cholic acid exerts the effects reported is not clear. Dietary lithocholic acid has been reported to induce liver cirrhosis in the rabbit (11) and Iguana iguana(12) and parenterally administered lithocholic acid has been shown to be pyrogenic in man(13). The increase in liver size observed appears to be the result of a biliary hyperplasia(1); however, the etiology of the lesion remains obscure.

The hypercholesterolemic effect of cholic acid when fed in conjunction with cholesterol and its lack of effect in a cholesterol-free diet, observed in these studies, is in accord with the reports of Hegsted *et al*(7) and Edwards (8). The indication from Experiment 2 that cholic acid might be hypercholesterolemic in older chicks fed a cholesterol-free diet was not borne out by the data obtained in an 8-week study (Exp. 3). Dietary cholic acid has been reported to increase liver cholesterol levels in mice(3) and rats(6); however, this bile acid did not significantly alter liver cholesterol levels in these studies with chicks.

Summary. Dietary lithocholic acid elevated plasma cholesterol and lipid phosphorus levels, and increased liver size of growing chicks. In cholesterol-fed chicks, lithocholic acid depressed liver lipid and cholesterol levels. The effects of lithocholic acid were partially reversed by cholic acid in chicks fed a cholesterol-free diet and almost completely reversed in chicks fed cholesterol. The plasma cholesterol level of cholesterol-fed chicks was increased by cholic acid feeding.

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1. Leveille, G. A., Sauberlich, H. E., Hunt, R. D., *Poultry Sci.*, 1962, v41, 1991.

2. Beher, W. T., Anthony, W. L., Baker, G. D., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 317.

3. Beher, W. T., Baker, G. D., Anthony, W. L., *ibid.*, 1960, v103, 385.

4. Hegsted, D. M., Andrus, S. B., Gotsis, A., Portman, O. W., *J. Nutrition,* 1957, v63, 273.

5. Howe, E. E., Bosshardt, D. K., Huff, J. W., *ibid.*, 1960, v72, 379.

6. Portman, O. W., Bruno, D., *ibid.*, 1961, v73, 329.

7. Hegsted, D. M., Gotsis, A., Stare, F. J., *ibid.*, 1960, v70, 119.

8. Edwards, H. M., Jr., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 742.

9. Leveille, G. A., Sauberlich, H. E., *J. Nutrition,* 1962, v76, 321.

10. ———, PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 300.

11. Holsti, P., *Nature,* 1960, v186, 250.

12. Stolk, A., *Experientia,* 1960, v16, 507.

13. Palmer, R. H., Glickman, P. B., Kappas, A., *J. Clin. Invest.*, 1962, v41, 1573.

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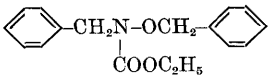
Effect of Benzyl N-Benzyl Carbethoxyhydroxamate (W-398) on Experimental Atherosclerosis and Hypercholesteremia. (28669)

F. M. BERGER, J. F. DOUGLAS, B. J. LUDWIG AND S. MARGOLIN
Wallace Laboratories, Division of Carter Products, Inc., Cranbury, N. J.

The compound benzyl N-benzyl carbethoxyhydroxamate (W-398) was originally described as a chemical intermediate by Jones and Fleck(1), but no reference to its biological effects has been found in the literature. The present paper describes the ability of this compound to reverse experimental atherosclerotic lesions and to reduce the levels of cholesterol and other fatty substances in the blood of certain species of animals. The compound has the following structural formula:



It is a bitter, oily liquid, poorly soluble in water (0.006% at 37°C) but readily miscible with most organic solvents. It is stable in dilute acids or alkali and is not altered by gastric or intestinal juices at 37°C.

Methods. Experimental atherosclerosis was induced in young adult male albino rabbits

by adding cholesterol 1% by weight to Wayne rabbit pellets. The cholesterol was dissolved in absolute ethanol, the pellets soaked with the solution and the ethanol removed in a drying oven. W-398 was applied to the food pellets as an alcoholic solution in the same manner as cholesterol. Body weights and food intake of the animals were recorded weekly. The lipid levels in serum were determined at 2-week intervals utilizing blood samples obtained from the ear vein. Serum cholesterol was determined by the method of Abell *et al*(2), serum phospholipids according to Youngburg and Youngburg(3), triglycerides according to Van Handel and Zilversmit(4), total lipids by the method of Blohm *et al*(5), and total fatty acids according to Drysdale and Billimoria (6). Gross signs of atherosclerotic changes were followed by observing the accumulation of lipid material in the iris of the rabbit, as described by Prior *et al*(7). Upon completion of the trials the animals were autopsied and various tissues preserved for histological examination. The extent of atherosclerosis in the aorta was scored according to the method of Fillios *et al*(8), assigning numerals from 1 to 4 to plaques according to their number, size and extent; zero indicating absence of plaques and 4 maximal involvement with large confluent plaques.

In rats, serum hypercholesteremia was produced by feeding the diet of Cuthbertson *et al*(9). Weanling rats about 56 days old were divided into 2 groups, one group given the Cuthbertson diet and the other this diet with added drug. The experiments were carried out for a period of 2 weeks and blood samples obtained by cardiac puncture at termination of the study. The statistical significance of differences between means was determined by Student's "T" test(10).

Results. Reversal of experimental atherosclerotic lesions. A group of 12 albino rabbits receiving a cholesterol diet for 7 weeks showed an average serum cholesterol level of 1735 $\pm$ 284 mg%. At this time, W-398 in a concentration of 2% was introduced into the diet of 6 of the rabbits. These animals, as well as the remaining 6 rabbits (cholesterol control), continued to receive cholesterol 1% in the food. A third group of 6 rabbits (normal control) received the normal diet without addition of cholesterol or drug. Fig. 1 gives average blood cholesterol values of the 3 groups of animals. It shows that administration of W-398 produced a reduction of blood cholesterol of about 30% within 2 weeks. Continued treatment further lowered blood cholesterol. These changes were observed in spite of the continued administration of cholesterol. There were no significant differences in body weight between the animals receiving the cholesterol diet without the drug and those receiving the cholesterol diet and W-398 although both these groups weighed less than control animals receiving a normal diet.

W-398 influenced not only the serum levels of cholesterol but also had a distinct effect on other serum and liver lipids of rabbits. These values, determined in both control and experimental groups after 9 weeks treatment with W-398, are given in Table I.

The livers of the drug-treated, cholesterol-fed animals showed a significant decrease in

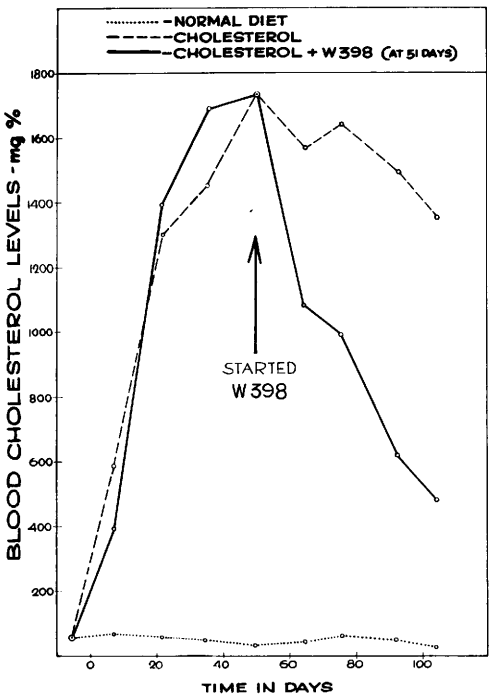
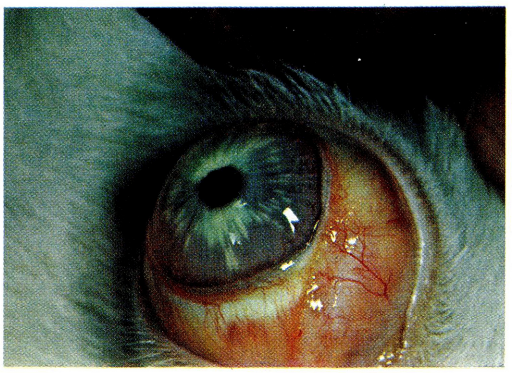
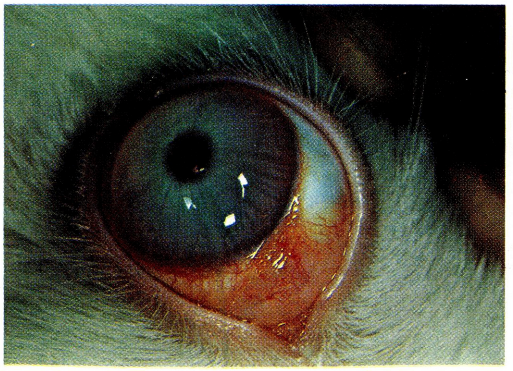


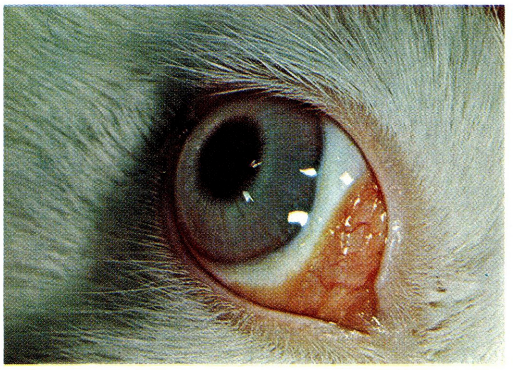
FIG. 1. Average blood cholesterol levels of rabbits receiving a normal diet, a diet with cholesterol 1% and a diet with cholesterol 1% and W-398 2%.



Cholesterol Diet

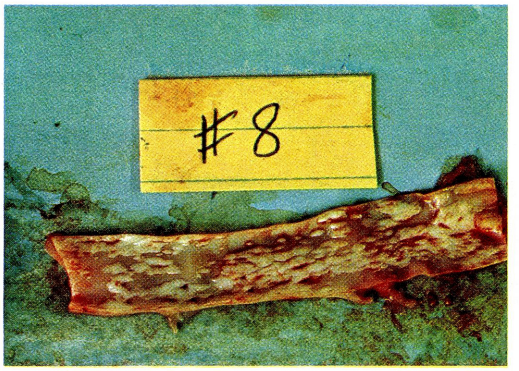


Cholesterol Diet + W-398



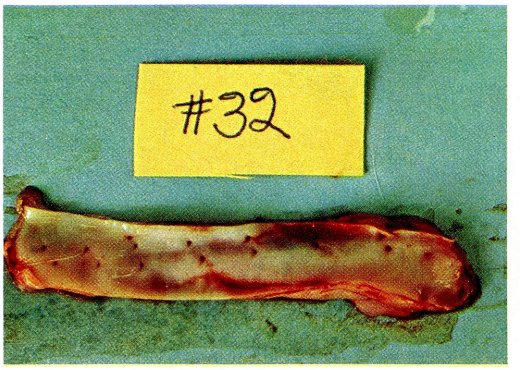
Normal Diet

FIG. 2. Photographs of albino rabbit irises illustrating disappearance of lipid deposits from the eye after W-398. Rabbits received 1% cholesterol diet for 51 days prior to administration of 2% W-398 in the diet.



Cholesterol Diet

Cholesterol Diet + W-398



Normal Diet

FIG. 3. Photographs of albino rabbit aortas, illustrating prevention of plaque formation by W-398. Rabbits received 0.5% cholesterol and concurrently 2% W-398 in the diet for 12 wk.

TABLE I. Influence of W-398 on Serum and Liver Lipids of Rabbits Fed a High Cholesterol Diet for 16 Weeks. Drug treatment was instituted on 51st day.

	Normal diet, no drug	Cholesterol diet	
		No drug	W-398, 2%
		Serum	
Total lipids, %	.26 ± .03	2.94 ± .63	1.76 ± .43
Cholesterol, mg %	29 ± 4	1477 ± 300	656 ± 207 †
Phospholipids, "	129 ± 13	919 ± 167	567 ± 111
Triglycerides, "	101‡	540‡	537‡
		Liver	
Total lipids, %	6.4 ± .4	11.6 ± .7*	6.9 ± .3
Cholesterol, mg/g	2.9 ± .1	12.6 ± 1.6*	10.9 ± 1.3*
Phospholipids, "	15.0 ± 2.1	17.0 ± 4.2	15.9 ± 3.6
Triglycerides, "	3.5 ± .7	6.9 ± 2.1	4.3 ± .7
Weight, g	106.0 ± 5.8	148.7 ± 6.5*	117.8 ± 9.6

* Differs significantly from normal diet controls ($P < .05$).
† Differs significantly from cholesterol diet controls ($P < .05$).
‡ Calculated values.

total lipids as compared to the livers of animals fed cholesterol alone, and approximated the levels observed in the livers of animals on normal diet. Phospholipid and triglyceride levels did not vary significantly among the livers of the 3 groups.

Prior *et al*(7) have shown that a high cholesterol diet fed to rabbits for several weeks will produce accumulation of lipid material in the iris that is visible to the naked eye. When W-398 was added to the diet after the fatty deposits became clearly visible, a gradual disappearance of the fatty deposits occurred even though the administration of cholesterol in the diet was continued. After 9 weeks of treatment with the drug (16th week of experiment), only little fatty material remained (Fig. 2). At this time, all animals were autopsied. Body weight, organ weight, and organ appearance of the control and experimental animals were not significantly different. Grossly, a clear-cut reduction in the size, extent and number of plaques in the aortas of the treated animals was observed.

TABLE II. Influence of W-398 and Other Drugs on Serum Cholesterol Values of Weanling Rats Fed a Hypercholesteremic Diet for Two Weeks.

Drug	Concentration in diet, %	No. rats	Serum cholesterol, mg %
None	—	24	748 ± 40
W-398	.25	24	463 ± 45*
W-398	.50	24	143 ± 11*
Triparanol	.25†	6	193 ± 60*
β-Sitosterol	1.0	6	295 ± 27*
Benzmalecene	1.0 †	6	250 ± 14*
Thyroxine	.0025	6	270 ± 46*

* Differs significantly from controls ($P < .01$).
† Marked suppression of body weight gains occurred with these concentrations of drug in the diet.

Prevention of experimental atherosclerotic lesions. A group of 26 rabbits was used in this experiment. Seven animals were fed the usual diet (Group 1), 10 received in addition 0.5% cholesterol (Group 2), and the remaining 9 animals were fed both 0.5% cholesterol and 2% W-398 (Group 3). The experiment was run for 12 weeks. A marked reduction of blood cholesterol levels in the animals receiving the drug was noted about 2 weeks after institution of treatment. At this time, the blood cholesterol levels of Group 1 were 50 ± 7, of Group 2, 395 ± 137 and of Group 3, 222 ± 64 mg%. Deposition of fatty material in the iris of animals receiving the cholesterol additive (Group 2) was clearly visible 7 weeks after the start of the experiment but was not visible in the animals that received the drug in addition to the cholesterol (Group 3). Blood cholesterol values at this time were 939 ± 164 mg% for the cholesterol control group and 479 ± 71 mg% for the W-398 treated animals. Before termination of the experiment, a bromsulfophthalein excretion test for liver function was carried out. No significant difference between the cholesterol control (Group 2) and the cholesterol-fed animals

TABLE III. Effect of W-398 on Liver Weight and Liver Lipid Content of Rats Fed a Hypercholesteremic Diet for 2 Weeks.

	Control	W-398, 0.25%	W-398, 0.5%
Total lipid, %	16.6 ± 1.4	19.0 ± 1.3	20.6 ± 1.9
Cholesterol, mg/g	12.9 ± 1.3	13.3 ± .6	10.3 ± 1.3
Phospholipids, "	18.1 ± .7	20.6 ± .6	17.6 ± 2.1
Triglycerides, "	12.3 ± 2.3	42.7 ±12.4*	71.6 ± 4.7*
Liver wt, g	7.8 ± .5	9.79 ± .7*	10.53 ± .5*

* Differs significantly from controls ($P < .05$).

treated with W-398 (Group 3) was noted, suggesting that in rabbits the drug did not interfere with liver function. Average body weights at the end of the experiment were 3.48 ± 0.23 kg for Group 1, 3.71 ± 0.09 kg for group 2 and 3.21 ± 0.07 kg for Group 3. The difference between the control and drug-treated animal was not statistically significant ($P > 0.2$).

At autopsy, W-398 treated animals had livers that in appearance and weight were more like those of normal animals. The cholesterol-fed animals had heavier livers (146 ± 8.1 g) than normal (103.5 ± 5.9 g) or cholesterol-fed and W-398 treated animals (115.9 ± 5.1 g). The aortas of cholesterol-fed animals showed numerous large plaques, particularly in the region of the aortic arch. Aortas of cholesterol-fed rabbits treated with W-398 showed only a few small plaques and were similar in appearance to the normal controls (Fig. 3). Thus, W-398 prevented or greatly retarded the development of atherosclerotic lesions. The average aortic plaque score for animals receiving the cholesterol diet was 2.2 ± 0.5 units, while animals receiving W-398 in addition had a score of only 0.44 ± .15 unit. This difference is statistically significant ($P > 0.01$).

Lipid changes in rats. Weanling rats fed a hypercholesteremic diet(9) for 2 weeks showed markedly elevated serum lipid values. When W-398 was added to the hypercholesteremic diet, serum cholesterol levels did not rise as much, and cholesterol serum level was inversely related to the concentration of W-398 in the diet.

Triglycerides, total fatty acids and total lipids in the serum were also lower in treated animals than in controls. Phospholipids were not affected. Measurements of daily food intake established no significant differences between control (10.6 ± 0.7 g/rat/day) and W-398 treated rats (10.6 ± 0.8 g/rat/day). Benzamalecene, β-sitosterol and triparanol also reduced cholesterol levels when administered in the diet in suitable concentrations (Table II).

W-398 at 0.25 and 0.5% in the diet did not significantly affect growth and body weight gains of rats. However, animals receiving the drug in concentrations of 1 or 2% did not gain weight satisfactorily but survived.

At autopsy no significant changes were noted in the gonads, adrenals, prostate, spleen, heart, kidney or thymus but some enlargement was noted in the liver. Table III gives the liver weight of the control and drug-fed animals and the amount of various lipids found in the livers of these animals. W-398 treatment did not significantly alter the cholesterol or phospholipid contents of the liver. The most striking change produced by the drug was the increase of the triglyceride contents of the livers.

Discussion. Several previously described substances are capable of decreasing serum cholesterol in animals. W-398 shares this property with these agents although it is entirely different in chemical structure. We do not know whether or not the mechanism of action of W-398 is similar to that of any of the previously described agents.

Preliminary experiments indicate that W-398 causes no reduction of lipid absorption from the gastrointestinal tract of rats or rabbits.

Other available evidence indicates that this compound inhibits the conversion of acetate to mevalonate without further blocking the conversion of mevalonate to cholesterol *in vitro*. The relation of the *in vitro* to the *in vivo* findings is difficult to assess because the

latter were obtained in animals consuming a diet high in exogenous cholesterol which by itself might have interfered with endogenous cholesterol synthesis.

The unique ability of W-398 to reverse established atherosclerotic lesions and to prevent their development is of great interest. The lack of adverse effects on all major organ systems, with the possible exception of the liver, points to a specific locus of action. Little is known concerning the mechanism by which this drug brings about the observed effects. A better understanding of these mechanisms is being sought in the hope that it might lead to the discovery of even more useful agents.

Summary. Benzyl N-benzyl carbethoxyhydroxamate (W-398) given orally to rabbits caused disappearance of atherosclerotic lesions previously established by administration of a diet containing 1% cholesterol. W-398 also prevented the occurrence of atherosclerotic lesions when administered jointly with a cholesterol containing diet. Elevated blood cholesterol level in adult rabbits or weanling rats fed a hypercholesteremic diet were also reduced by administration of W-398. The drug was effective in doses that were well tolerated by the animals.

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1. Jones, L. W., Fleck, E. E., *J. Am. Chem. Soc.,* 1928, v50, 2018.

2. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., *J. Biol. Chem.,* 1952, v195, 357.

3. Youngburg, G. E., Youngburg, M. V., *J. Lab. Clin. Med.,* 1930, v16, 158.

4. Van Handel, E., Zilversmit, D. B., *ibid.,* 1957, v50, 152.

5. Blohm, T. R., Kariya, T., Laughlin, M. W., *Arch. Biochem. Biophys.,* 1959, v85, 250.

6. Drysdale, J., Billimoria, J., *Clin. Chim. Acta,* 1960, v5, 828.

7. Prior, J. T., Kurtz, D. M., Ziegler, D. D., *Arch. Pathol.,* 1961, v71, 672.

8. Fillios, L. C., Andrus, S. B., Mann, G. V., Stare, F. J., *J. Exp. Med.,* 1956, v104, 539.

9. Cuthbertson, W. F., Elcoate, P. V., Ireland, D. M., Mills, D. C., Shearley, P., *Brit. J. Nutr.,* 1959, v13, 227.

10. Burn, J. H., Finney, D. J., Goodwin, L. G., *Biological Standardization,* London, Oxford Univ, Press, 1950, 2nd ed., 36.

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Sodium Iodide-Induced Submaxillary Sialadenitis in the Rat. (28670)

S. C. WOODWARD AND C. W. BERARD (Introduced by R. L. Mundy)

Department of Surgical Metabolism and Pathology, Division of Basic Surgical Research, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.

Inflammation of submaxillary glands has been described in man(1) and in the hamster (2) in response to oral or parenteral administration of massive doses of iodide. Previous reports have concerned those species which are known from radio-autographic and biochemical studies to concentrate iodide in submaxillary gland and its saliva(3,4). Attempts have been made to correlate glandular iodide-concentrating ability with the inflammatory and metaplastic process and to relate the inflammation to chemical effects of iodide upon duct structures(2).

The rat has been shown not to concentrate iodide in submaxillary gland parenchyma or its secretion(3,5); specific glandular inflammatory changes have not been noted after iodide administration. In our studies on antithyroid effects of iodides and sulfonamides, oral administration of massive doses of sodium iodide to rats resulted in marked submaxillary sialadenitis.

Materials and methods. Fifty-four male Walter Reed strain albino rats initially weighing 200-250 g were randomly assigned to 6 groups. All animals received tap water and ground D & G rat biscuits* (iodine con-

* G. L. Baking Co., Frederick, Md.