
		12	2450 ± 246	2600 ± 448	5380 ± 695‡	2280 ± 115 (8)	2260 ± 70
	F	6	2520 ± 199	2770 ± 65	3780 ± 458† (8)	—	—
		12	3050 ± 420	3070 ± 214	9510 ± 1630‡	2630 ± 272	2130 ± 136
Brain	M	6	5390 ± 373	5010 ± 137	5210 ± 123	—	—
		12	5190 ± 289	5770 ± 138	5350 ± 275	5980 ± 154	5560 ± 224
	F	6	5080 ± 223	5340 ± 332	5350 ± 181	—	—
		12	4910 ± 269	5580 ± 52†	5570 ± 196	5650 ± 109	5460 ± 90

Rats fed approximately 5 mg/kg (10 μmole/kg) or 18 mg/kg of AY-9944 per day for 12 months and sampled after 6 and 12 months, respectively. Phospholipids determined as described in the text. Results expressed as mg/100 ml of serum or as mg/100 g of tissue and given as mean ± SEM. Number of rats per group in parentheses.

* 6 months treatment with AY-9944 (5 mg/kg per day) and fed atherogenic diet followed by 6 months recovery on standard diet.

† $P < 0.05$ for difference from controls.

‡ $P < 0.01$ for difference from controls.

TABLE 7 BODY AND ORGAN WEIGHTS OF RATS AFTER LONG-TERM FEEDING OF AY-9944 AND STANDARD OR ATHEROGENIC DIET

Weight	Sex	Months of Treatment	Controls		AY-9944 Treatment		
			Standard Diet	Atherogenic Diet (9)	5 mg/kg per day, Standard Diet (10)	5 mg/kg per day, Atherogenic Diet (10)	18 mg/kg per day, Atherogenic Diet (10)
Body	M	6	506 ± 14 (9)	562 ± 12	477 ± 13	500 ± 13*	451 ± 13†
		12	633 ± 17 (6)	634 ± 19	420 ± 40† (5)	541 ± 31*	511 ± 20†
	F	6	313 ± 15 (9)	343 ± 13	276 ± 8*	299 ± 12*	274 ± 5†
		12	352 ± 15 (6)	366 ± 18	277 ± 17* (3)	304 ± 8†	290 ± 8*
Liver	M	6	13.9 ± 0.5 (9)	36.2 ± 2.8	17.5 ± 1.0*	34.6 ± 1.2	30.5 ± 1.5
		12	16.0 ± 0.6 (6)	47.9 ± 3.1	15.9 ± 1.3 (5)	37.9 ± 2.3*	31.9 ± 2.1†
	F	6	9.6 ± 0.3 (9)	23.8 ± 1.7	9.9 ± 0.3	24.6 ± 2.3	20.6 ± 1.4
		12	11.3 ± 0.3 (6)	30.6 ± 1.7	10.0 ± 1.3 (3)	25.3 ± 1.6*	26.8 ± 2.5
Lungs	M	6	1.76 ± 0.07 (9)	2.10 ± 0.10	2.44 ± 0.11*	2.09 ± 0.08	1.87 ± 0.14
		12	1.68 ± 0.16 (6)	2.61 ± 0.32	2.99 ± 0.16† (5)	2.08 ± 0.07	2.29 ± 0.18
	F	6	1.84 ± 0.40 (9)	1.58 ± 0.08	1.82 ± 0.08	1.53 ± 0.05	2.00 ± 0.23
		12	1.40 ± 0.04 (6)	1.70 ± 0.12	2.50 ± 0.28† (3)	1.62 ± 0.11	2.16 ± 0.18*
Adrenals‡	M	6	51 ± 2	87 ± 7	102 ± 10†	78 ± 5	
		12	—	91 ± 11	—	70 ± 5	
	F	6	68 ± 7	71 ± 4	196 ± 17†	110 ± 9†	
		12	—	73 ± 4	—	72 ± 4	

Rats were fed standard or atherogenic diet containing AY-9944 (approximately 5 mg/kg per day) for 12 months and sampled after 6 and 12 months. Values expressed as g/g of body weight and are given as mean ± SEM. Number of rats per group in parentheses.

* $P < 0.05$ for difference from controls.

† $P < 0.01$ for difference from controls.

‡ Expressed as mg/g of body weight.

treated adult and of newborn rats, and have emphasized the need of prolonged treatment with AY-9944 to produce accumulation of 7-dehydrocholesterol in adult rat brain.

The presence of 7-dehydrocholesterol, together with the fall in cholesterol, observed in the present study in the brain of rats treated with AY-9944 is of particular interest because it indicates the metabolic fate of sterols in the brain of mature rats. 7-Dehydrocholesterol found in the brain was either of extracerebral origin or synthesized in situ. It is very unlikely that, in the adult rat, circulating sterols penetrate into the brain (35) in quantities as high as those found for 7-dehydrocholesterol in the brain of rats treated with AY-9944. This is shown by our data obtained with a diet supplemented with cholesterol (Table 3): in rats fed such a diet for 12 months, the concentration of cholesterol in the liver and serum was up to 43 and 20 times higher than in rats maintained on the standard diet; in contradistinction, cholesterol levels in the brain were unchanged, hence independent of the amount of ingested cholesterol. Assuming that 7-dehydrocholesterol, like cholesterol (36), does not readily penetrate into the adult rat brain while AY-9944 does, the 7-dehydrocholesterol present in the brain of rats receiving AY-9944 was in all probability synthesized in situ.[5] Assuming further that, in a given period of time, the metabolic fate of 7-dehydrocholesterol in the brain of rats treated with AY-9944 is similar to that of cholesterol in the brain of untreated rats, the amount of 7-dehydrocholesterol in the brain would reflect the activity of cerebral cholesterol synthesis in the adult rat.

The changes observed in the brain of rats treated chronically with AY-9944, i.e. the fall in cholesterol together with the considerable accumulation of 7-dehydrocholesterol and its disappearance from the brain on termination of treatment, indicate active sterol metabolism in the adult rat brain; our conclusion is in agreement with similar conclusions made earlier by Kabara (37) and Nicholas (38).

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[5] The capacity of AY-9944 to penetrate the brain has been suggested earlier in pigs (4) and in rats (8, 10); it was demonstrated by radioautography in mice.[3]