Effect of *N*-γ-Phenylpropyl-*N*-benzyloxy Acetamide (W-1372) on Experimental Atherosclerosis in Rabbits[1] (34202)

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Berger and his co-workers (1, 2) reported that several *N*-substituted amides reduced atheromata in cholesterol-fed rabbits when administered at a level of 2% of the diet. One of these, *N*-γ-phenylpropyl-*N*-benzyloxy acetamide (W-1372) was also found to reduce atheromata in squirrel monkeys fed an atherogenic diet (2). We studied the effects of W-1372 on the establishment and progression of atherosclerosis in rabbits. Our findings are reported below.

Methods. Male, Dutch-belted rabbits (1600–1800 g) in groups of 10, 12, and 6, respectively were used throughout. In the experiments in which the effect of the test compound upon establishment of atheromata was investigated, the rabbits were fed a diet of rabbit chow augmented with 2% cholesterol suspended in 6% corn oil. The diets of the test animals were further augmented with 1 or 2% of W-1372. In the experiment in which the effect of W-1372 on progression of lesions was studied, a large group of rabbits was maintained on the atherogenic regimen. After 2 months, all the rabbits were bled and one group whose average serum cholesterol level was equal to that of a number of rabbits maintained on cholesterol and drug was killed. The average atheroma observed in these rabbits was taken as the base-line (2 month) control. The remaining rabbits were divided into two groups of equal serum cholesterol. One group was maintained on chow for another 2 months while the other group was fed chow plus 2% W-1372 for a similar period.

At the end of the feeding periods rabbits were bled from an ear vein, weighed, and killed, The livers were weighed and aliquots were taken for cholesterol determination. Aortas were graded for atheromata on a 0–4 scale (3) which has been shown to correlate with aortic cholesterol content (4). The aortic arch and thoracic aorta were graded separately. Serum and liver cholesterol levels were determined by the method of Mann (5); the liver cholesterol determinations were carried out on 1-g aliquots of liver which had been saponified in 15% alc. KOH. Serum triglycerides were assayed by the Van Handel and Zilversmit (6) procedure. The *N*-γ-phenylpropyl-*N*-benzyloxy acetamide was generously provided by Dr. F. M. Berger, Wallace Laboratories, Cranbury, New Jersey.

Results. The results of three experiments designed to study the influence of W-1372 upon establishment of atherosclerosis in rabbits are presented in Table I. In the first experiment, the drug was administered at a level of 1% of the diet. As shown, there was no difference in atheromata between the drug and control groups, but there was a significant reduction in liver cholesterol levels in the rabbits fed W-1372. In the next two experiments the W-1372 was fed at a level of 2% of the diet. In both experiments there was a reduction of atheromata in the drug-fed rabbits and in both experiments liver cholesterol levels were significantly lower in these animals.

In Expt. 2, as in Expt. 1, serum cholesterol levels were not different in the two groups; in the third experiment the control group was selected to have an average serum cholesterol level equal to that of the drug-fed group. Serum triglycerides were not determined in Expt. 1, but in the two subsequent experiments a significant increase in serum

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TABLE I. Influence of *N*-γ-Phenylpropyl-*N*-benzyloxy Acetamide (W-1372) on Experimental Atherosclerosis in Rabbits (2% cholesterol; 6% corn oil; 2 months).

Expt.:	1		2		3	
	W-1372	Control	W-1372	Control	W-1372	Control
Drug dosage (%)	1	—	2	—	2	—
Survival	10/10	10/10	12/12	12/12	6/8	6/6
Av wt gain (g)	166	327	19	182	5	400
Av liver wt (g)	114.1	124.4	84.5	117.8	89.7	107.7
Liver (% body wt)	5.79	5.83	4.64	5.91	5.56	5.59
Av liver cholesterol (g/100 g)	2.02 ± 0.43[d]	3.85 ± 0.53[a]	2.99 ± 0.40	5.43 ± 0.51[b]	1.47 ± 0.27	2.57 ± 0.36[a]
Av serum cholesterol (mg/100 ml)	760 ± 120	675 ± 115	1135 ± 136	1115 ± 49	1264 ± 311	1268 ± 280
triglyceride (mg/100 ml)	—	—	188 ± 34	108 ± 21[c]	703 ± 213	119 ± 17[a]
Av atheromata, arch	1.15 ± 0.26	1.20 ± 0.19	1.38 ± 0.23[c]	2.13 ± 0.25	0.83 ± 0.21	1.17 ± 0.21
thoracic	0.75 ± 0.21	0.65 ± 0.8	0.75 ± 0.22	1.29 ± 0.17	0.50 ± 0.13	0.92 ± 0.24

[a] $p < .02$. [b] $p < .001$. [c] $p < .05$. [d] Standard error.

triglyceride levels was observed in rabbits fed W-1372. The drug also affected weight gain in the rabbits. The rabbits maintained on 1% W-1372 for 2 months gained only half as much weight as did the controls, and those fed 2% of this compound gained practically no weight at all. The livers of the rabbits fed W-1372 were smaller in size than were those of the controls, and when calculated as percentage of body weight they were not enlarged.

The reduced extent of atheromatous involvement observed in the aortas of the rabbits fed 2% W-1372 was striking. In the aortic arch the severity of atherosclerosis was about 33% less and in the thoracic aorta it was about 40% less. When the atheromata observed in Expts. 2 and 3 are combined, we find the average atheromata in the arch are: W-1372, 1.19 ± 0.18 and control, 1.81 ± 0.20 ($p < .05$). Atheromata in the thoracic aorta are: W-1372, 0.67 ± 0.15 and control, 1.17 ± 0.14 ($p < .05$). Thus, W-1372 at 2% of the diet has a significant effect upon severity of cholesterol-induced atherosclerosis in rabbits.

The distribution of atheromata observed in Expts. 2 and 3 is shown in Table II. It is evident that few of the rabbits fed W-1372 had atheromata of severity greater than 1.0 (7/18 in the arch and 2/18 in thoracic aorta). In contrast severity greater than 1.0 was observed in the aortic arch of 12 of 18 control rabbits and in the thoracic aorta in 6 of 18 rabbits.

We have observed (7, 8) that when rabbits who have been rendered atherosclerotic by 2 months of cholesterol feeding are returned to a normal chow diet, the severity of their atheromata increases. Exacerbation of the severity of the lesions can be reduced by dietary unsaturated fat (7) or, under certain conditions, by drugs such as D-thyroxine (9). We tested W-1372 under similar conditions.

Table III shows that the drug again had an adverse effect upon weight gain. Serum cholesterol levels fell significantly in both groups (over 90%) as did liver cholesterol levels. The serum triglyceride levels of the W-1372 group, which were initially higher than those of the chow control, rose by 11%

TABLE II. Distribution of Atheromata in Aortas of Rabbits Fed 2% Cholesterol and 6% Corn Oil with and without W-1372 (2%) (Expts. 2 and 3).

Grade	W-1372 (18/20)[a]		Control (18/18)	
	Arch	Thoracic	Arch	Thoracic
4.0	—	—	—	—
3.5	—	—	2	—
3.0	—	—	1	—
2.5	3	1	1	—
2.0	1	1	5	5
1.5	3	—	3	1
1.0	4	2	5	7
0.5	7	11	1	5
0.0	—	3	—	—
Av	1.19 ± 0.18[b,c]	0.67 ± 0.15[d]	1.81 ± 0.20	1.17 ± 0.14

[a] Survival ratio.
[b] Standard error.
[c] $p < .05$.
[d] $p < .02$.

during the 2-month cholesterol-free period, whereas those of the controls fell by 19%.

The exacerbation of atheromatous lesions was retarded in the rabbits fed W-1372. In the aortic arch, the atheromata in the chow-fed group were 58% higher than in the cholesterol-fed controls, and in the thoracic aorta, severity of atherosclerosis was increased by 89%. The severity of lesions in the aortic arch and thoracic aorta of rabbits fed W-1372 was 42 and 33% higher than those observed in the cholesterol-fed controls and 11 and 30% lower than those of the chow-fed controls, respectively.

TABLE III. Influence of *N*-γ-Phenylpropyl-*N*-benzyloxy Acetamide (W-1372) on Preestablished Atheromata in Rabbits.

	Group		
	Cholesterol-fed[a]	Chow plus W-1372 (2%)	Chow
Drug dosage (%)	—	2	—
Survival	6/6	6/7	7/7
Av wt gain (g), 8 weeks	400	421	303
16 weeks	—	—12	381
Av liver wt (g)	107.7	99.5	93.7
Liver (% body wt)	5.59	5.20	4.17
Av liver cholesterol (g/100 g)	2.57 ± 0.36[b]	0.82 ± 0.27[c]	0.40 ± 0.17[d]
Av serum cholesterol (mg/100 ml), 8 weeks	1268 ± 280	1298 ± 351	1298 ± 331
16 weeks	—	102 ± 28[c]	113 ± 15[c]
Av serum triglyceride (mg/100 ml), 8 weeks	119 ± 17	104 ± 33	141 ± 29
16 weeks	—	116 ± 19	114 ± 24
Av atheromata, arch	1.17 ± 0.21	1.67 ± 0.51	1.93 ± 0.40
Thoracic	0.92 ± 0.24	1.17 ± 0.40	1.71 ± 0.34

[a] Identical with control group Expt. 3, Table I.
[b] Standard error.
[c] Compared with cholesterol fed: $< .01$; [d] $< .001$.

Discussion. When fed at sufficiently high levels (2% of the diet), *N*-γ-phenylpropyl-*N*-benzyloxy acetamide (W-1372) inhibits the establishment of cholesterol-induced atherosclerosis in rabbits. Rabbits fed 2% of W-1372 gain very little weight. This does not appear to be due to an anorectic effect of the drug, since food consumption was about equal in test and control groups. The drug does not affect serum cholesterol levels, but causes significant reductions in liver cholesterol. Even at a dose level (1%) which has no effect upon atheromata, the effects on weight gain and liver cholesterol are observed. Rabbits fed W-1372 also have significantly elevated serum triglyceride levels. This change in serum cholesterol/triglyceride ratios suggests a possible effect on lipoprotein levels with a shift towards lower density β lipoproteins. Pierce (10) observed that alloxan diabetic, cholesterol-fed rabbits when compared with cholesterol-fed controls exhibited a lower incidence of atheromata, despite elevated cholesterol levels. He attributed this to an observed shift in serum lipoprotein spectrum from a preponderance of S_f 10–30 molecules to S_f 80–100 lipoproteins. A similar difference in cholesterol/triglyceride ratios was observed by Adlersberg *et al.* (11) in rabbits fed cholesterol with and without cortisone. The serum cholesterol levels in the cholesterol–cortisone group were almost 50% higher than those observed in the cholesterol-fed group but triglycerides were elevated by 70% and atheromata were less severe.

The reduced liver cholesterol levels in the W-1372-fed rabbits also suggest a more efficient removal of cholesterol from the serum–liver pool. Whether the lowered liver cholesterol levels are the result of increased excretion of cholesterol or its metabolites or inhibition of deposition is not known at the present time. The effect of W-1372 upon fecal excretion of steroids and upon possible alterations in the cholesterol level in various tissues merits further study.

The mechanism of action of W-1372 in inhibiting atherosclerosis in cholesterol-fed rabbits may involve a change in β-lipoprotein spectrum. This may partially be the result of decreased cholesterol deposition in the liver.

Summary. The effect of N-γ-phenylpropyl-*N*-benzyloxy acetamide (W-1372) upon the establishment and progression of atheromata in cholesterol-fed rabbits has been investigated. At a level of 2% in the diet W-1372 inhibits weight gain and does not affect serum cholesterol levels but raises serum triglyceride levels. Liver cholesterol levels are significantly lower in rabbits fed W-1372. The average atheromata in the aortic arch and thoracic aorta of rabbits fed an atherogenic regimen augmented with 2% W-1372 are significantly lower than those observed in controls. When W-1372 (2%) is fed to rabbits with preestablished atheromata for 2 months, the exacerbation of severity of lesions is reduced when compared to controls. Atheromata in the aortic arch are 11% less severe and in the thoracic aorta 30% less severe.

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