Cholesterol-Lowering of Pantethine is Due to the Hydrolysis Product Cysteamine

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THE EFFECT OF PANTETHINE AND ITS METABOLITES ON SERUM CHOLESTEROL LEVELS IN CHOLESTEROL-FED RABBITS

by

Caran Graves

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Caran Graves
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Pantethine, a precursor of coenzyme A, has been shown to reduce serum cholesterol levels in hypercholesterolemic rabbits. The enzyme pantetheinase rapidly hydrolyzes pantethine to the vitamin pantothenic acid and the amino thiol cysteamine.

This study was designed to compare the effect of cysteamine and pantothenate supplementation with that of pantethine on hypercholesterolemic rabbits. New Zealand white rabbits were fed a 0.5% cholesterol diet for 5 weeks; treatment groups received only the high cholesterol diet (control), or a high cholesterol diet supplemented with 1% pantethine, or an equimolar amount of pantothenic acid or cystamine (the disulfide of cysteamine). Blood samples were drawn weekly and total serum cholesterol levels analyzed enzymatically.

Pantethine and cystamine both significantly reduced cholesterol levels in hypercholesterolemic rabbits.
serum cholesterol levels (p < 0.05); pantothenic acid had no effect. Separation of serum lipoproteins using a preparative ultracentrifuge showed an increase in very low density, intermediate density and low density lipoproteins.

A second experiment was conducted to compare the effect of cystamine with other small thiols; the protocol was similar to the first experiment with treatment groups consisting of a high cholesterol control, cystamine, cystine or 2-hydroxyethyl disulfide. There was no significant reduction in serum cholesterol levels between treatment groups, although the cystamine supplemented group tended to be lower than the other groups.

(76 pages)
INTRODUCTION

Cholesterol and Coronary Artery Disease

Coronary heart disease is the leading cause of death in the United States. Recent estimates indicate that 5.4 million Americans have symptomatic coronary heart disease (American Medical Association, 1985; Levy, 1986). While many factors have been associated with heart disease, high serum cholesterol levels are consistently identified with increased risk of developing coronary heart disease (Wilhelmset et al., 1973; Wright and Fredrickson, 1970; Keys, 1980).

Epidemiological studies show that the chance of developing coronary heart disease rises as serum cholesterol and low density lipoprotein (LDL) levels rise. The 20 year report (Kagan et al., 1962) of the Framingham Study noted that increasing serum cholesterol levels correlated with an increased incidence of coronary heart disease; participants with total serum cholesterol levels over 260 mg/dl had the greatest risk. The Seven Countries Study (Keys, 1980), involving over 10,000 men from Finland, Greece, Japan, Yugoslavia, Italy, Netherlands, and the United States, found that the incidence of coronary disease was directly related to increased serum cholesterol levels.
A prospective study of 855 Swedish men also identified high serum cholesterol levels (< 270 mg/dl) as a significant risk factor for coronary heart disease (Wilhelmset et al., 1973).

Recent clinical trials demonstrate the benefits of reducing serum cholesterol levels. The Leiden Intervention Trial (Arntzenius et al., 1985) reduced serum cholesterol levels by 10% over a two year period with dietary modifications. Coronary lesion growth (measured by coronary angiography) diminished significantly during this period and correlated with decreased serum cholesterol levels.

The Lipid Research Clinics Coronary Primary Prevention Trials (1984a, 1984b) used the bile acid sequestrant cholestyramine to study the effects of reduced serum cholesterol levels on serum lipids. Cholestyramine treatment was associated with an average reduction in serum cholesterol and LDL cholesterol of 13.4 and 20.3%, respectively. There was a 19% decrease in the occurrence of coronary heart disease death or non-fatal myocardial infarction associated with cholestyramine use.

Current epidemiological and clinical data indicate a strong correlation between high serum cholesterol levels and coronary heart disease. On the basis of this evidence the National Institutes of Health (Consensus Development Conference, 1985) and American
Heart Association (1984) recommend that the people of the United States actively try to reduce their risk factors for developing cardiovascular diseases, including reduction of serum cholesterol and LDL cholesterol levels. Aggressive treatment is recommended for those with serum cholesterol levels above the 90th percentile of the population. While dietary modifications may suffice for the majority, the use of therapeutics may be needed for high risk individuals (Levy, 1980; American Heart Association, 1983; Hoeg et al., 1986).

The major pharmacological treatments of hypercholesterolemia are bile-acid sequestrants and nicotinic acid (Levy, 1980; DeGraph, 1984; Hodges & Smith, 1984). These drugs reportedly decrease cholesterol levels 10% to 15% but have unpleasant side effects (Levy, 1980). Other therapeutic agents available in the U.S. include probucol and clofibrate; clofibrate lowers triglyceride levels but has limited effect on serum cholesterol. Pantethine, a derivative of the vitamin pantothenic acid, is being studied in Italy as a hypocholesterolemic agent (Maggi et al., 1982; Angelico et al., 1983); it is marketed as a hypolipidemic agent, Pantosin (Daiichi Seiyaku Co, Tokyo, Japan), in Japan.
Pantethine and its Metabolites

Pantetheine, the sulfhydryl form of pantethine, is composed of the vitamin pantothenic acid and the disulfide of the aminothiol, cysteamine. Pantetheine functions as the working arm of Coenzyme A and acyl carrier protein; as such, pantetheine is widely involved in the metabolism of carbohydrates, fat and proteins; cholesterol synthesis directly involves pantetheine (Abiko, 1975). The sulfhydryl moiety of cysteamine is the active site of the CoA molecule.

Pantethine and Serum Cholesterol

Human and animal studies show that pantethine treatment lowers serum cholesterol levels. Cattin and colleagues (1985) compared pantethine treatment with fenofibrate, a hypercholesterolemic drug, and found a comparable hypocholesterolemic effect. Hyperlipidemic patients given pantethine showed a 12% reduction in total serum cholesterol and a 33% decrease in serum triglycerides, but no changes in HDL cholesterol levels (Angelico et al., 1983). Other researchers report similar findings with hyperlipidemic diabetic patients (Miccoli et al., 1984) and dialysis patients (Donati et al., 1986).

Recent reports indicate that pantethine alters lipid profiles in cholesterol fed rabbits. Tomikawa and
colleagues (1982) fed rabbits a diet containing 0.5% cholesterol supplemented with 1% pantethine; control rabbits received a high cholesterol diet only. Total serum cholesterol and LDL cholesterol levels of pantethine supplemented rabbits decreased significantly (72% and 58%, respectively) after 4 weeks; very low density lipoprotein cholesterol was normalized, and high density lipoprotein cholesterol increased. These results were confirmed by Carrara and colleagues (1984) who also noted a decrease in the severity of atherosclerotic lesion formation in the pantethine fed rabbits.

Comparison of pantethine treatment with the hypocholesterolemic drug, probucol, shows that both are effective in lowering serum cholesterol levels (Tawara et al., 1986). The cholesterol-lowering effect was even more pronounced when these compounds were used simultaneously.

Oral administration of pantethine (70 to 1000 mg/kg/day) to cystinotic patients did not result in detectable levels of serum pantethine up to five hours after treatment (Wittwer et al., 1985). Serum levels of pantothenic acid and cysteamine did increase with pantethine administration. Blood samples from rats taken 30 minutes after intravenous administrations of pantethine showed over 50 percent present as free
pantothenic acid (Ono et al., 1974).

The enzyme pantetheinase hydrolyzes pantethine to pantothenic acid and cysteamine (Wittwer et al., 1983; Abiko, 1975). Pantetheinase activity has been identified in horse and pig kidney, rat liver and human serum (Abiko, 1975; Dupre and Cavallini, 1979; Wittwer et al. 1983, Wittwer et al., 1985). Rapid hydrolysis of orally administered pantethine is indicated by the lack of detectable serum pantethine in humans and rat and the concomitant rise in pantethine's metabolites (i.e. pantothenic acid and cysteamine).

Statement of the Problem

Pantethine reduces serum cholesterol levels in cholesterol-fed rabbits. The rapid hydrolysis of pantethine by serum pantetheinase results in high circulating levels of pantothenic acid and cysteamine and no measurable pantethine. It is possible that one of the hydrolysis products, pantothenate or cysteamine, produces the observed reduction in serum cholesterol levels. However, there have been no reports testing pantethine's metabolites for their hypocholesterolemic effect.

Objectives

The objective of this study is to examine the effects of pantethine and its metabolites on serum
cholesterol levels in cholesterol-fed rabbits. Currently there are few drugs which effectively treat hypercholesterolemia and those drugs which are available have side effects which interfer with compliance. Pantehine and its metabolites have few known side effects. If one of pantethine's hydrolysis products is an effective hypocholesterolemic agent, it may prove useful in clinically reducing high serum cholesterol levels.
REVIEW OF THE LITERATURE

Coronary heart disease kills 550,000 Americans annually, making it the leading cause of death in the United States (Center for Disease Control, 1986). An estimated 5.4 million Americans have coronary heart disease (Levy, 1986). Depending upon the severity of coronary heart disease, the direct medical costs and indirect costs (loss of income) range from $4 to approximately $9000 per person (Oster and Epstein, 1986). Atherosclerosis is the leading cause of myocardial and cerebral infarction which are the major causes of death from coronary heart disease (Ross, 1986). The lesions of atherosclerosis are characterized by large lipid and cholesterol deposits; the cholesterol is usually found as cholesterol esters (Goldstein and Brown, 1977; Ross, 1986). This observation stimulates interest in the relationship of cholesterol to atherosclerosis and coronary heart disease.

Epidemiological Studies

The high cholesterol content of atherosclerotic lesions is only one factor linking cholesterol with heart disease. High serum cholesterol levels are a major risk factor for developing coronary heart
disease (Wilhemset al., 1973). Epidemiological studies indicate an increase in the incidence of coronary heart disease as serum cholesterol rises.

The Framingham study, begun in 1949, prospectively analyzed coronary heart disease and hypertension in a group of 5,000 subjects. Subjects received biennial follow-up examinations which included a medical history, physical and laboratory tests including serum cholesterol levels. An eight year report of the Framingham Study (Kagan et al., 1962) noted that increased serum cholesterol levels correlated with an increased incidence of coronary heart disease; this risk was most pronounced in younger participants (40 to 49 years at the beginning of the study) with total serum cholesterol levels over 260 mg/dl. High blood pressure, smoking and obesity also correlated with increased rates of cardiovascular disease.

The Seven Countries Study (Keys, 1980) involved over 10,000 men from Finland, Greece, Japan, Yugoslavia, Italy, Netherlands, and the United States. These men were followed for 10 years to assess the relationship of blood pressure, serum cholesterol, smoking, activity level, weight, pulse rate, diet and respiratory function with coronary heart disease; only blood pressure and serum cholesterol were associated with an increased incidence of coronary disease. The
relationship between these variables and coronary heart disease was direct. Serum cholesterol levels correlated significantly with the ten year death rate from coronary heart disease ($r = 0.8$) and accounted for 64% of variability among cohorts in death rate from coronary heart disease. This high correlation was also identified with non-fatal coronary heart disease ($r = 0.79$).

The Pooling Project (Pooling Project Research Group, 1978) compiled and analyzed the results of five prospective studies on coronary problems. This retrospective study involved men aged 40 - 64 years and compared coronary heart disease rates at five quintiles of serum cholesterol (from $< 194$ mg/dl to $> 268$ mg/dl). The mean serum cholesterol levels of individuals with coronary disease was significantly higher than for those who were asymptomatic. The difference in means was most extreme at younger ages (19.7 mg/dl for 40 to 44 years) and least for older groups (9.8 mg/dl for 50 to 55 years). This relationship appears curvilinear with the risk of coronary heart disease rising dramatically in the upper quintiles. However, a recent study involving over 350,000 men found that the relationship between serum cholesterol and coronary heart disease is not curvilinear but linear (Stamler et al., 1986).
Intervention Trials

Prospective studies identified high serum cholesterol levels as related to the development of coronary artery disease. Intervention studies attempt to assess the effect of varying treatments, including decreasing serum cholesterol levels, on individuals with symptomatic coronary heart disease. Recent clinical trials demonstrate the benefits of reducing serum cholesterol levels.

The National Heart, Lung and Blood Institute analyzed the effect of diet and/or drug therapy on coronary arteriosclerosis in patients with hypercholesterolemia (Brensike et al., 1984). In this double-blind study, diet alone reduced serum cholesterol levels 11%; use of cholestyramine with the diet dropped serum cholesterol levels a total of 31%. Coronary angiography of arterial lesion growth (measured by changes in lumen diameter) following five years of treatment showed that the diet group had 49% progression of coronary artery disease versus only 35% (p < 0.05) in those receiving both dietary and pharmacologic treatment. Cholestyramine decreased serum cholesterol levels and slowed the progression of coronary heart disease.

The Lipid Research Clinics Coronary Primary Prevention Trials (1984a, 1984b) analyzed the long term...
benefits of drug therapy on serum lipids using the bile acid sequestrant cholestyramine. The clinics followed 3,810 hypercholesterolemic men from the United States who were at high risk for coronary heart disease based upon serum lipid levels above the 95th percentile (265 mg/dl). All participants followed a cholesterol-lowering diet and were assigned to either a cholestyramine or placebo treatment group. After ten years the cholestyramine group averaged cholesterol reductions of 13.4%; diet alone reduced serum cholesterol levels 8.5%. While there was no difference in total deaths between treatment groups, coronary heart disease death or non-fatal myocardial infarction decreased 19% with cholestyramine use.

The Leiden Intervention Trial (Arntzenius et al., 1985) analyzed the effect of a cholesterol-lowering diet on coronary lesion growth in 39 men with stable angina pectoris. Dietary modifications resulted in a 10% decrease in cholesterol levels over a two year period. Lesion growth was assessed by angiography pre- and post-treatment. Coronary lesion growth diminished significantly during this period and correlated with decreased serum cholesterol levels.

Along with the correlation of serum cholesterol to coronary heart disease development in humans, animal studies indicate that increasing cholesterol levels coincide with the development of arterial lesions.
Rabbits and nonhuman primates show evidence of lipid accumulation in the arterial lumen beginning at seven to ten days (Poole and Florey, 1958; Silkworth et al., 1975; Faggiotto et al., 1984a; 1984b). The accumulation of lipid results in the formation of atherosclerotic plaques within 12 months of beginning the diet (Faggiotto et al., 1984b).

Although high serum cholesterol levels have not been proven to cause coronary heart disease, the correlation is strong. On the basis of current epidemiological and clinical data, the American Medical Association, National Institutes of Health and the American Heart Association (Consensus Conference, 1985; National Institutes of Health, 1985; American Heart Association, 1984) recommend that the people of the United States actively try to reduce their risk factors for developing cardiovascular diseases. The recommendations include maintaining a desirable body weight and reducing serum cholesterol levels.

While dietary modifications may suffice for the majority, the use of therapeutics may be needed for high risk individuals (Levy, 1980; Hoeg et al., 1986). Aggressive treatment is recommended for those individuals with serum cholesterol levels above the 90th percentile of the population; for men 35 years and older, 270 mg/dl represents the 95th percentile.
(Rifkind and Segal, 1983).

Bile acid sequestrants and nicotinic acid provide the major form of drug therapy for hypercholesterolemic patients (Levy, 1980; Hunninghake, 1983; DeGraph, 1984). These drugs have unpleasant side effects that may limit patient compliance. Probucol also lowers serum cholesterol levels, mainly through the reduction of LDL cholesterol levels (Levy, 1980; DeGraph, 1984). However, it is also associated with a decrease in HDL cholesterol levels.

**Pantethine and its Metabolites**

**Pantethine**

The sulfhydryl of pantethine, pantetheine, is composed of the vitamin pantothenic acid and an aminothiol, cysteamine (see Figure 1). As part of Coenzyme A (CoA), the sulfhydryl moiety cysteamine is the active site of the molecule (Majerus et al., 1965; Pugh and Salih, 1965; Abiko, 1975). Because pantetheine can be synthesized *in vitro* from pantothenic acid and cysteine, it has not been identified as an essential nutrient. As the working arm of Coenzyme A and acyl carrier protein, pantetheine functions widely in the metabolism of carbohydrates, fat and proteins. Cholesterol synthesis directly involves pantetheine (Abiko, 1975; Sabine, 1977; Stryer, 1981). Synthesis
Figure 1: Pantethine and its metabolites
of cholesterol begins with two units of acetyl CoA, and CoA involvement continues through the first three steps of cholesterol synthesis to the formation of mevalonate from hydroxymethylglutaryl CoA (Sabine, 1977; Stryer, 1981).

Pantothenic acid

In 1933 Williams et al. (1933) first described and identified an essential growth factor for yeast which they named pantothenic acid (from the Greek meaning "everywhere"). The active agent in the chick anti­dermatitis factor studied by Woolley and co-workers (1938, 1939) was also identified as pantothenic acid (Jukes, 1939). The discovery by Lipmann (1945) of a coenzyme of acetylation (CoA) and identification of pantothenate's biochemical role as part of CoA (Lipmann et al., 1947) provided a biochemical basis for pantothenic acid's biological function. Pantothenate exists in tissue in a bound form (e.g. as CoA pantethine, Acyl Carrier protein); very little is present in a free form (Novelli, 1953; Abiko, 1975). Blood and serum contain high levels of pantothenic acid (Wyse et al., 1985). Pantothenic acid in serum is present only in an unbound form, while pantothenate in erythrocytes mainly occurs in bound forms.

While pantothenic acid is an essential nutrient, its ubiquitous distribution precludes widespread,
naturally occurring deficiencies. Deficiency has been experimentally induced in animals using pantothenate-deficient diets (Unna, 1940; Schaefer et al., 1942). Pantothenic acid deficiency in humans can be caused by providing a deficient diet or a pantothenate antagonist (Bean and Hodges, 1954; Lubin et al., 1956; Hodges et al., 1957; 1959). Deficiency symptoms include decreased resistance to infection, malaise, changes in sensation such as paresthesia, irritability, alterations in insulin sensitivity and lesions of the skin, nervous system and adrenal glands (Novelli, 1953; Fox, 1984). Most of these symptoms are non-specific and are assumed to result from a wide-spread deficiency of CoA and other active forms of pantothenic acid that thus affect a variety of organ systems (Novelli, 1953; Abiko, 1975).

Cysteamine

Cysteamine, the sulfhydryl moiety of pantethine, has no known nutritional significance. Cysteamine is effective in treating acetaminophen overdose (Hamlyn et al., 1981). Cysteamine and its disulfide, cystamine, also afford short term protection against ionizing radiation such as x-rays (Bacq et al., 1953; Bacq and Alexander, 1964). This protective effect occurs only when administered prior to exposure. Eldjarn and
Nygaard (1954) found that the period of effectiveness corresponds to peak serum cysteamine/cystamine concentrations following administration. Serum cysteamine concentration rises rapidly following oral administration and returns almost to normal after 2 hours (Eldjarn and Nygaard, 1954). The radioprotective action of thiols appears to be the result of rapid formation of mixed disulfides with proteins (Eldjarn and Pihl, 1956).

Cysteamine (300 mg/kg) is used experimentally to induce duodenal ulcers in rats (Selye and Szabo, 1973; Man et al., 1984); a dose of 1 gm/kg in rats results in a 70% mortality rate within 48 hours (Selye and Szabo, 1973). While the exact mechanism of ulcer formation is unknown, cysteamine administration is known to increase gastric acid output, pepsin activity, serum gastrin and to decrease gastric emptying time while altering the duodenal mucosa (Man et al., 1984).

Cysteamine is used clinically to treat cystinosis. Cystinosis is a rare inherited disease characterized by cellular accumulation of free cystine (Schneider and Schulman, 1983; Rosenberg and Scriver, 1974). Deposition of cystine crystals in the eyes, bone marrow, leukocytes and soft tissues results in growth retardation, vitamin D-resistant rickets, anemia, photophobia and renal failure; prior to dialysis, most children with cystinosis died of renal failure.
Cysteamine/cystamine depletes cystinotic cells, both in vivo and in vitro, of free cystine (Thoene et al., 1976; Yudkoff et al., 1981; Da Silva et al., 1985). Disulfide interchange of cysteine-cysteamine has been proposed as a depletion mechanism (Gahl and Bercu, 1985; Gahl et al., 1985b).

Rashes, lethargy, hyperthermia and hepatotoxicity are reported side effects of cysteamine treatment in cystinosis (Corden et al., 1981; Avner et al., 1983). The report of hepatotoxicity is limited to a single case and may not be due to cysteamine treatment but rather to hepatic veno-occlusive disease that occurs with long-standing cystinosis (Gahl et al., 1983). The other adverse reactions occurred only with initial administration of high doses of cysteamine, and in all patients cysteamine was successfully readministered (Corden et al., 1981). Despite concerns about the possible side effects of cysteamine there have been few reports of adverse side effects at doses of 3 - 90 mg/kg (Yudkoff et al., 1981; Da Silva et al., 1985; Schneider, 1985).

**Pantethine and Cholesterol**

Pantethine has been reported to alter lipid profiles in cholesterol fed humans and rabbits. The cholesterol-lowering effects of pantethine have been demonstrated in hyperlipidemic patients. Maggi and
colleagues (1982) studied the effect of pantethine treatment on hyperlipidemic patients who had showed limited response to low fat, low cholesterol diets. These patients complied with their diets and received 900 mg of pantethine daily; there was no control group. Treatment was associated with a 20% decrease in total serum cholesterol levels after 90 days (p < 0.01).

Angelico and partners (1983) fed hyperlipidemic patients on a low fat diet for two weeks (baseline), and then provided either 1200 mg pantethine or a placebo to each patient (patients continued on the diet). The cross-over design included two 30-day test periods (placebo and pantethine). Pantethine treatment resulted in a 12% reduction in total serum cholesterol (p < 0.01 versus baseline levels, and a 33% decrease in serum triglycerides, but no changes in HDL cholesterol levels.

Cattin and colleagues (1985) compared pantethine treatment with fenofibrate, a clofibrate analogue. Two groups of hypercholesterolemic patients received either fenofibrate or pantethine (900 mg/day) for 4 months; all patients consumed low fat diets. Both pantethine and fenofibrate resulted in a significant (p < 0.001) decrease in serum cholesterol levels after eight and 16 weeks of treatment. Serum cholesterol levels decreased 16 percent after 16 weeks of pantethine treatment versus 20% with fenofibrate. LDL cholesterol also
decreased significantly while HDL cholesterol remained unchanged.

Both diabetes and kidney failure are associated with increased cholesterol levels. Pantethine treatment decreases serum cholesterol levels in hyperlipidemic diabetic patients (Miccoli et al., 1984) and dialysis patients (Donati et al., 1986) treated with pantethine. In none of these studies were any adverse effects of pantethine treatment noted.

Studies with rabbits also indicate that pantethine lowers serum cholesterol levels. Tomikawa and co-workers (1982) fed three groups of rabbits diets of a) standard diet b) high cholesterol diet with 5% cholesterol) or c) the high cholesterol diet with 1% pantethine. Fasting blood samples were analyzed for lipid content using a density gradient. Total serum cholesterol and low density lipoprotein (LDL) cholesterol levels of pantethine supplemented rabbits began to decrease significantly after 10 days on the test diet. Cholesterol and LDL cholesterol levels reached their lowest levels (74% and 79%, respectively) after 14 days; these levels were maintained throughout the remaining 14 days of treatment. However, cholesterol levels in pantethine treated rabbits remained about four times those of control rabbits (103.5 mg/dl for the pantethine treated group vs 27.6
mg/dl for the high cholesterol group at day 14). Very low density lipoprotein cholesterol decreased significantly (p < 0.01) and high density lipoprotein cholesterol increased (p < 0.05).

Carrara and colleagues (1984) confirmed these results and also noted a decrease in the severity of atherosclerotic lesion formation in the pantethine fed rabbits. New Zealand rabbits were placed into treatment groups similar to those used by Tomikawa; fasting blood samples were analyzed for cholesterol using enzymatic methods. In addition, samples of aortic tissue were studied for atherosclerotic lesion formation. While pantethine was found to lower serum cholesterol levels significantly (p < 0.01) when compared with cholesterol only rabbits, this decrease was not significant until after 65 days of treatment and was about 50% of cholesterol only rabbits. After 90 days of treatment, pantethine resulted in a 65% decrease in total cholesterol levels (370 mg/dl vs 1050 mg/dl). Visual analysis of aortic and coronary artery tissue indicated a lowering of lesion formation in pantethine fed rabbits, but total cholesterol content of these tissues did not differ from those of the cholesterol only group.

A recent study analyzed the dose-response effect of pantethine, compared pantethine treatment with the hypocholesterolemic drug probucol, and studied the
effect of combined treatment of probucol and pantethine on the serum cholesterol levels of hypercholesterolemic rabbits (Tawara et al., 1986). Pantethine was as effective as probucol in reducing serum cholesterol levels; probucol was associated with a decrease in HDL cholesterol levels while pantethine was not. Pantethine exhibited a dose-response relationship with serum cholesterol levels with reductions of 25%, 32% and 70% (0.25%, 0.5% and 0.75% pantethine supplementation, respectively) at nine weeks. Combined pantethine and probucol treatment resulted in lower cholesterol levels than either drug achieved separately and resulted in higher HDL levels than did probucol treatment alone.

Studies on fibroblasts show a reduction in cholesterol synthesis with pantethine. Ranganathan and colleagues (1982) incubated human skin fibroblasts with pantethine; incorporation of radiolabeled acetate or mevalonolactone into sterols was measured. Pantethine incubation resulted in a 50% to 80% decrease in cholesterol synthesis (as measured by uptake of radiolabeled precursors) and a simultaneous increase in intermediate methyl sterols; the magnitude of pantethine's effect was dose related. Other sulfur-containing compounds (dithiothreitol, glutathione, CoA, and cystine) did not reduce cholesterol synthesis.
The mechanism of pantethine's hypercholesterolemic action is unknown. Because of pantethine's role in Coenzyme A and acyl carrier protein, changes affecting lipid metabolism and cholesterol levels may occur through alterations involving these compounds.

However, pantetheine is hydrolyzed to pantothenic acid and cysteamine by a specific enzyme, pantetheinase, (Wittwer et al., 1983; Abiko, 1975) and may not remain intact long enough to result in a therapeutic effect.

Pantetheinase is specific for pantetheine and is inhibited by high concentrations of pantothenate (Abiko, 1975; Wittwer et al., 1983). Pantetheinase has been purified from horse kidney (Dupre and Cavallini, 1979), rat liver and kidney (Abiko, 1975), and pig kidney (Wittwer et al., 1983). Pantetheine-hydrolyzing activity was noted in rat and human intestinal tissue (Wittwer et al., 1985). The level of pantetheinase activity in intestinal tissue appears to be sufficient to ensure nearly complete hydrolysis of pantethine during absorption (Shibata et al., 1983; Wittwer et al., 1983).

Evidence of plasma pantetheine-hydrolyzing activity exists in rats and humans (Ono et al., 1974; Wittwer et al., 1985). Blood samples from rats taken 30 minutes after intravenous administrations of pantethine showed over 50% present as free pantothenic acid, indicating
rapid hydrolysis of the injected dose. In cystinotic patients fed pantethine (70 - 1000 mg/kg/day), no serum pantethine was detected at any time after administration. However, serum levels of pantothenic acid and cysteamine increased dramatically (Wittwer et al., 1985). The authors also noted a 14% drop in serum cholesterol levels after two weeks of pantethine treatment.

The rapid hydrolysis of pantethine by pantetheinase leads to speculation that one of the hydrolysis products, rather than pantethine itself, may be the active hypercholesterolemic compound.
METHODOLOGY

Pantethine reduces serum cholesterol levels in both cholesterol-fed animals and hypercholesterolemic humans. Pantethine is rapidly hydrolyzed to its component metabolites, pantothenic acid and cysteamine, by the enzyme pantetheinase. It is hypothesized that one of these metabolites rather than pantethine produces the reported hypocholesterolemic effect. This study is designed to compare the effect of pantethine with the effects of pantothenic acid and cysteamine on serum cholesterol levels in hypercholesterolemic rabbits. To provide easy comparison, the experimental design is similar to that used by Tomikawa and colleagues (1982) in researching the effect of pantethine in experimentally hypercholesterolemic rabbits.

Experiment 1: Comparison of Pantethine, Cystamine and Pantothenic Acid on Serum Cholesterol in Cholesterol-Fed Rabbits

Fourteen male New Zealand white rabbits weighing 2.0 to 2.5 kg (4 to 6 weeks) were ordered from local suppliers. The number of rabbits in the study was limited by the space available at the Utah State University Lab Animal Research Center.
All rabbits were fed a high cholesterol diet. The cholesterol only group served as a control for cholesterol levels; the pantethine group served as reference group to compare the effect of pantothenic acid and cystamine. The rabbits were placed in one of four treatment groups: cholesterol, pantethine, pantothenic acid, and cystamine (the disulfide of cysteamine). The rabbits were divided into treatment groups by taking the four rabbits with the highest pre-treatment serum cholesterol levels (individual serum cholesterol level reported in Appendix 1) and randomly assigning them to one of the four treatment groups. This procedure was repeated with the rabbits having the four lowest serum cholesterol levels; the remaining rabbits were then randomly assigned to treatment groups.

Diets were custom made by ICN Nutritional Biochemicals (Cleveland, OH) and consisted of Purina Rabbit Chow with 0.5% cholesterol (supplied by ICN Biochemicals), 1% by weight pantethine or an equimolar amount of cystamine, or pantothenic acid (all purchased from Sigma Chemical Co., St. Louis, MO.). The rabbits were fed 150 g of food daily and residual food (if any) was weighed; water was supplied ad lib. Rabbits were housed and cared for in the Lab Animal Research Center.

Fasting blood samples were drawn weekly for analysis of total serum cholesterol levels. Serum
cholesterol levels were measured by the colorimetric method of Allain and co-workers (1974). This enzymatic procedure couples the hydrolysis and oxidation of cholesterol esters with a chromogenic system to produce a dye with a maximum absorbance at 500 nm. This determination employs the following reactions:

1) Cholesterol Esters $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty Acids

2) Cholesterol + $O_2$ $\xrightarrow{\text{Cholesterol Oxidase}}$ Cholest-4-en-3-one + $H_2O_2$

3) $2H_2O_2$ + 4-Aminoantipyrine + p-Hydroxybenzene-sulfonate $\xrightarrow{\text{Peroxidase}}$ Quinoneimine Dye + $4H_2O$

All reagents and standards were purchased as a kit from Sigma Chemical Co. (Cleveland, OH). The cholesterol assay is outlined in Table 1. Absorbance was measured using a Gilford 240 spectrophotometer.
Table 1: Procedures for total serum cholesterol assay.

<table>
<thead>
<tr>
<th>Reagent</th>
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<tr>
<td>dH$_2$O</td>
<td>10 ul</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>standard</td>
<td>--</td>
<td>10 ul</td>
<td>--</td>
</tr>
<tr>
<td>sample</td>
<td>--</td>
<td>--</td>
<td>10 ul</td>
</tr>
<tr>
<td>Cholesterol Reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

Mix and incubate at 37°C for 10 minutes.
Read $A_{500}$ using the blank as a reference.

Total serum cholesterol was calculated using the following formula:

$$\text{Total cholesterol (mg/dl)} = \frac{A_{500 \text{test}}}{A_{500 \text{standard}}} \times [\text{standard}]$$

In this study, the relationship between the absorbance at 500 nm and total serum cholesterol showed a linear relationship up to a concentration of 800 mg/dl.

Serum lipoprotein separation was performed prior to treatment and after 5 weeks. The separation was carried out using the density gradient method of Hinton et al. (1974); this method does not separate high density lipoproteins.

Chylomicrons were removed after centrifuging at
15,000 r.p.m. for 180 minutes (Nelson, 1972) using a Beckman model J-21C preparative centrifuge and a JA-20 rotor (Beckman Instrument Co., Palo Alto, CA).

Serum samples were adjusted to a density of 1.22 by adding 0.32 g/ml of solid NaBr. Three stock solutions were prepared as follows: A) 11.4 g NaCl + 0.1 g EDTA/L, d = 1.003; B) 263 g NaBr + 11.4 g NaCl + 0.1 g EDTA/L, d = 1.205; C) 582 g NaBr/L, d = 1.424. All chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI.). To form the density gradient, 0.7 ml of solution C was placed in a 12.6 ml nitrocellulose ultracentrifuge tube (Beckman Instrument Co.) and 0.7 ml of density adjusted plasma was layered on top. Linear density gradients were formed using 5.6 ml each of solutions A and B.

The density gradients were centrifuged at 30,000 r.p.m. for 2 hours in a Beckman model L3-50 preparative ultracentrifuge using a SW-41 swinging bucket rotor.

The density gradients were analyzed using a Beckman Fraction Recovery System and Isco absorbance monitor model UA-4 (Isco Instrument Specialties Co., Lincoln, NE.) at $A_{240}$. There was no quantitative analysis performed on the separated fractions.
Experiment 2: Comparison of Pantethine, Cystamine, Cysteamine, and 2-Hydroxyethyl Disulfide on Serum Cholesterol in Cholesterol-Fed Rabbits

A second experiment was performed to compare the effects of other low-molecular weight thiols on serum cholesterol levels in hypercholesterolemic rabbits. The disulfide forms of the following thiols were used: cysteamine (cystamine), cysteine (cystine), 2-mercaptoethanol (2-hydroxyethyl disulfide); all chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) and are shown in Figure 2.

Figure 2: Structures of cysteamine, cysteine, and 2-mercaptoethanol.

The experimental design was similar to that used in the first experiment. Sixteen male New Zealand White rabbits weighing 1.8 to 2.0 kg (4 to 6 weeks) were obtained from R & R Rabbitry (Stanwood, WA). The
rabbits included four sets of littermates. The rabbits were divided into groups so that there was one member of each litter per group. Baseline cholesterol levels were obtained on each rabbit.

Four treatment groups, with four rabbits per group, were established as follows: cholesterol, cystamine, cystine, 2-hydroxyethyl disulfide. Treatment groups were fed a 0.5% cholesterol diet supplemented with molar equivalents of one of the test compounds at levels equal to 1% pantethine. Total serum cholesterol levels were analyzed using the assay previously described; serum cholesterol concentrations were calculated from a standard curve run with the serum samples. Lipoprotein separation was not performed.

**Analysis of Data**

Weekly, individual cholesterol levels were averaged within treatment groups. Analysis of variance to test for significance among mean treatment cholesterol levels was conducted weekly using Minitab Statistical Package (Pennsylvania State University, University Park, PA) according to the following model:

\[
Y_{ij} = T_i + e_{ij}
\]

where \( Y_{ij} \) is cholesterol level for each observation, \( T_i \) is the variation associated with each treatment, and
$e_{ij}$ is variation due to individual differences.
For any week in which significant differences among means were found, treatment means were analyzed for least significant differences using the Fisher Least Significant Difference test (Ott, 1977) to determine which specific treatments affected serum cholesterol levels.
RESULTS

The purpose of this study was to compare the effect of pantethine and its metabolites, pantothenic acid and cysteamine, on serum cholesterol levels in hypercholesterolemic rabbits. The results of a second experiment comparing the effects of small thiols on serum cholesterol levels are also reported.

Experiment 1: Comparison of Pantethine, Cystamine and Pantothenic Acid on Serum Cholesterol in Cholesterol-Fed Rabbits

All experimental diets were well tolerated, with each group consuming an average of 150 gm/day; this provided 750 mg cholesterol per day and 1500 mg pantethine, 600 mg cystamine or 1300 mg pantothenic acid daily. All rabbits gained weight during the test period. Analysis of variance on the serum cholesterol levels of treatment means showed a significant difference (p < 0.005) after three weeks of supplementation (Figure 3). Treatment means were analyzed for least significant differences. This analysis indicated that cystamine and pantethine supplemented groups were significantly different from the cholesterol only and pantothenic acid groups (p < 0.05). Serum cholesterol levels in the pantethine and
Figure 3: Total serum cholesterol response of cholesterol-fed rabbits to pantethine and its metabolites. (*, p < 0.05; **, p < 0.005).
cystamine fed rabbits were reduced an average of 21% and 30%, respectively when compared to the cholesterol only group (Table 2).

Table 2: Total serum cholesterol levels (mg/dl) by treatment group--Experiment 1 (mean ± standard deviation).

<table>
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<tr>
<th>Week</th>
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<th>Pantothenate</th>
<th>Cystamine</th>
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<td></td>
<td>X</td>
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<td>X</td>
<td>SD</td>
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<tr>
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<td>18</td>
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<td>76</td>
<td>778</td>
<td>186</td>
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<tr>
<td>5</td>
<td>1121</td>
<td>81</td>
<td>864</td>
<td>205</td>
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Serum lipoprotein separation indicated a marked increase in the level of very low density, intermediate density and low density lipoproteins with cholesterol feeding; the increase in intermediate density lipoproteins was most pronounced. An example of the changes seen in serum lipoprotein fractions pre- and post treatment is presented in Figure 4. There appeared to be no noticeable difference in lipoprotein profiles associated with any treatment.
Figure 4: Pre- (………) and post-treatment (——) serum lipoprotein profiles of cholesterol treated rabbits.

Experiment 2: Comparison of Cystamine, Cystine, and 2-Hydroxyethyl Disulfide on Serum Cholesterol

The significant reduction in serum cholesterol levels with cystamine supplementation led to speculation regarding the specificity of this thiol in reducing serum cholesterol levels. A second experiment was conducted comparing cystamine with the disulfides of two structurally similar, low-molecular weight thiols, cystine and 2-hydroxyethyl disulfide.
The 2-hydroxyethyl disulfide diet was not as well-accepted as the other diets. In order to avoid any differences in serum cholesterol levels due to food intake, all groups were fed to match the intake of the 2-hydroxyethyl group. The average intake was 110 gm/day, resulting in a total of 550 mg cholesterol daily and either 450 mg cystamine, 480 mg cystine or 300 mg of 2-hydroxyethyl disulfide. This intake level resulted in a 27% decrease in cholesterol and supplement intake compared with that of experiment 1. There were no adverse effects noted from any of the diets.

None of the test compounds significantly reduced serum cholesterol levels, nor did cystamine treatment significantly reduce cholesterol levels. There was considerable variation within the groups that may have made achieving significance difficult (Table 3, Appendix 2).
Table 3: Total serum cholesterol levels (mg/dl) by treatment group—Experiment 2 (mean ± standard deviation).

<table>
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<tr>
<th>Week</th>
<th>Cholesterol X</th>
<th>SD</th>
<th>Cystamine X</th>
<th>SD</th>
<th>Cystine X</th>
<th>SD</th>
<th>2,Hydroxyethyl X</th>
<th>SD</th>
</tr>
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<td>60</td>
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<td>60</td>
<td>4</td>
<td>78</td>
<td>49</td>
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<tr>
<td>1</td>
<td>254</td>
<td>186</td>
<td>249</td>
<td>290</td>
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<td>141</td>
<td>308</td>
<td>183</td>
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<td>545</td>
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<td>610</td>
<td>223</td>
<td>750</td>
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<td>179</td>
<td>1294</td>
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<td>1548</td>
<td>211</td>
</tr>
</tbody>
</table>

The serum cholesterol levels of the control group were similar to those of experiment 1 after five weeks of treatment; however, at week five, the cystamine group had serum cholesterol levels 20% higher than in experiment 1. Despite the lack of significant reductions, cholesterol levels in the cystamine treated group tended to be lower than for the other groups (Figure 5). There was no significant difference in serum cholesterol levels among the litters (Figure 6).
Figure 5: Total serum cholesterol response of cholesterol-fed rabbits to cystamine, cystine, and 2-hydroxyethyl disulfide.
Figure 6: Total serum cholesterol response of cholesterol-fed rabbits by litters—Experiment 2.
DISCUSSION

Many researchers have identified pantethine as an effective hypocholesterolemic agent; however, there have been studies reported in which pantethine did not significantly reduce serum cholesterol levels. A study comparing pantethine to tiadenol (a hypocholesterolemic drug) treatment found that pantethine treatment did not reduce total serum cholesterol nor LDL cholesterol levels in 30 hypercholesterolemic patients (Da Col et al., 1984).

Rubba and colleagues compared the effectiveness of short-term (1 month) treatment of pantethine versus fenofibrate (1985). They found that while fenofibrate treatment resulted in significant reductions in serum cholesterol levels, pantethine treatment lowered serum cholesterol levels a non-significant 4%.

Other researchers have noted significant decreases with pantethine treatment of some serum lipid fractions, namely triglycerides and VLDL cholesterol, but not in total serum cholesterol (Maggi et al., 1982).

The cholesterol-lowering effect of pantethine in cholesterol-fed rabbits was confirmed in this study, although the reductions were considerably less than those reported by Tomikawa et al. (1982). However, in
experiment 1, cystamine, a metabolite of pantethine, provided a reduction of serum cholesterol levels similar to that of pantethine. Pantothenate did not lower serum cholesterol levels. The second experiment was inconclusive regarding the specificity of cysteamine's action.

In experiment 2, cysteamine did not have the same hypocholesterolemic response as in experiment 1. This may be due to different chemical sources (Sigma vs. Aldrich) or to the wide variation within treatment groups. It is also possible that the decreased food intake in experiment 2, and the concomitant decrease in cystamine intake, may have lowered the given dose to a non-therapeutic level. Figure 7 compares the average serum cholesterol levels of rabbits fed 750 mg cholesterol/day (experiment 1) versus 550 mg cholesterol/day (experiment 2) averaged across the cholesterol only and cystamine groups during the first 5 weeks of treatment. These two treatments occurred in both experiments and provide an indication of the response of serum cholesterol levels to changes in the amount of cholesterol consumed (750 mg vs. 550 mg).
The response to any drug is dependent upon dose. If the level is too low, there is no response; if it is too high, there is an unwanted or toxic response (Roberts, 1982; Gibaldi, 1984). The effective dose is based upon many interrelated variables of pharmacodynamics and pharmacokinetics (Gibaldi, 1984). Some hypocholesterolemic drugs, such as cholestyramine, show a graded response beginning at even low doses (DeGraph, 1984). However, nicotinic acid only begins

Figure 7: Weekly total serum cholesterol levels of rabbits fed 750 mg cholesterol/day (Experiment 1) or 550 mg cholesterol/day (Experiment 2).
to effectively reduce cholesterol levels at 50 - 500 times the recommended vitamin requirements (Hodges and Smith, 1984).

Pantethine treatment has shown a dose-response relationship in some studies. Tawara and colleagues (1986) compared the effect of varying doses of pantethine on serum cholesterol levels and found that the response was dose related. A dose of 0.25% pantethine resulted in a 25% decrease in serum cholesterol levels versus a 32% decrease at a dose of 0.5%. Ranganathan et al. (1982) found a similar relationship for pantethine on cholesterol synthesis of human skin fibroblasts.

The hypocholesterolemic effect of pantethine has been attributed to alterations in CoA levels. CoA shunts acetate molecules into either the tricarboxylic acid cycle (TCA cycle) for oxidation or into lipid and sterol synthesis (Maggi et al., 1982; Gaddi et al., 1984). If increased CoA preferentially shifted acetate into the TCA cycle and away from lipid and cholesterol synthesis, then cholesterol levels would be lowered. CoA can be synthesized from pantothenic acid; or pantetheine, through direct phosphorylation, can bypass the first three steps in CoA biosynthesis and rapidly increase CoA levels in vitro (Shimizu and Abiko, 1965; Abiko, 1975). In view of the presence of pantetheinase
activity in serum, pantethine may not enter the CoA biosynthetic pathway intact; it may be hydrolyzed to pantothenic acid prior to CoA biosynthesis. However, in this study pantothenic acid had no effect on serum cholesterol levels, indicating some mechanism other than increased levels of CoA may be responsible for the hypocholesterolemic effect of pantethine.

The mechanism of pantethine's effect on serum cholesterol is unknown. Shinomiya and co-workers (1980) found that cholesterol esterase activity increased in rat arterial walls on a high cholesterol, pantethine supplemented diet when compared with an unsupplemented diet. Cholesterol esterase hydrolyzes cholesterol esters, the cellular storage form of cholesterol, following binding to lipoprotein receptors and uptake by cells. Increasing cholesterol esterase activity helps remove circulating cholesterol and decrease serum cholesterol levels.

Pantethine inhibits cholesterol synthesis in vitro. Incorporation of radiolabeled acetate into cholesterol markedly decreases in human skin fibroblasts incubated with pantethine (Ranganathan et al., 1982). The inhibition occurs in the conversion of lanosterol to cholesterol; there are several intermediate steps in this conversion and the exact point of inhibition is unknown. The effect of other sulfur-containing compounds (dithiothreitol, glutathione, and cystine)
were compared with pantethine and had no effect on cholesterol biosynthesis; cystamine was not tested.

The hypocholesterolemic effect of pantethine and cysteamine may result from the effect of thiol:disulfide exchanges on enzyme systems. Ziegler (1985) states that thiol:disulfide exchange may alter enzyme activity and serve to regulate enzyme activity. One enzyme system in which this mechanism appears to work is 3-hydroxy-3-methylglutaryl-CoA (HMGCoA) reductase. HMGCoA catalyzes the first committed step in the biosynthesis of cholesterol by reducing HMGCoA to mevalonate. Gilbert and Stewart (1981) found that thiols such as HMGCoA and CoA rapidly inactivate HMGCoA reductase. Activity can be restored with dithiothreitol. Dithiothreitol inhibits disulfide formation, so these researchers speculate that disulfide formation may be responsible for inactivation of HMGCoA reductase. The effect of cystamine on HMGCoA reductase has not been studied.

Familial dysbetalipoproteinemia (familial Type III lipoproteinemia) is characterized by increased levels of very low density and intermediate density lipoproteins (Havel et al., 1980). Clearance of chylomicrons and very low density lipoproteins is impaired and leads to an increase in cholesterol-rich remnant particles (Hazzard and Bierman, 1976; Chait et
al., 1978; Havel, 1982). Cholesterol-fed rabbits, including the cholesterol treated rabbits in this study, exhibit similar increases in very low density and intermediate density lipoproteins (Shore et al., 1974; Kushwaha and Hazzard, 1978). Ross and Zilversmit (1977) found that increased chylomicron remnants constituted the majority of these lipoproteins.

While cholesterol-fed rabbits exhibit a lipoprotein profile similar to that in Type III hypercholesterolemia, the mechanism of increased cholesterol levels differ. Hypercholesterolemic rabbits result not from alterations in apoprotein E, but from saturation of hepatic lipoprotein receptors and a simultaneous decrease in the number of hepatic lipoprotein receptors (Kita et al., 1981; Kovanen et al., 1981). The mechanism of the hypocholesterolemic observed in rabbits may not be the same as that observed in humans.

Humans with familial dysbetalipoproteinemia (familial Type III hyperlipidemia) possess a variation in apolipoprotein E which is needed for recognition of remnant particles by hepatic lipoprotein receptors. The alteration in apoprotein E reduces recognition, binding and uptake of very low density lipoprotein remnants by hepatic cells and results in greatly increased serum cholesterol levels (Havel, 1982).

The varient apoprotein E in Type III
hyperlipoproteinemia, i.e apolipoprotein E2, results from a cysteine for arginine substitution at residues 112 and 158 (Weisgraber et al., 1981). Another form, apoprotein E3, has one cysteine for arginine substitution at residue 112. These substitutions result in altered lipoprotein charges and are associated with decreased in binding to LDL receptors (Schneider et al., 1981), with apoprotein E2 showing the most dramatic decrease in binding capacity.

Cysteamine and cystamine have been proposed as hypocholesterolemic agents for patients with familial dysbetalipoproteinemia (Fisher and Gahl, 1982; Weisgraber et al., 1982; Hui et al., 1984; Innerarity et al., 1984; Gahl et al., 1985a). Cysteamine and cystamine alter the charge on apolipoprotein E in vitro by forming a mixed disulfide with cysteine and converting it to a lysine analogue (Fisher and Gahl, 1982; Weisgraber et al., 1982; Rall et al., 1983). Cysteamine treatment increases LDL receptor binding activity but does not return binding capacity to normal levels. Innerarity et al. (1984) confirm the increased receptor binding of apoprotein E2 with cysteamine treatment but note that the increased activity is retained following reversal of the charge modification. Positive charge may not be the only cause of improved binding activity.
Alteration of defective apolipoproteins with cysteamine in serum from Type III hyperlipidemic patients suggests a possible mechanism for cysteamine's hypolipidemic effect in rabbits, but, to date, the effect of cysteamine treatment in patients with Type III hyperlipoproteinemia or with other types of lipid disorders has not been studied.

Another possible mechanism for cystamine's hypocholesterolemic action is through alterations in hormone regulation. Cystamine treatment in rats is associated with alterations of some hormones. Cysteamine depletes tissues of somatostatin (Seiler et al., 1983; Szabo and Reichlin, 1985) and lowers the noradrenaline of the hypothalmus in rats (Vecsei et al., 1985). Cystinotic children receiving cysteamine show altered prolactin response (Millard et al., 1982).

Conclusions

Pantethine and cystamine appear equally effective at lowering serum cholesterol levels in hypercholesterolemic rabbits. Current research on the use of pantethine has not considered its metabolites as possible hypocholesterolemic agents. In view of results presented in this study, previous research designs should be repeated on animals and hyperlipidemic patients using cystamine as one test agent. Of particular interest would be studies
involving the effect of pantethine and cystamine treatment in patients with Type III hyperlipoproteinemia. The possibility of a dose-related response for cystamine also needs further testing.

Possible cholesterol-lowering mechanisms for cysteamine include inhibition of HMGCoA, alterations in apoprotein levels or binding capacity, or changes occurring through endocrine regulation.

Cysteamine's major physiological actions result from its ready ability to form mixed disulfides with many compounds. Future research into cystamine's hypocholesterolemic action might focus on the formation of disulfides as a possible mechanism.
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APPENDICES
Appendix 1: Serum Cholesterol Levels of Individual Rabbits by Group--

Experiment 1.

### Cholesterol Only Group

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### Pantethine Group

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Appendix 2: Serum Cholesterol Levels of Individual Rabbits by Treatment Groups—Experiment 2.

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