DNA Synthesizing Cells in Rabbit Heart Tissue After Cholesterol Feeding

By Sanford C. Spraragen, M.D., Victor P. Bond, M.D., Ph.D., and Lewis K. Dahl, M.D.

Studies on the role of cell proliferation in the development of atheromatous lesions in the aorta of rabbits, using thymidine-H³ (H³Th) and autoradiography1,2 have been reported recently from this laboratory.3 While observing the coronary arteries in connection with the above study, many labeled cells were noted in the adjacent myocardial tissues of both control and cholesterol-fed animals. This fact plus the observation that cholesterol feeding appeared to influence the degree of labeling in rabbit heart tissue prompted a more critical evaluation of these chance findings. This evaluation along with selected photomicrographs is reported here.

Methods

EXPERIMENTAL DESIGN

New Zealand white, female rabbits approximately one year of age, weighing from 3 to 5 Kg.,4-6 were caged separately in a temperature-controlled, air-conditioned room. They were assigned at random to one of two groups: the control or cholesterol-fed (test group). Rabbits, one from each group, were paired at random and sacrificed, one pair at a time, at weekly intervals through the fifth week of the trial and then at the seventh, thirteenth, seventeenth, twentieth, and twenty-first weeks.

The control animals were offered 150 Gm. of Purina Rabbit Chow per day. The test animal diet differed only in that 250 mg. of cholesterol (U.S.P.) were admixed with the last 50 Gm. of chow added to the feeding trough. Addition of the cholesterol was accomplished by sprinkling a cholesterol-ether solution of known concentration over evenly spread chow pellets and then allowing the ether to evaporate. Water was permitted ad libitum. Weights were recorded weekly.

CHELSTEROL ANALYSES

Plasma total cholesterol values were determined during the course of the experiment using a modified Abell7 method. An estimate of each animal's usual range of plasma cholesterol was obtained from analyses performed at weekly intervals during the four weeks immediately preceding the initiation of the dietary regimens. Subsequently, cholesterol levels were ascertained one week after the animals were started on their respective diets and again just prior to the administration of the H³Th. Animals were fasted overnight before blood was drawn.

ADMINISTRATION OF ISOTOPE

The animals each received 0.5 mc. of thymidine-H³* intravenously (physical half life of tritium, 12.26 years; beta energy, 0.018 Mev) per Kg. of body weight one hour prior to sacrifice.

SELECTION OF TISSUE AND PREPARATION OF STAINED AUTORADIOGRAMS

Death was precipitated rapidly by the intravenous injection of air. The heart was excised, and a block taken from the left anterior ventricular wall was fixed in 10 per cent buffered formal. They were assigned at random to one of two groups: the control or cholesterol-fed (test group). Rabbits, one from each group, were paired at random and sacrificed, one pair at a time, at weekly intervals through the fifth week of the trial and then at the seventh, thirteenth, seventeenth, twentieth, and twenty-first weeks.

The best-fitting rectangle was measured by means of an ocular micrometer. The rectangles were inked on the coverslips over the sections and checked microscopically for position. All dimension measurements were made in triplicate. The defined areas on the sections were then scanned systematically using an ocular (10X) and a high dry objective (43X). An oil immersion

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*1.9 curies per mM, Schwarz Laboratories, Mt. Vernon, New York.

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DNA SYNTHESIZING CELLS

TABLE 1

Plasma Cholesterol Values and Labeling Data

<table>
<thead>
<tr>
<th>Week sacrificed</th>
<th>Control diet (1 rabbit/group)</th>
<th>Cholesterol-supplemented diet (1 rabbit/group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma cholesterol (mg./100 ml.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Usual* (mean)</td>
<td>1-wk. value</td>
</tr>
<tr>
<td>1st</td>
<td>78</td>
<td>62</td>
</tr>
<tr>
<td>2nd</td>
<td>59</td>
<td>74</td>
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</tr>
<tr>
<td>21st</td>
<td>56</td>
<td>57</td>
</tr>
</tbody>
</table>

*Mean of four pretrial plasma cholesterol determinations.

FIGURE 1

H¹¹Th-labeled mesenchymal cells (arrows) in a section cut tangential to the long axis of the muscle fibers. Exposure time six days. H and E stain, × 800.

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FIGURE 2

H¹¹Th-labeled mesenchymal cell (arrow) adjacent to muscle fiber cell cut perpendicular to its long axis. Exposure time six days. H and E stain, × 800.
FIGURE 3
Examples of $^3$H-labeled capillary endothelial cells (arrows). Exposure time six days. H and E stain, $\times 480$.

object was used to confirm the existence of a suspected labeled cell before recording its presence. At least one hour was taken to scan each section.

Results

CHOLESTEROL LEVELS

Test animals (table 1) manifested elevated plasma cholesterol levels by the end of the first week of dietary feeding and significant hypercholesterolemia by the time of sacrifice.

HISTOLOGICAL

No histological difference was found in the myocardial tissue of the two groups. Specifically, no evidence of focal necrosis, degeneration, or inflammation in either set of tissues was encountered. As reported elsewhere, the daily 250 mg. cholesterol supplement failed to produce vascular lesions in the coronary vessels during the course of study. Indeed, the coronary vessels of the test animals were not found to differ histologically from those of the control animals.

AUTORADIOGRAPHIC

An appreciable number of labeled cells was observed in the heart tissues of both groups of animals. The number of labeled cells and the labeling index (cells/mm$^2$ of heart tissue) for each animal are presented in table 1. As judged from the H and E stained autoradiograms used in this study, it appeared the mesenchymal cells (figs. 1 and 2) accounted for most of the labeled cells observed and, in the test animal, were the cells which seemed to be influenced by dietary cholesterol. Labeled capillary endothelial cells (fig. 3) were also commonly seen in both tissue groups, and rarely, labeled cells considered to be cardiac muscle cells (figs. 4 and 5) were seen. Figure 6 depicts labeled epithelial cells of the control duodenal tissue.

STATISTICAL

In the seven sets of paired data, the labeling index (cells/mm$^2$ of heart tissue) was higher in the cholesterol-fed animal than in the control animal in five of the pairs, showed no difference in one, and was less than the control in one (fig. 7). Although the available data do not warrant comparison of experimental and control animals at any one point in time, the results suggest that cholesterol feeding positively influences the labeling in-
DNA SYNTHESIZING CELLS

FIGURE 5
Same cell (arrow) as seen in figure 4. In this instance, the camera was focused below the label to demonstrate the nucleus better. Note relative size, shape, and chromatin configuration of the nucleus and polar halo. H and E stain, X 800.

FIGURE 6
H$^3$Th-labeled epithelial cells in a section taken from the duodenum. This highly proliferative tissue was used to serve as a control for processing the autoradiograms. Exposure time six days. H and E stain, X 480.

dex during the first four weeks of the trial and perhaps again around the seventeenth week. The labeling indices of the seven pairs of rabbits were compared by a paired t-test, which yielded a t value of 2.71 and a P value of 0.03. If the indices for all the rabbits, 7 control and 10 test animals, were compared by the standard t-test, assuming independent samples, a t value of 1.81 and a P value of 0.09 were obtained. These tests were interpreted as suggestive, but not conclusive evidence that the cholesterol feeding prompted an increase in the number of cells producing DNA in the myocardial tissue of the test rabbits.

It is of interest to note that the control rabbits with the lowest plasma cholesterol values also have the lower labeling indices. However, no definite relationship between plasma cholesterol levels and labeling indices could be found when all the data were considered by either charting of the data or by analysis of covariance calculations. The final weights of the rabbits and the size of the section (table 1) appeared unrelated to the heart tissue findings.

Discussion

The interesting aspect of this preliminary study is the suggestive evidence that, in the absence of recognizable histopathology, cholesterol feeding influences the number of cardiac mesenchymal cells engaged in the synthesis of DNA in the rabbit. Clearly, further investigations specifically designed to test this possibility are needed. The results reported here indicate that the first few weeks of cholesterol feeding deserve particular attention and that perhaps more conclusive results might have been obtained if initial plasma cholesterol values were used as the basis for pairing the animals.

Using similar techniques, other investigators have made observations pertaining to DNA activity in heart tissue of the adult rat and mouse. Schultz and Oehlerl7 and Edwards and Klein both reported an absence of labeling of cardiac muscle cells. The former investigators commented on having observed labeled mesenchymal cells in the heart while the latter reported labeling of up to 1.9 per cent of the capillary endothelial cells in mouse heart tissue. Leblond et al. listed cardiac muscle among those tissues comprised of stable cell populations. They observed, however, that cardiac muscle cells depending upon their location might have different proliferative potentials. They found evidence of cells in DNA synthesis in the outer one-third of the ventricular walls but not in the atria, ventricular sep-
turn, and inner two-thirds of the ventricular wall. The findings in this study are in general agreement with these published data in that mesenchymal, capillary endothelial, and, rarely, what were interpreted as myocardial cells were observed to be labeled.

Summary
Suggestive evidence that cholesterol feeding may increase the number of thymidine-H\textsuperscript{3} labeled cells in the heart tissues of rabbits was presented. This increment appeared to be primarily restricted to the interstitial cells and was not related to associated degenerative, necrotic, or inflammatory changes.

Evidence of DNA synthesis was also found in capillary endothelial cells and, rarely, in what were considered to be myocardial muscle cells.

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References
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