Effect of *N*-γ-Phenylpropyl-*N*-benzyloxy Acetamide (W-1372) and of Clofibrate on the Lipids of Normal and Hypercholesteremic Rats (34200)

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It has previously been shown that certain substituted hydroxamates can prevent or retard the formation of fatty deposits in the aortas of rabbits and squirrel monkeys fed a high cholesterol diet (1, 2). The present paper describes the effect of *N*-γ-phenylpropyl-*N*-benzyloxy acetamide (W-1372) and of clofibrate (ethyl-2-(*p*-chlorophenoxy)-2-methyl propionate) on the serum and liver cholesterol of rats fed the usual, normocholesteremic, or a high fat, hypercholesteremic, diet. The observed results are of interest because of the marked differences in effect of the two drugs depending on the diet consumed.

Materials and Methods. Weanling CFE rats, weighing 60–95 g were fed Wayne ground mouse breeder blox (Allied Mills, Inc., Chicago, Illinois) or the Cuthbertson *et al.* (3) high fat diet of the following composition (g): crude casein, 25.0; corn starch, 45.5; arachis oil (hydrogenated), 22.0; cholesterol, 2.0; choline chloride, 1.0; cholic acid, 0.5; salt mixture (4), 4.0; vitamin mixture (Nutritional Biochemicals Corporation), 2.2.

W-1372 in concentrations of 0.25, 0.125, and 0.063%, or clofibrate in concentrations of 1.0, 0.5, and 0.25% were mixed with the diet and fed to the animals *ad libitum* for 2 weeks. Blood samples were obtained by cardiac puncture at the termination of the study.

Extraction of cholesterol from the aortas and livers was carried out according to the method of Folch *et al.* (5). Serum cholesterol was determined on the AutoAnalyzer by the method of Levine and Zak (6). Plasma glutamic oxaloacetic transaminase (SGOT) and plasma glutamic pyruvic transaminase (SG PT) were measured by the Harleco modification of the procedures of Reitman-Frankel (7) and Wroblewski and Cabaud (8); alkaline phosphatase was determined by the technique of Klein *et al.* (9) and creatine phosphokinase by the method of Hughes (10). Liver protein was measured by the method of Lowry *et al.* (11). The total serum–liver cholesterol pool was calculated according to Kritchevsky *et al.* (12). The mean values, the standard errors, the statistical significance and differences between means as determined by the Student's *t* test were carried out as described by Burn *et al.* (13). Ten animals were used in each experimental group.

Results. Table I gives the results obtained with the drug compounds administered to rats fed the usual, normocholesteremic diet. Neither of the drugs caused a significant change in the body weight gain or daily food intake of the animals with the exception of clofibrate when given in a concentration of 1%. The reduction of serum cholesterol levels in animals receiving W-1372 was slight and of borderline significance. Clofibrate, on the other hand, produced a marked and statistically significant reduction of blood cholesterol levels at all concentrations given. Both drugs increased liver weights which were significantly greater than those of the control animals. This increase was marked in all groups except the one receiving the lowest concentration of W-1372. Administration of W-1372 did not cause significant changes in the plasma SGOT, SGPT, alkaline phosphatase or creatine phosphokinase. Clofibrate, at the highest concentration only, somewhat increased the plasma SGOT and alkaline phosphatase values, but SGPT and creatine phosphokinase were not affected at any dose level used.

TABLE I. The Effect of W-1372 and of Clofibrate on Rats Receiving the Usual Diet (2-week study).

Treatment	% Drug in diet	Final mean body wt (g)	Serum cholesterol (mg/100 ml)	t	Liver wt (g)	t
Control	—	153.0 ± 5.1	101.3 ± 3.4	—	5.07 ± 0.25	—
W-1372	0.25	135.5 ± 7.1	95.6 ± 4.3	1.05	8.64 ± 0.44	7.06[c]
	0.125	149.5 ± 4.7	91.6 ± 3.3	2.06	7.86 ± 0.33	6.74[c]
	0.063	135.8 ± 8.2	91.2 ± 2.6	2.35[a]	5.83 ± 0.18	2.47[a]
Clofibrate	1.0	130.7 ± 5.7[b]	75.4 ± 4.0	4.96[c]	9.08 ± 0.39	8.66[c]
	0.5	153.8 ± 6.0	83.2 ± 3.1	3.92[b]	7.77 ± 0.31	6.78[c]
	0.25	151.7 ± 6.1	81.3 ± 3.2	4.30[c]	7.03 ± 0.31	4.92[c]

[a] Differs significantly from control ($p < 0.05$); [b] ($p < 0.01$); and [c] ($p < 0.001$).

The two drugs fed to animals on the high fat diet did not significantly affect body weight gain or food intake of the animals. W-1372 at the two higher concentrations produced a dramatic drop of serum cholesterol levels of the order of 50% (Table II). Clofibrate was also effective in reducing serum cholesterol levels at the two higher concentrations but markedly less so than W-1372. Both compounds increased liver weight. In this respect, clofibrate produced a greater increase of liver weight at the two lower concentrations than that produced by the two lower concentrations of W-1372. Neither of the drugs caused a significant increase in the plasma SGOT, SGPT, alkaline phosphatase or creatine phosphokinase levels. Additional experiments carried out on two other occasions yielded similar results.

Administration of the high fat diet for 2 weeks did not significantly increase the cholesterol content of the aortas. The mean amount of cholesterol of aortas of rats on the usual diet was 7.0 ± 0.25 mg/kg as compared with 7.9 ± 0.25 mg/kg in rats fed the Cuthbertson high fat diet for 2 weeks. Neither W-1372 nor clofibrate administration decreased the aorta cholesterol in animals on either diet.

The two drugs, however, exerted a different effect on the liver lipid contents of the animals. In rats fed the usual diet (Table III), W-1372 produced an increase in the total lipids, cholesterol, phospholipids, and triglycerides. The increase in the amount of fatty substances in the liver depended on the amount of drug given. Clofibrate, in animals receiving the usual diet, significantly lowered the cholesterol concentration in the liver without affecting any of the other lipid concentrations measured.

Rats on a hypercholesteremic diet (Table

TABLE II. The Effect of W-1372 and of Clofibrate on Rats Receiving a Hypercholesteremic Diet (2-week study).

Treatment	% Drug in diet	Final mean body wt (g)	Serum cholesterol (mg/100 ml)	t	Liver wt (g)	t
Control	—	131.7 ± 4.6	429.7 ± 25.4	—	8.21 ± 0.46	—
W-1372	0.25	136.4 ± 3.7	151.3 ± 6.1	10.66[a]	13.96 ± 0.53	8.19[a]
	0.125	128.5 ± 6.7	224.6 ± 15.6	6.88[a]	9.72 ± 0.59	2.02
	0.063	135.5 ± 5.0	449.1 ± 22.7	0.57	9.35 ± 0.56	1.57
Clofibrate	1.0	128.4 ± 2.3	304.5 ± 15.3	4.22[a]	11.04 ± 0.40	4.64[a]
	0.5	138.3 ± 3.7	296.4 ± 10.7	4.84[a]	10.91 ± 0.40	4.43[a]
	0.25	136.8 ± 3.3	381.0 ± 25.6	1.35	10.00 ± 0.26	3.39[b]

[a] Differs significantly from control ($p < 0.001$); and [b] ($p < 0.01$).

TABLE III. Effect of W-1372 and Clofibrate on the Liver Lipids of Rats Fed the Usual Diet.

Treatment	% Drug in diet	Total lipid (%)	Total lipid t	Cholesterol (%)	Cholesterol t	Triglycerides (%)	Triglycerides t
Control	—	3.6 ± 0.22	—	0.47 ± 0.02	—	0.28 ± 0.05	—
W-1372	0.25	10.2 ± 0.98	6.6[a]	1.21 ± 0.18	4.1[b]	4.5 ± 0.69	6.1[a]
	0.125	8.1 ± 0.47	8.8[a]	0.95 ± 0.14	3.4[b]	3.1 ± 0.41	6.7[a]
	0.063	4.2 ± 0.20	2.2	0.45 ± 0.03	0.7	0.50 ± 0.09	2.0
Clofibrate	1.0	3.8 ± 0.14	0.96	0.26 ± 0.01	9.6[a]	0.32 ± 0.03	0.6
	0.5	3.6 ± 0.14	0.19	0.28 ± 0.02	7.6[a]	0.26 ± 0.05	0.3
	0.25	3.7 ± 0.14	0.58	0.27 ± 0.01	10.0[a]	0.26 ± 0.06	0.3

[a] $p < 0.001$.
[b] $p < 0.01$.

IV), in response to W-1372 administration at the two higher doses, had higher total liver lipids than control animals. This increase was predominantly due to a higher content of liver triglycerides. Cholesterol and phospholipids were not significantly affected by W-1372. These findings are similar to those previously observed with compound W-398 (1, 2). Clofibrate given to rats receiving a hypercholesteremic diet did not markedly affect the lipid contents of the liver. The only exception was the somewhat higher cholesterol concentration in the liver of rats receiving clofibrate in a concentration of 0.25%.

In order to ascertain the effect of the two drugs on the total body cholesterol, the total serum–liver cholesterol pool was calculated according to the method described by Kritchevsky *et al.* (12) (Table V). In animals on the usual diet, W-1372 in the two higher concentrations significantly increased the pool while the low concentration of the drug had no effect. Clofibrate slightly decreased the serum–liver cholesterol pool in these animals but the change was not significant.

Both drugs produced the opposite effects when administered to animals on a high fat diet. Under these conditions, W-1372 did not significantly affect the serum–liver cholesterol pools. Clofibrate, on the other hand, produced an increase in the cholesterol pool. This increase was significant at the low concentration of the drug.

The liver protein content of rats on the usual diet was not affected by either of the drugs. The drugs, however, had an effect on the protein content of the livers in animals

TABLE IV. Effect of W-1372 and Clofibrate on Liver Lipids of Rats Fed a Hypercholesteremic Diet.

Treatment	% Drug in diet	Total lipid (%)	Total lipid t	Cholesterol (%)	Cholesterol t	Triglyceride (%)	Triglyceride t
Control	—	10.1 ± 0.72	—	3.2 ± 0.34	—	1.2 ± 0.25	—
W-1372	0.25	23.3 ± 1.30	8.8[a]	2.1 ± 0.39	2.1	13.6 ± 0.11	47.7[a]
	0.125	15.8 ± 1.15	4.2[b]	3.2 ± 0.45	—	6.3 ± 0.63	7.6[a]
	0.063	11.0 ± 0.73	1.0	3.3 ± 0.51	0.2	1.3 ± 0.25	0.29
Clofibrate	1.0	9.5 ± 0.81	0.56	2.8 ± 0.37	0.8	0.90 ± 0.14	1.1
	0.5	10.2 ± 1.12	0.07	3.7 ± 0.62	0.7	0.93 ± 0.10	1.0
	0.25	12.1 ± 0.93	1.7	4.1 ± 0.10	2.5[c]	0.73 ± 0.22	1.1

[a] $p < 0.001$.
[b] $p < 0.01$.
[c] $p < 0.05$.

TABLE V. Effect of W-1372 and Clofibrate on Serum–Liver Cholesterol Pool in Rats.

		Serum–liver cholesterol pool (mg)			
		Usual diet		High fat diet	
Treatment	% Drug in diet	Mean	*t*	Mean	*t*
Control	—	31.4 ± 3.4	—	284.3 ± 27.3	—
W-1372	0.25	106.4 ±15.1	3.8[a]	320.7 ± 44.8	0.69
	0.125	75.3 ± 9.4	4.4[a]	319.7 ± 42.2	0.70
	0.063	28.9 ± 2.1	0.63	321.8 ± 46.8	0.69
Clofibrate	1.0	26.4 ± 0.62	1.5	329.6 ± 48.4	0.82
	0.5	26.4 ± 1.3	1.4	404.0 ± 51.6	2.1
	0.25	24.5 ± 0.4	2.0	403.5 ± 16.5	3.7[a]

[a] $p < 0.01$.

receiving the hypercholesteremic diet. In these animals clofibrate increased the protein content as previously described (14). W-1372, on the other hand, in the concentration of 0.25%, produced a decrease in liver protein concentration from 15.8 ± 0.61 observed in the controls to 12.5 ± 1.09 in the treated animals. Lower concentrations of the drug did not produce significant effects.

To determine whether the liver enlargement produced by W-1372 was reversible, the drug was administered to 45 rats receiving the usual diet for a period of 3 months. The drug was given by intubation at a dose of 300 mg/kg/day as a 3% suspension in 1% gum acacia in distilled water, once daily, 5 times a week. Control animals received the gum acacia suspension only. After 3 months, when the drug treatment was terminated, one group of 10 animals was sacrificed. Other groups of 5 animals were sacrificed at various intervals after discontinuation of the W-1372 administration.

Administration of W-1372 for 3 months significantly increased liver weights but had no effect on serum cholesterol concentrations, or SGOT, SGPT, or alkaline phosphatase values. The liver enlargement produced by W-1372 was rapidly and completely reversible. As early as 9 days after discontinuation of the drug, the liver weight did not significantly differ from that of the untreated control animals.

Discussion. Both W-1372 and clofibrate, under appropriate conditions, are capable of lowering serum cholesterol and of affecting liver lipids. The effects produced by the two drugs are, however, dependent on the composition of the diet fed the animals.

Clofibrate appeared to be particularly effective in lowering serum cholesterol in rats fed the usual diet which is relatively low in fat contents (10.4%). W-1372, on the other hand, was much more effective in lowering blood cholesterol levels in rats fed the hypercholesteremic diet than in animals fed the normocholesteremic diet.

The effect of benzyl *N*-benzyl carbethoxyhydroxamate (W-398), a compound chemically related to W-1372, on serum and liver cholesterol of rats fed a sterol-free semisynthetic diet was studied by Kritchevsky and Tepper (15). Although the compound produced marked weight loss, it had no effect on serum cholesterol levels. It caused liver enlargement, however, and produced a large increase of liver cholesterol. In weanling rats fed a hypercholesteremic diet, the compound lowered serum cholesterol, increased liver triglycerides, but did not affect liver cholesterol content (1).

Clofibrate was reported to reduce serum cholesterol levels in rats on a normocholesteremic diet (16). This effect was, however, not readily reproducible (17). Kokatnur *et al.* (18) studied the effect of clofibrate on serum cholesterol in rats given several different high fat diets containing corn oil, beef tallow, or medium-chain triglycerides at a level of 20%, supplemented with cholesterol

2% and a 1:1 mixture of cholic acid–sodium cholate (1%). Clofibrate 0.3% (the only concentration tested) failed to show a serum cholesterol-lowering effect in animals fed these diets. On the contrary, a marked elevation of serum cholesterol was noted with certain diets. These results, which are at variance with those reported above, indicate that the type of fat present in the diet may be an important factor in the serum cholesterol lowering effect of clofibrate.

At the present time, little is known about the mechanism by which clofibrate or W-1372 lower blood cholesterol levels. The results described in the present paper indicate that the two drugs affect the fat metabolism of the rat in different ways and suggest that the hypocholesteremic action of the two drugs is brought about by different mechanisms.

Summary. *N*-γ-Phenylpropyl-*N*-benzyloxy acetamide (W-1372) and clofibrate were given to weanling rats in the diet for 2 weeks. The hypocholesteremic and other effects of the drugs differed greatly depending on the kind of diet which was provided. Clofibrate reduced serum cholesterol of animals on the usual, normocholesteremic diet, but had much less effect in animals on a hypercholesteremic, high fat diet. W-1372, in contrast, was much more effective in lowering blood cholesterol levels in animals fed a high fat diet. The two drugs also produced different effects on liver cholesterol, liver triglycerides, the serum liver cholesterol pool and the protein content of the livers. Both drugs produced liver enlargement but did not affect plasma SGOT, SGPT, and alkaline phosphatase. These findings suggest that different mechanisms are involved in the hypocholesteremic action of these compounds.

1. Berger, F. M., Douglas, J. F., Ludwig, B. J., and Margolin, S., Proc. Soc. Exptl. Biol. Med. **114**, 337 (1963).

2. Berger, F. M., Douglas, J. F., Ludwig, B. J., and Margolin, S., J. Pharmacol. Exptl. Therap. (1969), in press.

3. Cuthbertson, W. F., Elcoate, P. V., Ireland, D. M., Mills, D. C., and Shearley, P., Brit. J. Nutr. **13**, 227 (1959).

4. Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., J. Nutr. **14**, 273 (1937).

5. Folch, J., Lees, M., and Sloane Stanley, G. H., J. Biol. Chem. **226**, 497 (1957).

6. Levine, J. B. and Zak, B., Clin. Chim. Acta **10**, 381 (1964).

7. Reitman, S. and Frankel, S., Am. J. Clin. Pathol. **28**, 56 (1957).

8. Wroblewski, F. and Cabaud, P., Am. J. Clin. Pathol. **27**, 235 (1957).

9. Klein, B., Read, P. A., and Babson, A. L., Clin. Chem. **6**, 269 (1960).

10. Hughes, B. P., Clin. Chim. Acta **7**, 597 (1962).

11. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

12. Kritchevsky, D., Moynihan, J. L., and Sachs, M. L., Proc. Soc. Exptl. Biol. Med. **108**, 254 (1961).

13. Burn, J. H., Finney, D. J., and Goodwin, L. G., "Biological Standardization," 2nd ed., p. 36. Oxford Univ. Press, London and New York (1950).

14. Platt, D. S. and Thorp, J. M., Biochem. Pharmacol. **15**, 915 (1966).

15. Kritchevsky, D. and Tepper, S. A., Proc. Soc. Exptl. Biol. Med. **121**, 1162 (1966).

16. Best, M. M. and Duncan, C. H., Am. J. Cardiol. **15**, 230 (1965).

17. Thorp, J. M., J. Atherosclerosis Res. **3**, 351 (1963).

18. Kokatnur, M. G., Malcom, G. T., and Martinez, R. D., Metabolism **18**, 73 (1969).

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