CONTRASTING EFFECTS OF ETHANE-1-HYDROXY-1,1-DIPHOSPHONATE (EHDP) ON THE REGRESSION OF TWO TYPES OF DIETARY-INDUCED ATHEROSCLEROSIS

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Summary

Atherosclerosis was induced in White Carneau pigeons by feeding cholesterol either continuously or intermittently during a 14-month period. Animals were then treated daily with either saline, or 0.5 or 2.5 mg EHDP/kg body wt during which time a cholesterol-free "regression" diet was fed. Subgroups of pigeons were studied after four and eight months of treatment. After the progression period, aortas and brachiocephalic arteries of those pigeons that were subjected to intermittent hypercholesterolemia accumulated more cholesterol, were more complicated, and showed a different pattern of lesion regression than arteries in animals continuously hypercholesterolemic. EHDP treatment had no effect on plasma cholesterol, triglyceride, calcium, or phosphorus concentrations. In animals with atherosclerosis induced by intermittent hypercholesterolemia, EHDP treatment resulted in a reduction in intimal plaques in the aorta and brachiocephalic arteries while in animals with atherosclerosis induced by continuous hypercholesterolemia EHDP treatment resulted in more extensive intimal plaques. In animals fed cholesterol intermittently and treated with EHDP, aortic cholesterol concentrations were lower; but animals with atherosclerosis induced by continuous cholesterol feeding and treated with EHDP had higher aortic cholesterol concentrations than controls. Among pigeons with atherosclerosis augmented by intermittent cholesterol feeding, there was increased mineralization during regression and this complication was found to be prevented by EHDP treatment. No effect was seen on collagen concentrations in either the intermittent or the continuous cholesterol fed animals. The beneficial effect of EHDP on the more advanced atherosclerosis in the pigeons fed cholesterol intermittently may be due to an action on lipid-

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calcium-connective tissue complexes within the atherosclerotic plaque. The opposite effect of EHDP in pigeons with relatively uncomplicated atherosclerosis induced by continuous cholesterol feeding is less easily understood, but a possible role implicating lysosomes is suggested.

Key words: Atherosclerosis — Atherosclerosis regression — Ethane-1-hydroxy-1,1-diphosphonate — Pigeons

Introduction

On the basis of recently published observations on atherosclerotic lesion regression, important questions have arisen concerning whether the potential for regression by diet and/or drug therapy is the same for all types of lesions. Most inferences to the human atherosclerotic plaque have been made by evaluating lesions induced by continuous hypercholesterolemia during the progression period followed by a period of normocholesterolemia during “regression” [1-3]. We have reported previously on the differences in atherosclerotic plaques of pigeons induced by continuous or intermittent hypercholesterolemia [4]. Continuous hypercholesterolemia induced relatively uncomplicated plaques while plaques induced by intermittent hypercholesterolemia were more complicated as evidenced by increased concentration of cholesterol ester, calcium, and collagen. Differences were also noted in potential for regression with the more uncomplicated lesions undergoing a greater amount of regression. The purpose of this report is to describe the contrasting effects of a drug, ethane-1-hydroxy-1,1-diphosphonate (EHDP), on the regression of atherosclerosis induced by either continuous or intermittent hypercholesterolemia.

Several recent studies have provided evidence that EHDP, a synthetic phosphonate, which regulates calcium and phosphate metabolism may affect atherosclerosis. The compound has been reported to be effective in inhibiting arterial mineralization and thromboarteritis in rabbits [5]. In other studies, less extensive and less severe atherosclerosis has been reported in EHDP-treated rabbits [6,7]. The reported characteristics of EHDP indicated to us the suitability of selecting this compound to compare drug effects on uncomplicated as opposed to relatively complicated atherosclerosis.

Materials and methods

The animals used in this study were obtained from the breeding colonies of Bowman Gray School of Medicine Arteriosclerosis Research Center and housed at the Center's Research Farm. Three hundred White Carneau pigeons of either sex and 6-10 months of age were divided randomly into three groups. Two groups were fed an atherogenic diet * for eight months. The method in which the atherogenic diet was fed differed between these two groups. During a 14-month period, one group was fed the diet intermittently for two-month periods

* Purina pigeon pellets : lard : cholesterol (weight ratio: 89.5/10.0/0.5).
interrupted by control diet (Purina Pigeon Pellets) for two month periods. The other group was fed the atherogenic diet continuously for the last eight months of the 14-month period while control diet was fed during the first six months. A third group of animals was fed a control pellet diet to assess the effects of age and naturally-occurring atherosclerosis in the White Carneau pigeon.

At the end of the 14-month period for progression of diet aggravated atherosclerosis each of the three main dietary groups, the "intermittent-cholesterol fed", the "continuous-cholesterol fed", and the "control" groups were divided into four subgroups with the conscious bias of equalizing plasma cholesterol concentrations and male : female ratios among groups. One subgroup from each of the three dietary groups was used to evaluate the atherosclerosis developed during the progression period and to serve as a basal or baseline observation for the regression period. The animals comprising the remaining subgroups were fed control diets during an atherosclerosis lesion regression period.

EHDP treatment was begun at the start and continued throughout the entire regression period. The drug was administered daily by injections into the pectoralis muscle. Within each of the main dietary groups, the subgroups of animals received either saline, about 0.5 (0.124 mg P/kg) or 2.5 mg EHDP/kg (0.62 mg P/kg). The effect of EHDP on lesion regression was evaluated by examining animals at four and at eight months of treatment.

**EHDP preparation**

The EHDP * was in the form of disodium salt (97.2% Na₂EHDP). The impurities included phosphite (0.34% as H₃PO₃), phosphate (0.03% as H₃PO₄) and water (2.0%). A stock solution for each dosage was made so that the injection volume was 0.2 ml per bird. Each solution was adjusted for phosphorus concentration, prepared in sterile saline and adjusted to pH 7.4 with 1 N NaOH.

**Plasma determinations**

Plasma cholesterol and triglyceride concentrations were determined monthly during the progression and regression period. Plasma calcium, and inorganic phosphorus concentrations were determined at four and eight months regression. Calcium, inorganic phosphorus, and triglyceride concentrations were determined only on male pigeons. Plasma cholesterol and triglyceride concentrations were determined on isopropanol extracts of serum [8]. Serum samples were diluted with 0.5% LaCl₃ and analyzed for calcium concentration by atomic absorption spectrophotometry **. Inorganic phosphorus concentrations were measured on serum by an AutoAnalyzer adaptation of the method of Fiske and SubbaRow [9].

**Necropsy procedures**

At necropsy, total body, liver and heart weights were recorded. The ascend-
ing and thoracic portions of the aorta and the brachiocephalic arteries were removed, cleaned of adventitial tissue, opened longitudinally and flattened. The extent of atherosclerosis of the thoracic aorta, and the right brachiocephalic artery was obtained by calculating an atherosclerotic index (AI). The aortic AI corresponded to the visual estimation of the percentage of intimal surface from the left brachiocephalic artery to the origin of the celiac artery covered with raised atherosclerotic plaques. The brachiocephalic AI corresponded to the visual estimation of the percentage of the intimal surface from the aortic arch to the origin of the axillary, carotid and vertebral arteries that was covered with raised plaques. The arteries were then measured, weighed and frozen for subsequent angiochemical analyses.

**Angiochemical procedures**

The arteries were homogenized in chloroform-methanol (2:1, v/v) and extracted for lipids according to the method of Folch et al. [10]. The lipids were separated by thin-layer chromatography on silica gel using a solvent system of Skellysolve B:ethyl ether:acetic acid (146:50:4, v/v/v). The cholesterol and esterified cholesterol fractions were localized by staining with iodine and were eluted from the silica gel with chloroform. The concentrations of the individual lipids were determined by the AutoAnalyzer method of Rush et al. [8]. The results of all lipid determinations were corrected for any loss by calculating the recovery of an added internal standard of radioactive cholesterol.

The lipid-free aortic residue remaining after chloroform—methanol treatment was dried to a constant weight in a vacuum dessicator and treated with 0.1 N hydrochloric acid at 4°C for 48 h. A portion of the acidic extract was mixed with lanthanum chloride to a final concentration of 0.5% to overcome phosphorus interference and analyzed for calcium concentration by atomic absorption spectrophotometry. Inorganic phosphorus concentrations were measured on the acidic extracts by an AutoAnalyzer adaptation of the method of Fiske and SubbaRow [9].

The lipid-free and mineral-free aortic residue was treated with 0.1 N sodium hydroxide at 98°C for 50 min in order to separate the elastin and collagen fractions of the arterial tissue. The alkali-insoluble material corresponding to the elastin fraction of the artery was washed with sodium hydroxide and water. The alkali-soluble material and washes, which included the collagen fraction, was hydrolyzed in 6 N hydrochloric acid for 18 h and analyzed for hydroxyproline by the method of Bergman and Loxley [11]. Collagen concentrations were calculated assuming the aortic collagen contained 12.9% hydroxyproline [12].

All angiochemical data were expressed on a concentration (mg/g wet or dry artery) and on a content basis (mg/artery normalized for individual animal differences in body weight; mg/cm² intimal surface analyzed). Where similar conclusions were reached we have presented results expressed only on a concentration basis. All data were analyzed by the analysis of variance or Student's t-test.

**Bone analysis**

Male animals in the intermittent-cholesterol-fed group receiving 2.5 mg
EHDP/kg were compared with the male controls for that group in order to determine any effects of EHDP on bone. Tibiotarsal bones were cleaned of adhering extraneous tissue and delipidated with three changes of warm acetone. The bones were dried, and ashed at 500°C for 24 h in a muffle furnace *. The ash was weighed and dissolved in 0.1 N HCl. A portion of the acidic solution was mixed with 0.5% lanthanum chloride and analyzed for calcium concentration by atomic absorption spectrophotometry. The inorganic phosphorus concentrations were determined on the acidic solution by an Auto-Analyzer adaptation of the method of Fiske and SubbaRow [9].

Results

The effect of intermittent or continuous cholesterol feeding on the characteristics of atherosclerosis of pigeons are presented in a previous report [4]. In brief, the aortas and brachiocephalic arteries of those animals that were subjected to intermittent hypercholesterolemia accumulated more cholesterol, were more complicated, and showed a different pattern of lesion regression than those of continuously hypercholesterolemic animals.

Figures 1 and 2 show the plasma cholesterol concentrations throughout the study. The treatment groups were equalized for mean plasma cholesterol concentrations after the 14-month atherosclerosis progression period. Since the birds were divided into treatment groups on the basis of their plasma cholesterol concentration during progression, no significant differences existed among the groups in the intermittent cholesterol fed animals (Fig. 1). During the progression phase of the study, when the animals were fed control diets,

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* Laboratory Box Furnace, Model 51848, SB Lindberg, Watertown, Wis.
the mean plasma cholesterol concentration decreased to a level similar to that for the basal state. During the regression period, no effect of EHDP on plasma cholesterol concentration was seen and the three treatment groups had similar concentrations comparable to pre-experimental levels.

The plasma cholesterol concentrations in the continuous-cholesterol-fed pigeons are shown in Fig. 2. Again, since the treatment groups were selected on the basis of their plasma cholesterol concentration no significant differences existed among any of the four groups during cholesterol feeding. EHDP did not effect plasma cholesterol concentrations and during the treatment period the three groups had similar plasma cholesterol concentrations which were not significantly different from basal levels. The plasma cholesterol concentrations of the animals fed control pellet diets throughout the experiment varied little and ranged from 246 ± 9 (mean ± SEM) to 395 ± 12 mg/dl.

Throughout the treatment period, considerable variation in plasma triglyceride concentrations was seen among the animals of all groups; however, the mean triglyceride concentrations were not affected by pretreatment diets or by EHDP (Fig. 3).

The aortic AI of the control pigeons (saline treated) and pigeons treated with EHDP is shown in Fig. 4. After four months of treatment, a downward linear trend with increasing dosage of EHDP was seen in pigeons fed cholesterol intermittently. After eight months of treatment of the same group, a similar downward trend was seen although the change from four months was not sig-
Fig. 3. A: plasma triglyceride concentrations during the treatment period of the male pigeons fed cholesterol intermittently prior to initiation of treatment period. O—O Saline, O—-—-O 0.5 mg EHDP/kg body wt, O—-—-O 2.5 mg EHDP/kg body wt. Points from 1–4 months represent 9–11 birds and points from 5–8 months represent 4–6 birds for each of the treatment groups. The SEM ranged from 3–7 in the high EHDP-treated group; 4–12 in the low-EHDP treated group; and 4–16 in the controls.

B: plasma triglyceride concentrations during the treatment period of the male pigeons fed cholesterol continuously prior to initiation of treatment period. O—O Saline, O—-—-O 0.5 mg EHDP/kg body wt, O—-—-O 2.5 mg EHDP/kg body wt. Points from 1–4 months represent 9–12 birds and points from 5–8 months represent 4–7 birds for each of the treatment groups. The SEM ranged from 2–13 in the high EHDP-treated group; 5–16 in the low EHDP-treated group, and 7–17 in the controls.

C: plasma triglyceride concentrations during the treatment period of male pigeons fed the control diet prior to initiating the treatment period. O—O Saline, O—-—-O 0.5 mg EHDP/kg body wt, O—-—-O 2.5 mg EHDP/kg body wt. Points from 1–4 months represent 8–10 birds and points from 5–8 months represent 4–5 birds from each of the treatment groups. The SEM ranged from 3–9 in the high EHDP-treated group; 2–11 in the low EHDP-treated group and 4–16 in the controls.
The aortic AI of 9.2 ± 1.5 for the high (2.5 mg/kg) EHDP treated group was significantly (P < 0.05) lower than the mean of 16.4 ± 2.4 for the 0.5 mg/kg dosage group at eight months. Surprisingly, a contrasting effect of EHDP was found in the group of animals with continuous hypercholesterolemia prior to drug treatment. After eight months of treatment, both the low and the high EHDP-treated groups had a significantly higher (P < 0.05) aortic AI (20.6 ± 4.3 and 25.3 ± 7.3 respectively) than the control group given saline (9.7 ± 1.6).

The effect of EHDP on brachiocephalic artery atherosclerosis was quite similar to that seen in the aorta (Fig. 5). Among the birds fed cholesterol intermittently prior to drug treatment, an EHDP dose-related downward linear trend can be seen that was significant at eight months. As with the aorta, among the birds fed cholesterol continuously prior to drug treatment, there was a contrasting EHDP dose-related upward linear trend apparent at eight months.

The aortic total cholesterol concentration of pigeons fed cholesterol intermittently before a period of treatment with EHDP is shown in Fig. 6. Compared to baseline, the control and two treated groups of animals had significantly (P < 0.05) less total aortic cholesterol at four and eight months. No effect of the drug was apparent after four months of treatment; however, after eight months of treatment both the low and high dose groups had less (P < 0.07) aortic cholesterol (5.30 ± 0.93 and 4.64 ± 0.96 mg/g respectively) than the saline treated controls (11.42 ± 3.34 mg/g).

Contrastingly, among pigeons fed cholesterol continuously prior to the...
Fig. 5. Brachiocephalic atherosclerosis index in White Carneau pigeons treated with EHDP. Bars represent the mean ± SEM. □ Atherosclerosis induced by intermittent hypercholesterolemia, □ Atherosclerosis induced by continuous hypercholesterolemia. The superscript above the bars indicate significant differences within groups. A mean with "a" is significantly different (P < 0.05) from those not having "a"; those with "b" are significantly different from those not having "b" etc.

Fig. 6. Aortic total cholesterol concentration of White Carneau pigeons fed cholesterol intermittently before treatment with EHDP. The bars represent means ± SEM. The superscripts above the bars indicate significant differences within groups. A mean with "a" is significantly different (P < 0.05) from those not having "a"; those with "b" are significantly different from those not having "b" etc.
EHDP treatments no significant differences between saline treated controls and the two treated groups were apparent at four months (Fig. 7). After eight months treatment, however, the total cholesterol concentrations did show a dose-related trend to be greater with increasing dosage of EHDP.

The relationships between the method of atherosclerosis induction and the effect of EHDP treatment on the aortic nonesterified and esterified cholesterol fractions of aorta are shown in Figs. 8 and 9 respectively. Among the pigeons fed cholesterol continuously before drug treatment, the nonesterified cholesterol concentrations increased slightly but not significantly with time (Fig. 8). In contrast, the group fed cholesterol intermittently before treatment showed a downward trend with time for both dosages of EHDP. These results indicate an augmentation by the drug of the removal of nonesterified cholesterol from the atherosclerotic lesion in the intermittently-fed group and no effect or a slight increase in the lesion-nonesterified-cholesterol if the atherosclerosis was induced by continuous cholesterol feeding.

Changes from baseline in aortic esterified cholesterol concentration are shown in Fig. 9. Regardless of the method of atherosclerosis induction and at both doses of EHDP, esterified cholesterol concentrations decreased with time. The results shown in Fig. 9 indicate that between 40 and 76% of the accumulated aortic esterified cholesterol was removed within four months. Four additional months of treatment effected either the removal of only a small portion of additional esterified cholesterol in the case of the animals fed cholesterol intermittently, or no further removal in the case of those animals fed cholesterol continuously.

The concentrations of total cholesterol in the brachiocephalic artery of pigeons with atherosclerosis induced by intermittent feeding of cholesterol are shown in Fig. 10. After eight months of treatment, the group receiving 2.5 mg EHDP/kg body weight had the lowest concentration of cholesterol in the brachiocephalic artery. An apparent dose-related effect of EHDP on brachio-

![Fig. 7. Aortic total cholesterol concentration of White Carneau pigeons fed cholesterol continuously before treatment with EHDP. The bars represent means ± SEM. The superscripts above the bars indicate significant differences within groups. A mean with “a” is significantly different (P < 0.05) from those not having “a”; those with “b” are significantly different from those not having “b” etc.](image-url)
cephalic artery atherosclerosis was suggested and the changes followed the similar trend that was seen for the aorta in this group of pigeons (see Fig. 6).

As in the aorta, a contrasting effect of the drug was seen when given to birds previously fed cholesterol continuously. Increasing concentrations of brachiocephalic artery cholesterol were seen with increasing dosage of EHDP (Figs. 11, 7).

The aortic calcium concentrations of pigeons treated with EHDP are shown in Fig. 12. Generally, most of the mean calcium concentrations were lower for those groups treated with EHDP compared to their saline controls. Only the pigeons fed cholesterol intermittently had significantly ($P < 0.05$) higher aortic calcium accumulations ($2.97 \pm 0.36$ mg/g) after eight months of lesion regression. Animals from that diet group that were given 2.5 mg EHDP had significantly lower aortic calcium concentration ($1.54 \pm 0.08$ mg/g).

The animals fed pigeon pellets throughout the study and given the high dosage of EHDP had significantly less aortic calcium than their saline control group. No significant drug effect on aortic calcium concentration was seen among pigeons that previously had been fed cholesterol continuously. When the calcium data were expressed on a content basis ($\mu g$/aorta/kg body weight, or $\mu g$/aorta/cm$^2$ intimal surface area analyzed) similar conclusions were reached on the effect of EHDP treatment.
Fig. 9. Aortic esterified cholesterol concentrations in pigeons treated with either 2.5 (A) or 0.5 (B) mg EHDP/kg body weight. Points represent means and lines SEM's. ○ Birds fed cholesterol intermittently, ▲ Birds fed cholesterol continuously.

Fig. 10. Brachiocephalic artery total cholesterol concentration among pigeons fed cholesterol intermittently before treatment with EHDP. Bars represent means ± SEM for observations at baseline and four months lesion regression. Bars at eight months regression represent the average concentration determined for samples which were pooled within a treatment group.
Fig. 11. Brachiocephalic artery total cholesterol concentration of pigeons fed cholesterol continuously before treatment with EHDP. Bars represent means ± SEM for observations at baseline and four months regression. Bars at eight months regression represent the average concentration determined for samples which were pooled within a treatment group.

Fig. 12. Aortic calcium concentration in pigeons treated with EHDP. Bars represent means ± SEM. ◊ Fed cholesterol intermittently. □ Fed cholesterol continuously. ■ Controls. The superscripts above the bars indicate significant differences within groups. A mean with "a" is significantly different ($P < 0.05$) from those not having "a"; those with "b" are significantly different from those not having "b" etc.
Groups of pigeons from both means of atherosclerosis induction had higher mean collagen concentrations than the control group, but these differences were not large (Fig. 13). No significant effects of EHDP were seen in any of the three groups of animals. Expressing the results of the collagen analysis to represent the content within an aorta resulted in similar conclusions.

Whole pigeon body weights, heart weights, plasma concentrations of calcium and inorganic phosphorus were not significantly different among the three groups of animals at baseline and did not appear to be affected by drug treatment at four or eight months. The liver weights of the two cholesterol-fed groups were significantly higher than controls at baseline but decreased to control levels by four months during which period the animals were fed a low-fat control diet. At either of the treatment times studied, no significant effects of EHDP were seen on the liver weights.

When male pigeons fed cholesterol intermittently and treated for eight months were examined for alterations in bone metabolism, no significant effects of EHDP were seen. Weights of the delipidated bone, the bone ash, and the concentrations of bone calcium and phosphorus were similar in animals treated with 2.5 mg EHDP and in the controls.

Discussion

Although a few studies have been done on the effects of drugs on atherosclerotic lesion regression, none have considered contrasting means for the induction of the atherosclerosis to be studied. Intravenous polyunsaturated soya phosphatides have been used and some studies have provided favorable evidence for their efficacy [13,14], but other studies have not [15]. A number of investigators have reported the use of colchicine [7] and antimetabolic drugs such as mercaptopurine, hydroxyurea, and pyridinal carbamate [16], but, these
compounds have obvious limitations in long-term therapeutic interventions.

In the present study using White Carneau pigeons, we have shown EHDP to improve the atherosclerosis augmented by intermittent feeding of dietary cholesterol. In contrast we have shown an opposite effect of EHDP when the atherosclerosis had been augmented by feeding cholesterol continuously. Among the birds with atherosclerosis augmented by intermittent cholesterol feeding there was increased mineralization during regression and this complication was found to be prevented by EHDP treatment.

Several recent studies have provided evidence of a promising effect of EHDP on the progression and regression of atherosclerosis. Rosenblum et al. [5] reported that EHDP was effective in inhibiting arterial mineralization and thromboarteritis in rabbits fed an atherogenic diet in conjunction with moderately high doses of vitamin D and nicotine. In another study, Hollander et al. [6] reported less severe atherosclerosis in rabbits treated with 5 mg EHDP/kg after eight weeks of regression of lesions that were induced by feeding an atherogenic diet for eight weeks. The authors reported that EHDP treatment resulted in a decreased sudanophilia, decreased aortic content of nonesterified and esterified cholesterol, collagen, elastin, and calcium. Another study by Kramsch and Chan [7] reported similar findings for regression and also reported that EHDP administered during an eight week atherosclerosis progression period prevented the accumulation of aortic cholesterol, collagen, elastin, calcium, and inorganic phosphorus. In a recent report, Kramsch and Chan [17] used [3H] lysine and [3H] proline markers to show that EHDP inhibited the increased arterial synthesis of collagen, but not elastin, in rabbits fed an atherogenic diet for eight weeks.

In two previous studies from this institution, the morphological, chemical and metabolic characteristics of regressed atherosclerotic lesions was investigated. The studies were done by examining subgroups of White Carneau pigeons fed an atherogenic diet for one year followed by control diet for 16 months [3]. The results indicated that regression effected a removal of approximately 50% of the cholesterol in the aortic lesions. The metabolic studies on these regressed pigeon atherosclerotic lesions have shown them to contain a pool of cholesterol which is nonmiscible or only very slowly miscible with plasma cholesterol [18]. These results implicate the importance of the slowly miscible pool of cholesterol in the lesion. If the size of this pool of cholesterol increases as the severity of the lesion increases, the regression of human atherosclerosis could be critically limited by diet and drug therapy directed only toward plasma cholesterol concentrations and the resulting labile plaque cholesterol and not to the slowly miscible pool of plaque cholesterol.

In the present study, the positive beneficial effect of EHDP on the more advanced atherosclerosis in the intermittent cholesterol-fed pigeons was seen in two different arterial beds, the aorta and the brachiocephalic artery. Since the regressed pigeon lesions can have as much as 50% of their cholesterol concentration as a constituent of a nonmiscible or slowly miscible pool, it can be speculated that this cholesterol could be in the form of a lipid-calcium-connective tissue complex. If such a complex has a tendency to form and stabilize in the regressing pigeon lesion then EHDP may act to interfere with the availability of calcium or other divalent ions necessary for complex formation in the artery.
The opposite effect of EHDP in the group of pigeons with relatively uncomplicated atherosclerosis produced by continuous cholesterol feeding is less easily understood. The lesions produced in the two groups of animals were chemically distinct in that the animals with the more advanced atherosclerosis had significantly greater esterified cholesterol and calcium concentrations [4]. If one assumes that the uncomplicated atherosclerotic lesions consisted mainly of fat-filled cells with very little extracellular lipid and the more advanced atherosclerotic lesions had a larger portion of plaque cholesterol in an extracellular pool, the effect on the two groups indeed might be different. Since the lesions induced by intermittent hypercholesterolemia had accumulations of calcium during regression but those induced by continuous hypercholesterolemia did not, it is conceivable that if the mode of action of the compound involves an effect on a lipid-calcium-connective tissue complex in the atherosclerotic plaque this difference would be evident.

It is also possible that EHDP altered the activity of one or more of the lysosomal enzymes, such as cholesteryl ester hydrolase. Although these areas remain to be studied, there is one report by Doty et al. [19] who showed a significantly reduced bone cell lysosomal activity in rats following EHDP doses as low as 0.5 mg P/kg body weight.

Since EHDP was initially investigated in calcium and phosphate metabolism, many of the previous studies have presumed that the compound acted via its effect on calcium. Perhaps the effect of EHDP seen on the more advanced lesions in this study was due to its action on calcium metabolism, but, because of the multiplicity of factors operating in an atherosclerotic plaque, one must also consider a possible role associated with the efflux of cholesterol from plaques.

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