Effect of 2-Acetoamidoethyl (*p*-Chlorophenyl) (*m*-Trifluoromethylphenoxy) Acetate (Halofenate) on Cholesterol Oxidation by Rat Liver Mitochondria[1] (36348)

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Gilfillan *et al.* (1) have reported on the hypolipidemic properties of a new compound, 2-acetoamidoethyl (*p*-chlorophenyl) (*m*-trifluoromethylphenoxy) acetate (halofenate). At dietary levels as low as 0.00625%, halofenate was reported to lower serum cholesterol, triglycerides, and phospholipids and its hypolipemic activity was compared to that of ethyl *p*-chlorophenoxyisobutyrate (clofibrate). In an earlier study (2), we reported on the effect of clofibrate on the oxidation of 26-^{14}C-cholesterol by rat liver mitochondria. This report deals with the influence of dietary halofenate (0.05%) on the oxidation of 26-^{14}C-cholesterol to $^{14}CO_2$ by suitably fortified preparations of rat liver mitochondria.

Methods. Male, Wistar rats (150–160 g) were maintained for 2 or 3 weeks on a synthetic test diet containing mixed cereal (70%), skim milk powder (22%), wheat germ (7%) and vitamin mix (1%). The halofenate (0.05%) or clofibrate (0.3%) were added at the expense of the cereal. The rats were killed by decapitation. The serum cholesterol (3) and triglyceride (4) levels were determined. A portion of liver was homogenized in chloroform–methanol, 2:1, and the liver cholesterol and triglyceride levels were determined with aliquots of the lipid extract. The mitochondrial oxidation was carried out according to published methods (5, 6).

Incubations were carried out in stoppered 125-ml Erlenmeyer flasks containing center wells. The incubation mixture consisted of 1 ml of mitochondrial preparation; 1 ml of a solution containing adenosine triphosphate (ATP, 25 mg), nicotinamide adenine dinucleotide (NAD, 5 mg), adenosine monophosphate (AMP, 8 mg), reduced glutathione (15 mg), sodium citrate monohydrate (30 mg), magnesium nitrate hexahydrate (10 mg), potassium penicillin G (2000 units) and streptomycin sulfate (1 mg); 5 ml of labeled substrate in 0.25 *M* tris(hydroxymethyl)aminomethane HCl, pH 8.5; and 5 ml of boiled supernatant.

Incubations were carried out at 37° for 18 hr. At the end of this period 2.5 ml of a 1 *M* methanolic solution of Hyamine 10X (*p*-diisobutyl-cresoxyethoxyethyl)dimethylbenzylammonium hydroxide) was injected into the center well. The solution was acidified with 1 *N* H_2SO_4 (2.5 ml) and the flasks were shaken for 3 hr at 37° to displace $^{14}CO_2$. The Hyamine solution was removed from the center well and a sample was taken for radioactive assay by liquid scintillation spectrometry.

The 26-^{14}C-cholesterol, purchased from the New England Nuclear Corporation, Boston, MA, was purified by thin-layer chromatography prior to use.

The halofenate was generously provided by Dr. C. W. Mushett, Merck & Co., Rahway, NJ, and the clofibrate was a gift of Dr. J. Noble, Ayerst Co., New York, NY.

Results and Discussion. Table I presents the effects of halofenate (0.05%) and clofibrate (0.3%) in the diet on the serum and liver lipids of rats fed for either 2 or 3 weeks. At the end of the 2-week feeding (Expt. 1), both drugs had exerted a hepatomegalic effect, as had been reported for both compounds by other workers (1, 7). Gilfillan

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TABLE I. Influence of Halofenate (0.05%) and Clofibrate (0.3%) in Diet on Serum and Liver Lipids in Rats.

Group	Survival ratio	Wt gain (g)	Liver wt (g)	Liver wt (% body wt)	Serum: (mg/100 ml)		Liver: (mg/100 ml)		Serum + liver: (mg)	
					Chol.	Trig.	Chol.	Trig.	Chol.	Trig.
Expt. 1 (2 wk)										
Halofenate	4/6	15 ± 8[a]	6.8 ± 0.3	3.76 ± 0.18	51.3 ± 12.1	66.0 ± 8.6	400 ± 21	408 ± 42	30.0	31.3
Clofibrate	6/6	29 ± 3	7.7 ± 0.4	4.03 ± 0.12	55.9 ± 2.5	56.5 ± 4.8	380 ± 23	382 ± 36	32.5	32.6
Control	6/6	9 ± 3[b]	5.8 ± 0.4[c]	3.39 ± 0.08[b]	54.8 ± 3.3	56.3 ± 5.6	332 ± 13[d]	332 ± 37	22.2	22.2
Expt. 2 (3 wk)										
Halofenate	5/6	93 ± 10	9.9 ± 0.8	4.11 ± 0.20	24.3 ± 1.1	57.2 ± 4.0	376 ± 7	476 ± 14	39.0	51.3
Clofibrate	6/6	81 ± 6	12.7 ± 0.7[c,e]	5.54 ± 0.16[f]	26.0 ± 1.8	50.0 ± 4.5	362 ± 11	408 ± 35	47.8	55.3
Control	6/6	95 ± 7	8.4 ± 0.7	3.46 ± 0.19[d]	44.5 ± 3.4[g]	63.0 ± 5.9	345 ± 11[d]	450 ± 19	32.2	42.4

[a] Standard error.
[b] vs clofibrate, $p < .001$.
[c] vs control, $p < .01$.
[e] vs halofenate, $p < .05$.
[e] vs halofenate, $p < .05$.
[f] vs halofenate or control, $p < .001$.
[g] vs halofenate or clofibrate, $p < .001$.

TABLE II. Oxidation of 26-^{14}C-Cholesterol to $^{14}CO_2$ by Mitochondrial Preparations from Rats Fed 0.05% Halofenate or 0.3% Clofibrate for 2 or 3 Weeks.

	No.	Cytosol	Oxidation[a] (%)
Expt. 1 (2 weeks)			
Halofenate	4	+	13.3 ± 2.6[b]
Clofibrate	6	+	13.9 ± 2.3
Control	6	+	15.7 ± 3.7
Expt. 2 (3 weeks)			
Halofenate	5	+	2.9 ± 0.3[c]
Clofibrate	6	+	3.8 ± 0.4[d]
Control	6	+	1.9 ± 0.3
Halofenate	5	—	5.2 ± 1.0[e]
Clofibrate	6	—	4.1 ± 0.4[d]
Control	6	—	1.8 ± 0.5
Expt. 3 (3 weeks)			
Halofenate	5	+	4.5 ± 0.8
Control	6	+	3.6 ± 0.9
Halofenate	5	—	3.1 ± 0.6
Control	6	—	1.9 ± 0.4

[a] Calculated as (26-^{14}C-cholesterol/$^{14}CO_2$) × 100. Corrected for milligrams of *N*.

[b] Standard error.

[c] Halofenate vs control, $p < .05$.

[d] Clofibrate vs control, $p < .01$.

[e] Halofenate vs control, $p < .02$.

et al. (1) had reported that halofenate or clofibrate, fed for 9 days at the levels used in this experiment, had increased liver weights by 21 and 24%, respectively. In our 2-week study the increases in liver weight were 17% for the group fed halofenate and 33% for that fed clofibrate. In contrast to their findings, both test groups gained weight.

There was no significant effect on serum lipids in the 2-week study. Liver cholesterol and triglyceride levels were increased in both test groups but only the liver cholesterol increase in the halofenate group was significant. These increases led to higher serum-plus liver-cholesterol pools in both the halofenate and clofibrate groups.

In the 3-week feeding experiment, we again observed hepatomegaly. In this experiment, liver weight was increased by 19 and 51% in the halofenate and clofibrate groups, respectively. Serum cholesterol and triglycerides were reduced in both drug groups, but the triglyceride reductions were not significant. Liver cholesterol levels were lower in the control rats than in either test group; but in this experiment, the liver triglyceride levels of the clofibrate group were lower than those of the controls, whereas those of the halofenate group were higher. We have no ready explanation for the discrepancies between our data and those of Gilfillan *et al.* (1), although our findings and theirs coincide on many points. One source of difference may be the high sucrose diet used by Gilfillan *et al.* which may enhance lipemia. We have observed that high carbohydrate diets elevate serum cholesterol and triglyceride levels in rats (8). It is hard to judge the effects of the diet in the Gilfillan and co-workers' experiment, since percentages of increase and decrease from control, rather than actual values, were reported.

In earlier work with clofibrate (2), we found that although gross oxidation of 26-^{14}C-cholesterol to $^{14}CO_2$ by rat liver mitochondria of test groups was higher than that observed for controls, when calculated as percentage oxidation per milligram of mitochondrial nitrogen, there was no difference. However, in the absence of the boiled supernatant cofactor (cytosol), the oxidation of cholesterol by liver mitochondrial preparations from rats fed clofibrate was significantly higher than that of controls. We speculated that the increase in oxidation might be associated with hepatomegaly—possibly with changes in mitochondrial protein. Since then we have carried out experiments with three other hypolipemic agents [Su 13,437 (Ciba); DH-581 (Dow); and W1372 (Wallace)]; and, while they all cause hepatomegaly, only the Ciba compound (the one most nearly resembling clofibrate in structure) showed a similar effect, namely, increased cholesterol oxidation in the absence of cytosol (9–12).

The results of three experiments are detailed in Table II. In the first, we did not assay oxidation in the absence of cytosol and found no differences in cholesterol oxidation. In the second experiment we observed significant increases in oxidation of cholesterol both in the presence and absence of cytosol. The third experiment involved only control and

halofenate (0.05%) groups. After 21 days on the diet, rats were killed; and the livers were taken for preparation of mitochondria. No serum or liver lipid determinations were carried out. In this experiment, we again observed the increased oxidation by liver mitochondria from the halofenate group in the absence of cytosol. Differences in levels of cholesterol oxidation between Expt. 1 and Expts. 2 and 3 are not due to duration of feeding, but are in the range of differences often observed in this type of experiment, even with preparations of normal rat liver. The data suggest that the effect of halofenate and clofibrate on cholesterol oxidation in the absence of cytosol is not merely associated with increased liver size, but may be a function of the specific chemical structure involved.

Summary. We have compared the effects of halofenate [2-acetoamidoethyl (*p*-chlorophenyl) (*m*-trifluoromethylphenoxy) acetate] and clofibrate (ethyl *p*-chlorophenoxyisobutyrate) on rat serum and liver lipids and on oxidation of cholesterol by suitably fortified preparations of rat liver mitochondria. The halofenate was fed as 0.05% of the diet and the clofibrate as 0.3%. Both compounds caused increases in liver size and liver lipids. In the 3-week feeding experiment, both test compounds showed a significant hypocholesteremic effect.

When complete incubation mixtures were used, the oxidation of 26-^{14}C-cholesterol to $^{14}CO_2$ was no different for liver mitochondrial preparations from halofenate, clofibrate, or control rats. However, in the absence of boiled supernatant factor (cytosol), oxidation of cholesterol was significantly higher with liver mitochondrial preparations from the drug-treated rats.

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