Cardiovascular Interaction of Nicotine, Ergonovine, and Hypercholesterolemia in the Rabbit

By Duane G. Wenzel, Ph.D., James A. Turner, M.D., Scott W. Jordan, M.D., and Jasbir Singh, M.S.

A high incidence of myocardial necrosis and lesions of the feet and lower legs has been demonstrated in rabbits treated with nicotine and a hypercholesterolemic diet. Peripheral circulatory changes were marked in all rabbits on the hypercholesterolemic diet, but the effects were more severe and occurred earlier in the course of treatment for those animals also receiving nicotine. Coronary vessels of the cholesterol-fed animals were thickened with advanced atherosclerotic changes, but, in those also treated with nicotine, a consistent development of necrosis was observed in addition to these changes.

The purpose of this paper is to describe a study designed to determine possible causes for the observed interaction of nicotine and a hypercholesterolemic diet in both the myocardial and the peripheral vascular effects. As an initial probing experiment, an attempt was made to relate several physiological parameters to the pathological effects. The nicotine treatment had not previously influenced plasma cholesterol or phospholipid levels on the high (1 per cent) cholesterol diet and thus these determinations were not repeated. From histopathological evidence obtained at the termination of this initial study, however, it was apparent that the anticipated myocardial necrosis had not developed. To determine the cause for this apparent discrepancy, the experiment was repeated with the addition of ergonovine to the usual regimen. Ergonovine was the only additional factor known to be present in the earlier study demonstrating myocardial pathology. It had been employed in order to demonstrate alterations in the electrocardiogram indicative of coronary artery disease.

Methods

An initial experiment employed four groups of 12 female, albino, New Zealand rabbits per group. The rabbits were eight weeks of age and weighed between 2.8 and 3.5 Kg. Groups received the following treatments: (1) untreated control, Purina rabbit chow and water ad libitum; (2) 1.14 mg./kg./day of nicotine as the alkaloid in the drinking water; (3) 1 per cent cholesterol and 5 per cent cottonseed oil in the diet; (4) combined treatments of groups 2 and 3.

Systolic blood pressures were measured with the use of a Grant ear capsule. The thermal circulation index (TCI) was employed to assess the effects of the treatments upon peripheral circulation. This index is the ratio of the difference between body surface temperature and external (room) temperature, and the difference between the internal body temperature and the skin temperature. It is based upon the rate at which internal body heat is transferred to the body surface and thus indirectly determines peripheral blood flow.

TCI = \frac{\text{Skin temperature - room temperature}}{\text{Rectal temperature - skin temperature}}

Temperatures were measured with a YSI Model 41-Tele-thermometer employing thermistors as temperature-sensing elements. A flexible waterproof probe, YSI no. 8430, was used to determine the rectal temperatures, and a YSI no. 8442 pancake-type probe was employed for determining skin temperatures. Room temperature was measured with an ordinary laboratory thermometer.

Blood coagulation time was determined by the capillary tube method. Blood was withdrawn from a stab wound in the tail. Because of the short coagulation time, the tubes were broken as rapidly as possible or about every two seconds.

Control values of all measurements were obtained before the treatments were initiated and at four-week intervals thereafter for the remainder of the 24-week test period. Coagulation times were not obtained at the four- and eight-week periods.

Following the 24-week tests, the remaining...
animals were sacrificed and the hearts examined grossly and microscopically. Multiple cross sections were made perpendicular to the basal-apical axis at the level of the aortic valve ring and midway between the aortic valve ring and apex. Two sections were also taken through the leg vessels to correspond with the femoral artery and the anterior tibial.

Because of the lack of marked microscopic changes in the cholesterol and nicotine-cholesterol hearts, the reason for this difference was sought. Ergonovine had previously been used to treat the animals in which myocardial necrosis had been demonstrated; therefore, this procedure was repeated. Two additional groups of rabbits were established using the following regimens: The first group received the hypercholesterolemic diet plus the injection of 0.05 mg./Kg. of ergonovine maleate into the marginal ear vein at four-week intervals. This dose of ergonovine and the frequency of administration was the same as that previously employed.1 The second group of rabbits was administered 1.14 mg./Kg./day of nicotine alkaloid in the drinking water in addition to the regimen of the first group. The hearts and eyes were again studied at 24 weeks. The eyes had been previously noted to have a severe lipid infiltration of the iris.5

**Results and Discussion**

The mean thermal circulation indices (TCI's), as shown in figure 1 and table 1, indicated that the peripheral circulation of the control animals had not been appreciably changed, but, from the fall in the mean TCI's of the nicotine-treated groups, it is apparent that the peripheral circulation had been reduced by the administration of nicotine and that the effect was cumulative throughout the 24-week period. When the 20-week values were subjected to an analysis of variance, it was found that the control group values differed significantly from those of the nicotine group ($P<0.01$) and the nicotine-cholesterol group ($P<0.05$). The 20-week values were employed throughout for the analysis of variance because of the high mortality in the cholesterol-treated groups during the last four weeks of the study. The immediate increase in the mean TCI of the cholesterol group, while not significant in the statistical sense, displays a definite trend indicating an initial increase in peripheral circulation followed by a gradual return to the control level. It is quite apparent that the peripheral pathological changes that resulted from the high cholesterol-oil diet were not directly related to the effect upon peripheral circulation, at least as expressed by the TCI. These lesions have been previously described.1 Both groups receiving cholesterol had demonstrated the lesions of the feet and legs, and once again these were observed to occur earlier and with greater severity in the nicotine-cholesterol animals. The peripheral pathological changes, apparently the result of the cholesterol-fat diet, were merely intensified by the reduction in circulation or other concomitant action of nicotine but did not directly result from its circulatory effect.

It is not surprising that the administration of nicotine should lower the skin temperature relative to that of the body interior and thus depress the TCI. Reductions in the skin temperature induced by smoking or by the administration of nicotine are well known, but such studies have related only to the acute effects of nicotine administration. There was no evidence in this study to indicate the production of acquired tolerance to any of the cardiovas-
cicular functions tested. The steady decline of the TCI values of rabbits continuously treated with nicotine suggests the need for similar carefully conducted chronic nicotine studies in the human.

The pressor effect of nicotine is illustrated by the mean systolic pressures as shown in figure 2 and table 1. It is evident that all groups demonstrated some increase in the systolic pressure. Analysis of the 20-week values revealed the systolic pressure of both nicotine groups to be significantly raised ($P<0.01$). While it is well known that nicotine may induce a transient and rather erratic pressor action in humans, the cumulative rise observed with rabbits may be of practical significance. It would be of interest to know whether the pressure could be still further elevated by prolongation of the treatment and whether it was fixed. The fact that the combination of nicotine and hypercholesterolemic diet resulted in a pressor interaction is also of practical interest. At the 20-week period the combination induced a significantly higher blood pressure than either the cholesterol or the nicotine treatments ($P<0.01$).

It is frequently postulated that hypercoagulability of the blood may be associated with the pathogenesis of atherosclerosis. Coronary thrombosis is one manner by which subclinical atherosclerosis may be converted to a clinical disease, but the precise role that the clotting rate of the blood plays in the production of thrombi is open to question. As may be observed in figure 3 and table 1, all groups of the experimental animals demonstrated an increased rate of blood coagulation over the 24-week period ($P<0.01$). While the coagulation rate of the control animals had also increased by the twentieth week, the rates of all treated groups had become significantly faster ($P<0.01$).

Histopathological examination of the lower legs indicated that the peripheral vessels of the nicotine-cholesterol group may have had a minimal increase in involvement over the cholesterol group. This was not enough to warrant a clear-cut statement of any major difference, however.

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**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Systolic pressure</th>
<th>Coagulation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>70.17±</td>
<td>0.86±</td>
</tr>
<tr>
<td>Nicotine</td>
<td>78.00±</td>
<td>0.86±</td>
</tr>
<tr>
<td>Nicotine + Cholesterol</td>
<td>78.00±</td>
<td>0.86±</td>
</tr>
<tr>
<td>Nicotine + Hyperebolesterolemic Diet</td>
<td>78.00±</td>
<td>0.86±</td>
</tr>
</tbody>
</table>

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There was a marked discrepancy in the cardiac pathological changes when compared to those of the earlier experiment. As anticipated, both cholesterol-treated groups demonstrated thickening of the coronary vessels with perivascular aggregates of foam-filled macrophages. These changes, however, were essentially the same in both groups with no evidence of greater pathology in the group receiving both nicotine and cholesterol. In the previous study, not only did those animals treated with nicotine and cholesterol demonstrate more severe histopathological changes, including fatty metamorphosis and necrosis, than was observed with the cholesterol group, but even the group that received nicotine alone as a treatment had been found to have the small branches of the coronaries thickened and fibrotic. In the present study, the vessels of the nicotine-treated rabbits were essentially normal.

Treatments and tests were repeated in a new series of rabbits with the addition of the intravenous administration of ergonovine maleate, 0.05 mg./Kg., at four-week intervals. This was the only known additional factor in the first study and had been ignored in this preliminary evaluation because of the infrequent administration. Ergonovine was previously employed in order to demonstrate electrocardiographic evidence of coronary insufficiency. The animals were sacrificed at 24 weeks and the hearts and eyes examined.

Essentially the same changes were found in the eyes of the cholesterol and nicotine-cholesterol groups. Although slight differences could be grossly observed in the extent of iris infiltration, there was no real histological variance. Apparently, any vascular interaction occurring within the nicotine-cholesterol-ergonovine treatment did not include involvement of the vessels of the eye.

The systolic blood pressures, coagulation times, and thermal circulation indices did not differ significantly from those values previously measured in corresponding groups without ergonovine.

All of the hearts of those animals treated with the hypercholesterolemic diet plus ergonovine grossly showed fine white streaks in the ventricular myocardium. Microscopically, the coronary arteries demonstrated cells with an abundant foamy cytoplasm, presumably fat, in the intima. These deposits varied from localized plaques to lesions that completely occluded the arterial lumen. The media of all coronary arteries was normal. These findings are essentially the same as those that had been observed in the rabbits treated with the hypercholesterolemic diet alone. There were, however, striking differences between the hearts from cholesterol-ergonovine and nicotine-cholesterol-ergonovine treated animals. Foam cells were present in greater number, but more significant was the presence of myocardial necrosis in all hearts of this latter group. This necrosis varied from small foci of inflammatory cells to large areas of coagulation necrosis with infiltrates of polymorphonuclear leukocytes, lymphocytes, and plasma cells. It was most evident in the papillary muscle of the left ventricle and to a lesser extent in the trabeculae carneae of some hearts. These findings demonstrated that the pathological changes in the heart previously attributed to a nicotine-cholesterol interaction were, in actuality, the result of three factors: nicotine, hypercholesterolemic stimulus, and ergonovine.

It has been generally noted that cholesterol feeding per se in animals has not resulted in coronary thrombosis or cardiac infarcts. While experimental hypertension has been found to accelerate and aggravate cholesterol-induced atherosclerosis in rabbits, there is likewise no evidence to indicate that this combination of treatments will result in myocardial necrosis. This belief is borne out in the first portion of the present study by the fact that a nicotine-cholesterol treatment resulted in both hypertension and cholesterol-occluded coronaries, but not in myocardial necrosis.

While the role of an increased rate of blood coagulation in myocardial infarction is open to debate, the results obtained under the conditions of the experiments would indicate that it, like the hypertension, is not a major con-
Control nicotine hypercholesterolemic diet

Nicotine + hypercholesterolemic diet

**Figure 2**

Mean systolic blood pressures.

**Figure 3**

Mean coagulation times.

A contributory factor in the production of the observed myocardial necrosis.

Although the limited evidence would make any explanation for the interaction of the nicotine-cholesterol-ergonovine treatment largely speculative, certain definite possibilities are presented for consideration. It is generally recognized that hypercholesterolemia will lead to the production of experimental atherosclerosis, but it is doubtful whether hypercholesterolemia alone can account for the malignant pathology of atherosclerosis in animals or man. As the combination of nicotine and ergonovine has been found to induce thickening and fibrosis of the small branches of the coronaries, a reasonable assumption might be that the observed myocardial necrosis resulted from a damaged vascular bed on which the effects of hypercholesterolemia were superimposed. The postulated requirement of pre- or concomitantly existent vascular damage for the overt clinical involvement related to hypercholesterolemia has been suggested by numerous investigators and appears to be justified by the absence of coronary thrombosis or infarcts in rabbits receiving a hypercholesterolemic diet as the sole treatment.

Neither nicotine nor ergonovine treatments alone, together, or singly in combination with cholesterol resulted in myocardial pathology greater than that induced by the cholesterol. It is, therefore, obvious that the combined effects of the nicotine, ergonovine, and cholesterol are necessary for the observed damage to the myocardium. While the ergot alkaloids in excessive or continued dosage are known to damage the vascular endothelium, neither such effects nor changes in the blood pressure or coagulation times were observed with the dosage and frequency of administration of ergonovine employed in this study. Ergonovine is more potent than ergotoxine when tested on the cock’s comb and has been reported to cause gangrene in humans. Similarly, under the conditions in which nicotine was used as a single treatment, pathological changes were not apparent. As most of the vascular actions of small to moderate doses of nicotine are related to the release of cata-
cholamines, it is probable that epinephrine and (or) norepinephrine account for the action of this drug. Ergonovine, in contrast to the other ergot alkaloids, is not sympatholytic in its action and has been demonstrated to enhance the amplitude and duration of the actions of epinephrine and norepinephrine. As the repeated administration of epinephrine has been found to damage the intima and is stored in vascular tissue, it is suggested that the role of ergonovine may be both indirect, through enhancement and prolongation of the nicotine-released catecholamines, and direct, through a vascular-damaging effect of its own. A further possible factor in this complex interrelationship may be related to an increased cardiac work load resulting from the release of catecholamines in the presence of acute ergonovine-induced coronary constriction. Such an interaction could increase the metabolic demands of certain critical areas of the myocardium beyond the capacity of the constricted vessels to supply the metabolic requirements.

Irrespective of the mechanism, it may be concluded that the nicotine-ergonovine combination in the rabbitacts synergistically as a premissive agent for the myocardial damage induced through hypercholesterolemia.

**Summary**

Rabbits were treated with nicotine and a hypercholesterolemic diet for 24 weeks. At the initiation of the experiment and at four-week intervals thereafter, the following factors were measured: peripheral circulation through the use of the thermal circulation index (TCI), systolic blood pressure, and coagulation time. After 24 weeks, the heart and vessels of the hind legs were examined for pathological changes. Throughout the course of the experiment, the peripheral circulation was depressed, the systolic pressure elevated, and the coagulation rate speeded. Peripheral pathological changes were minimal and areas of cardiac necrosis observed in a similar previous study were absent. In order to determine the cause of this apparent discrepancy, a second group of rabbits was similarly treated except that ergonovine was added to the regimen. The 24-week histological examination this time revealed cardiac necrosis in all animals receiving the combined nicotine-cholesterol-ergonovine treatment. This effect had not been obtained with any single member or pair of components of the treatment. Possible explanations for the observed interaction are presented.

**Acknowledgment**

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**References**

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