Effectiveness of 1-Dimethylaminoethyl-4-benzylpiperidine (IN379) in Prevention and Regression of Experimental Atherosclerosis in the Rabbit

By Thomas B. O'Dell, Ph.D., Martha D. Napoli, B.S., Lowell D. Miller, Ph.D., and Chester J. Cavallito, Ph.D.

In the search for effective treatment or prevention of atherosclerosis, a wide variety of substances, drugs, and procedures have been investigated in both animal and human studies. Examples of some of the types of compounds which have been employed include the lipotropic agents such as choline, betaine, heparin, sex hormones, alpha tocopherol, detergents, plant sterols, cortisone, and ACTH. It also was found that certain procedures such as the production of experimental alloxan diabetes could influence the course of atherosclerosis in animals. Major emphasis has been placed on the influence of diet and the possible merits of low intake of fat and cholesterol. A recent article by Stamler reviews this field very well.

The primary approach to development of specific drugs appears to have been directed toward agents which can lower blood cholesterol. This interest is based on the assumption that hypercholesterolemia is an essential component for the production of atherosclerosis. Some drugs currently being intensively studied are nicotinic acid or its derivatives, triparanol or MER-29 and d-thyroxin. For several years, we have conducted a program of screening in which the guiding criterion was not the blood cholesterol level but rather the intensity of induced atherosclerotic-like plaque deposition in rabbit aortas. The selection of compounds for this rather tedious screening approach has been largely intuitive and based on a tentative assumption that plaque deposition might be related more to stability in solution of lipoprotein complexes rather than to absolute cholesterol levels.

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Among the compounds screened, the most consistent favorable influence in reducing plaque formation has been provided by 1-dimethylaminoethyl-4-benzylpiperidine (IN379) conveniently prepared as the dihydrochloride salt.

\[
\text{CH}_2 - \text{N} - \text{CH}_2\text{CH}_2 \text{N} (\text{CH}_3)_2 \cdot 2 \text{HCl}
\]

This report summarizes the animal experimental findings determined over a period of several years. It has been of particular interest that the antiatherogenic properties of the compound are unrelated to blood cholesterol levels, and in fact, hypercholesterolemia is not depressed by this drug.

Methods

The methods and procedures employed in these investigations were chosen or designed for the primary purpose of determining the influence that an agent might have on the development or regression of aortic atherosclerotic plaques.

The animals used in these studies were New Zealand white rabbits with starting body weights of approximately 2.5 Kg. The oral route of administration (via stomach tube) was used primarily, although other routes were employed in certain instances. The agent under study was administered once daily, seven days per week, and the duration of drug administration varied with the test procedure employed. The number of rabbits per group for each dose level varied from 5 to 12, and placebo control groups were always run concurrently with drug-treated groups.

At the termination of the tests, the rabbits were sacrificed and the aortas removed for evaluation of plaque formation. A state of plaque deposition will be referred to as atherosclerotic without implication that the condition in rabbits is the same as that in man. In an effort to make the results obtained as accurate and valid as possible, the degree of atherosclerosis in each aorta was graded independently by at least two and usually three individuals. Each individual graded on the basis of 0 (none) to 5+ (complete) plaque deposition and evaluated the arch, thoracic, and abdominal portions of each aorta separately. The degree of atherosclerosis for the total aorta was the
average of the values found for the three portions. To delineate more clearly the grading system employed, hypothetical aortic plaque depositions, and the values that would be assigned when grading them, are shown by the drawings in figure 1.

Two methods were employed to develop the experimental atherosclerosis. One was patterned after that published by Friedman, Oester, and Davis. The condition was produced by daily administration of large doses of thyroxine (0.12 to 0.15 mg./Kg., subcutaneous) plus epinephrine according to the following schedule:

<table>
<thead>
<tr>
<th>Day</th>
<th>Epinephrine dose (μg./Kg., intravenous)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>4 to 10</td>
<td>25 to 50</td>
</tr>
</tbody>
</table>

As was pointed out by Friedman and co-workers,23 the arteriopathy produced by this procedure is essentially a medial sclerosis and necrosis with, in some instances, also a concomitant intimal proliferation. With this method, it was essential that the epinephrine be injected very slowly. No special diet was required and the animals were maintained on a standard ration (Purina).

The second method was based on production of an experimental atherosclerosis by feeding rabbits a high cholesterol diet. This was accomplished by utilizing regular rabbit chow pellets (Purina) in which was incorporated cholesterol to the extent of 1.5 per cent.

Using the two methods, experiments were conducted to evaluate both prophylactic and therapeutic regression activities of the test compound. The general procedures followed in each instance will be described.

**SHORT-TERM, EPINEPHRINE-THYROXINE METHOD**

**Prophylactic studies.** The drug being evaluated or placebo was administered once daily throughout the entire epinephrine-thyroxine treatment period. The duration of this period was usually 9 to 10 days. At the end of this time, all surviving rabbits were sacrificed and the aortas of the drug-treated animals compared with those of the controls.

**Regression studies.** The usual epinephrine-thyroxine schedule was followed for a total of nine days, then discontinued. At this time, the rabbits were divided into groups and treatment with drug or placebo once daily was instituted. After treatment for three weeks, the rabbits were autopsied and the control group compared with the drug-treated groups as to the degree of atherosclerosis found in the aortas.

**Circulation Research, Volume X, June 1962**
**TABLE 1**

Prophylactic Effectiveness of IN379 Against Epinephrine-Thyroxine-induced Atherosclerosis

<table>
<thead>
<tr>
<th>IN379 dose (mg./Kg.)</th>
<th>Route of administration</th>
<th>Average degree of total atherosclerosis in aorta (0-5+)</th>
<th>Number of rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>2.5</td>
<td>intraperitoneal</td>
<td>1.6 ± 0.4</td>
<td>10</td>
</tr>
<tr>
<td>Placebo‡</td>
<td>intraperitoneal</td>
<td>3.0 ± 0.6</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>oral</td>
<td>1.5 ± 0.4</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>oral</td>
<td>1.4 ± 0.2</td>
<td>8</td>
</tr>
<tr>
<td>20</td>
<td>oral</td>
<td>1.5 ± 0.3</td>
<td>8</td>
</tr>
<tr>
<td>40</td>
<td>oral</td>
<td>1.6 ± 0.3</td>
<td>8</td>
</tr>
<tr>
<td>50</td>
<td>oral</td>
<td>0.8 ± 0.2</td>
<td>8</td>
</tr>
<tr>
<td>Placebo‡</td>
<td>oral</td>
<td>3.1 ± 0.3</td>
<td>10</td>
</tr>
</tbody>
</table>

*Average total atherosclerosis ± standard error.

†The difference between the initial and final numbers of rabbits indicates the number of animals that died as a result of the large doses of epinephrine and thyroxine used to induce the experimental atherosclerosis.

‡Placebo (control) medication consisted of saline (intraperitoneal), or water (oral).

**TABLE 2**

Influence of IN379 on Regression of Epinephrine-Thyroxine-induced Atherosclerosis

<table>
<thead>
<tr>
<th>IN379 dose (mg./Kg.)</th>
<th>Average degree of atherosclerosis in aorta (0-5+)</th>
<th>Average body weight (Kg.)</th>
<th>Number of rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arch Thoracic Abdominal Total†</td>
<td>Initial 10 Days‡ Final</td>
<td>Initial Final</td>
</tr>
<tr>
<td>5</td>
<td>0.4 3.5 2.4 2.1 ± 0.6</td>
<td>2.52 2.01 2.93</td>
<td>10 6</td>
</tr>
<tr>
<td>Placebo‡</td>
<td>2.7 4.3 2.0 3.0 ± 0.7</td>
<td>2.66 2.15 2.81</td>
<td>10 6</td>
</tr>
</tbody>
</table>

*Route of administration = oral.

†Average total atherosclerosis ± standard error.

‡Epinephrine-thyroxine discontinued, treatment with IN379 started.

§The difference between the initial and final numbers of rabbits indicates the number of animals that died as a result of the large doses of epinephrine and thyroxine used to induce the experimental atherosclerosis.

drug or placebo treatment. This was done to establish the cholesterol-induced experimental atherosclerosis. Treatment was then started, and the drug or placebo was administered once daily for an additional eight weeks. By the first procedure, the high cholesterol diet was continued throughout the period of treatment, whereas in the second procedure, the high cholesterol diet was discontinued and the animals were placed on a regular diet when drug treatment was started.

Blood cholesterol values were determined at various time intervals before and after drug treatment. At the conclusion of the tests, in addition to grading the aortas for the degree of atherosclerosis, the following determinations were made: visual grading of lipid deposition in the eyes and liver, blood coagulation time, and lipid, cholesterol, and calcium content of the aortas.

**DETERMINATION OF CALCIUM IN THE AORTAS**

The lyophilized aortas were first ashed in silica crucibles and then extracted with dilute HCl solution. After adjusting the pH of the acid extract to between 9 and 10 with 25 per cent NaOH solution, the calcium was precipitated with 5 per cent trisodium phosphate. The remainder of the determination was essentially the same as that described in Hawk et al.24

**TOTAL LIPID DETERMINATION**

The lyophilized aortas were extracted for total lipids as described by J. Folch et al. An aliquot of the lipid extract was quantitatively measured into a preweighed aluminum weighing dish and the solvent evaporated under a stream of nitrogen. The amount of lipid material was then determined gravimetrically.
CHOLESTEROL DETERMINATIONS

Blood plasma. Free cholesterol and cholesterol ester determinations were made using the methods as described in Schoenheimer and Sperry,28 and Hawk et al.24 with minor modifications.

Aorta. The lyophilized aortas were cut into small pieces and extracted with ethanol:ether (3:1). The total cholesterol and cholesterol esters were then determined as described under “Blood plasma.”

Results

GENERAL PROPERTIES OF IN379

Since this compound is new, it would be desirable to present a brief summary of the
TABLE 3
Prophylactic Effectiveness of IN379 Against Cholesterol-induced Atherosclerosis*

<table>
<thead>
<tr>
<th>IN379 dose (mg./Kg.)</th>
<th>Route of administration</th>
<th>Average degree of atherosclerosis in aorta (0-5)</th>
<th>Total serum cholesterol (mg./100 cc.)</th>
<th>Average body weight (Kg.)</th>
<th>Average food intake pounds/weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>intra-peritoneal</td>
<td>Arch: 3.0, Thoracic: 1.4, Abdominal: 0.2, Total: 1.5 ± 0.4</td>
<td>Total 660-960</td>
<td>2.1, 3.0</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>oral</td>
<td>Arch: 1.0, Thoracic: 0.5, Abdominal: 0.0, Total: 0.5 ± 0.1</td>
<td>Total 970-1,150</td>
<td>2.1, 2.8</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>oral</td>
<td>Arch: 1.3, Thoracic: 0.4, Abdominal: 0.6, Total: 0.6 ± 0.2</td>
<td>Total 920-1,120</td>
<td>2.5, 2.8</td>
<td>1.64</td>
</tr>
<tr>
<td>Placebo</td>
<td>oral</td>
<td>Arch: 3.5, Thoracic: 2.1, Abdominal: 0.7, Total: 2.1 ± 0.4</td>
<td>Total 580-870</td>
<td>2.1, 2.9</td>
<td>1.74</td>
</tr>
</tbody>
</table>

*Number of rabbits per group = 8.
1Average total atherosclerosis ± standard error.
2Range of individual variations within each group. Values determined at end of test period.

general pharmacological and toxicological characteristics of IN379 before discussing the specific results obtained against experimental atherosclerosis.

Toxicological studies, both acute and subacute, were conducted. In mice, the acute LD_{50} values for IN379 were found to be as follows: intravenous, 47 mg./Kg.; intraperitoneal, 215 mg./Kg.; oral, 1,950 mg./Kg. Parenteral 30-day subacute studies were carried out in dogs using the intravenous route and in mice using the intraperitoneal route. Maximum doses used were 10 mg./Kg. for the dogs and 100 mg./Kg. for the mice with no evidence of toxicity noted. Long-term, 6 to 12 month, toxicity studies were conducted using dogs (beagles) and rats (Wistar). The oral route of administration was used, and the daily doses employed ranged from 15 to 100 mg./Kg. for the dogs and from 15 to 300 mg./Kg. for the rats. The only deleterious effect noted was some apparent decrease in weight gain after prolonged daily administration of doses greater than 75 mg./Kg.

The general pharmacological evaluation of this compound revealed that it had negligible effects on the blood pressure per se, but did potentiate the hypotensive activity of cryptenamine, a Veratrum alkaloid preparation. However, IN379 did prove to have considerable peripheral (femoral) and coronary arterial vasodilating properties when injected directly into the arteries. This activity was much less pronounced after intravenous administration. At this point, it should be mentioned that there is probably no direct correlation between these effects and any antiatherogenic properties of IN379 in that other compounds or drugs which were equal or superior to IN379 as vasodilators were devoid of antiatherogenic activity.

IN379 did not appear to have any pronounced effect on the central nervous system and showed no anticoagulant activity in vitro or in vivo. The compound did offer protection against electrically induced atrial fibrillation in cats at intravenous doses of 0.5 to 2.0 mg./Kg. Mild to moderate antispasmodic activity was found when IN379 was tested on the guinea pig ileum and dog tracheal chain using a modified Magnus procedure.

ANTIATHEROGENIC ACTIVITY

In general, wherever possible, the results obtained will be summarized graphically to facilitate evaluation and comparison of the drug-treated and control groups.

EPINEPHRINE-THYROXINE-INDUCED ATHEROSCLEROSIS

Prophylactic effectiveness of IN379. The results obtained following parenteral and oral administration of IN379 are shown in table 1. The compound appeared to be as effective orally as when administered by the intraperitoneal route. Also, it was noted that there was no apparent dose-response variation with this compound in the oral dose range of from 5 to 40 mg./Kg. The protection shown in the drug-treated groups was significant, and there was no obvious correlation between the degree of atherosclerosis found in the aortas.
Graphic summary of the results obtained from experiments designed to determine the influence of IN379 on regression of previously established, high cholesterol-induced atherosclerosis in which the high cholesterol diet was continued throughout the period of drug treatment. Also shown are the average plasma cholesterol levels and the average values found for total cholesterol, lipid, and calcium in the aortas from the drug-treated and placebo control groups.
TABLE 4
Effectiveness of IN379 in Regression of Cholesterol-induced Atherosclerosis (Cholesterol Continued During Treatment)

<table>
<thead>
<tr>
<th>IN379 dose (mg./Kg.)</th>
<th>Average degree of atherosclerosis in aorta (0-5+)</th>
<th>Average plasma cholesterol (mg./100 cc.)</th>
<th>Average body weight (Kg.)</th>
<th>Average food intake pounds/week</th>
<th>Number of rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arch Thoracic Abdominal Total*</td>
<td>Initial 8 weeks 16 weeks</td>
<td>Initial Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.3 1.1 0.7 1.7 ± 0.5</td>
<td>48 1524 2765</td>
<td>2.06 3.02</td>
<td>1.80</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>3.2 1.1 1.0 1.8 ± 0.4</td>
<td>56 1715 2251</td>
<td>2.33 3.16</td>
<td>1.82</td>
<td>11</td>
</tr>
<tr>
<td>Placebo</td>
<td>3.8 2.2 1.25 2.4 ± 0.7</td>
<td>51 1601 1806</td>
<td>2.45 3.20</td>
<td>1.84</td>
<td>10</td>
</tr>
</tbody>
</table>

*Average total atherosclerosis ± standard error.
†Drug treatment started, high cholesterol diet continued.

TABLE 5
Effectiveness of IN379 in Regression of Cholesterol-induced Atherosclerosis (Cholesterol Continued During Treatment)

<table>
<thead>
<tr>
<th>IN379 dose (mg./Kg.)</th>
<th>Average degree of atherosclerosis in aorta (0-5+)</th>
<th>Average plasma cholesterol (mg./100 cc.)</th>
<th>Average body weight (Kg.)</th>
<th>Average food intake pounds/week</th>
<th>Number of rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arch Thoracic Abdominal Total*</td>
<td>Initial 8 weeks 16 weeks</td>
<td>Initial Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.0 1.2 0.2 1.1 ± 0.2</td>
<td>60 1240 166</td>
<td>2.36 3.02</td>
<td>2.12</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>1.5 1.1 0.6 1.1 ± 0.3</td>
<td>51 1141 149</td>
<td>2.18 3.31</td>
<td>2.04</td>
<td>11</td>
</tr>
<tr>
<td>Placebo</td>
<td>3.0 1.5 1.0 1.8 ± 0.5</td>
<td>62 1246 336</td>
<td>2.27 3.13</td>
<td>1.06</td>
<td>11</td>
</tr>
</tbody>
</table>

*Average total atherosclerosis ± standard error.
†Drug treatment started, high cholesterol diet discontinued.

and the body weight, food consumption, or water intake of the various groups.

Regression following IN379. Table 2 contains the data comparing the findings from the placebo control group of rabbits and the group treated with daily oral doses of 5 mg./Kg of IN379. Although the degree of atherosclerosis found in the drug-treated animals was significantly less than that in the controls, the difference was not as marked as that obtained in the prophylactic experiments. Again, the antiatherogenic activity of the compound could not be accounted for by influence on weight gain, food consumption, or water intake.

The average degree of total aortic atherosclerosis found for each of the treated and control groups in both the prophylactic and regression studies is summarized graphically in figure 2.

CHOLESTEROL-INDUCED ATHEROSCLEROSIS

Prophylactic effectiveness of IN379. Table 3 presents the data obtained from the prophylactic studies conducted. The compound proved to be highly effective in preventing the formation of atheromatous plaques in the aorta. This was evident when either the total aortas or the separate arch, thoracic, and abdominal areas of the aortas for the drug-treated and control groups were compared. As in the short-term epinephrine-thyroxine experiments, IN379 was active orally and there seemed to be no significant differences in effects of the two dosages used.

It was of interest to note the complete lack of correlation between the atherosclerotic sparing action of IN379 and any antihypercholesterolemic effect. In fact, the data suggested that the reverse might be the case. The slight difference in food intake found for the control and treated groups was not sufficient to account for the protective effect of the compound, and this was verified in the regression studies.

To facilitate the comparison of the results obtained when the drug or placebo was administered orally, the data are presented graphically in figure 3.
FIGURE 5

Graphic summary of the results obtained from experiments designed to determine the influence of IN379 on regression of previously established, high cholesterol-induced atherosclerosis in which the high cholesterol diet was discontinued when drug treatment was begun. Also shown are the average plasma cholesterol levels and the average values found for total cholesterol, lipid, and calcium in the aortas from the drug-treated and placebo control groups.

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Regression following IN379. The first procedure carried out in the regression studies was probably the most severe test for the compound since the high cholesterol diet was continued throughout the entire period of drug treatment. As is evident from the data presented in table 4, there was less atherosclerosis found in the IN379-treated rabbits than among the placebo controls. The table also shows the average plasma cholesterol values obtained initially; after 8 weeks, when drug treatment was started; and at 16 weeks, the end of the test period. As in the prophylactic studies, the groups treated with IN379 had higher average plasma cholesterol levels after treatment, even though the degree of hypercholesterolemia at the end of the pretreatment period (8 weeks) was essentially the same as the control group. This might indicate a mobilization of the aortic plaque cholesterol or a suppression, by IN379, of the deposition of circulating cholesterol. There was no significant difference between the average body weights of the animals in the treated and control groups. This was also reflected in the food consumption found with the various groups.

Figure 4 summarizes graphically the findings in this study and compares the average control values obtained with the combined average values of the IN379-treated groups. In addition to the degrees of atherosclerosis and plasma cholesterol levels, this figure also shows the amount of total cholesterol, total lipid, and total calcium found in the aortas of the control and treated animals. These will be discussed subsequently.

In the second procedure for the regression studies, in which the high cholesterol diet was discontinued when treatment with IN379 was begun, the influence of the compound on the degree of atherosclerosis was somewhat more pronounced. The overall results are shown in table 5 and figure 5 in the same manner as for the first regression procedure. In contrast to the results obtained by the first procedure, it was noted that in this group the average plasma cholesterol level of the IN379-treated animals was consistently less than that of the controls during the post-treatment period. However, it is doubtful if the differences are significant. As in the previous studies presented, the average body weights, rate of growth, and food consumption were essentially the same for the treated and control animals. Thus, the action of IN379 could not logically be attributed to a change in food consumption.

Table 6 presents a comparison of the average total atherosclerosis found in the two high cholesterol diet regression experiments with the average values obtained from grading the lipid deposition in the eyes and livers, clotting time of the blood, and average values for

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**TABLE 6**

Comparison of Total Atherosclerosis with Other Values Obtained from IN379 Regression Studies

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>IN379 dose (mg./Kg.)</th>
<th>Average degree of total atherosclerosis (0-5+)</th>
<th>Average degree of lipid deposition in Eyes (0-5+)</th>
<th>Blood clotting time (min.)</th>
<th>Average aorta values (mg./Gm. dried aorta)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cholesterol Lipid Calcium</td>
</tr>
<tr>
<td>Regression cholesterol diet continued</td>
<td>5</td>
<td>1.7 ± 0.5</td>
<td>1.7</td>
<td>3.0</td>
<td>3.25</td>
</tr>
<tr>
<td>Placebo</td>
<td>5</td>
<td>1.8 ± 0.4</td>
<td>2.2</td>
<td>3.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Regression cholesterol diet discontinued</td>
<td>5</td>
<td>2.4 ± 0.7</td>
<td>2.4</td>
<td>3.4</td>
<td>3.5</td>
</tr>
<tr>
<td>Placebo</td>
<td>5</td>
<td>1.1 ± 0.2</td>
<td>1.4</td>
<td>1.6</td>
<td>3.0</td>
</tr>
</tbody>
</table>

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TABLE 7

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Dose (mg./Kg.)</th>
<th>Route of administration</th>
<th>Method employed</th>
<th>Type of experiment</th>
<th>% Reduction of atherosclerosis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin</td>
<td>5</td>
<td>intraperitoneal</td>
<td>epinephrine-thyroxine</td>
<td>prophylactic</td>
<td>48</td>
</tr>
<tr>
<td>IN379</td>
<td>2.5</td>
<td>intraperitoneal</td>
<td>epinephrine-thyroxine</td>
<td>prophylactic</td>
<td>52</td>
</tr>
<tr>
<td>Betaine</td>
<td>5, 10, 20</td>
<td>oral</td>
<td>epinephrine-thyroxine</td>
<td>prophylactic</td>
<td>55</td>
</tr>
<tr>
<td>Cholic acid</td>
<td>40, 50</td>
<td>oral</td>
<td>epinephrine-thyroxine</td>
<td>prophylactic</td>
<td>50</td>
</tr>
<tr>
<td>β-Sitosterol</td>
<td>250</td>
<td>subcutaneous</td>
<td>high cholesterol</td>
<td>prophylactic§</td>
<td>7.1</td>
</tr>
<tr>
<td>+ nicotine acid</td>
<td>25</td>
<td>oral</td>
<td>epinephrine-thyroxine</td>
<td>prophylactic</td>
<td>28</td>
</tr>
</tbody>
</table>

*Based on the degree of atherosclerosis found in the aortas of untreated (control) animals run concurrently.
†Drug administered once daily throughout entire epinephrine-thyroxine treatment period.
§Drug started after nine days of epinephrine-thyroxine treatment. Epinephrine-thyroxine discontinued.
||Drug started concurrently with high cholesterol diet and administered once daily over entire test period. Duration of test, 10 weeks.
|||Drug started after rabbits had been on high cholesterol diet for eight weeks. The high cholesterol diet was continued and drug administered once daily. Duration of drug treatment was eight weeks. (Total test period, 16 weeks.)
**Same as footnote ||, except that high cholesterol diet was discontinued and animals placed on regular diet when drug treatment started.

Of particular interest were the values found for the calcium content of the aortas in that the amount of calcium appeared to be greater in those groups in which the degree of atherosclerosis was more pronounced. This was especially apparent in the regression experiment in which the high cholesterol diet was continued throughout the period of drug treatment.

Thus, the lipid deposition in the eyes and livers and the calcium content of the aortas were found to have a possible correlation with the degree of atherosclerosis, whereas there was no apparent correlation between the atherosclerosis and the cholesterol levels in the blood or aortas.

All of the results obtained with IN379 us-
ing the various experimental procedures have been summarized in table 7, where the results are expressed as per cent reduction of the atherosclerosis based on that found in the placebo controls. The effects obtained with known drugs which were also tested in our laboratories have been included for comparison.

Discussion

The paucity of new, reliable leads in atherosclerosis therapy is, in part, a reflection of the difficulties in design and execution of an experimental approach in animals to a reliable means for the detection and evaluation of agents possibly useful as antiatherosclerotic agents in man. As a screening approach, it would usually be impractical to carry out all of the studies presented for the subject compound of the present report (includes 164 rabbit tests with completed autopsies). Since any one set of data from the groups presented might be subject to questioning as to validity of experimental design and significance of findings, it was felt that because of the difficulties in this field inherent with evaluation in humans, a larger number of group trials with experimental variations in technique would be needed to justify clinical consideration. We believe it to be particularly significant that although one might question the reliability of any one test procedure alone, yet the consistent qualitatively favorable influence of the test compound enhances the significance of the quantitative differences.

The mechanism of action of the test drug remains obscure. In any event, there certainly is no blood-cholesterol level reduction propensity. The most significant difference observed between control and treated animals, other than plaque deposition, was the calcium content of the aortas. If one considers the possibility that plaque deposition is a manifestation of interaction of lipoprotein and anionic mucopolysaccharides (ground substance polygalacturonate) with possible inter-\v
vention of calcium ions,\textsuperscript{27, 28} it may well be that the action of IN379 may involve an interference with formation of the insoluble calcium complex. This might result from a stabil-
PREVENTION AND REGRESSION OF ATHEROSCLEROSIS


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