Effect of Vitamin D, Sucrose, Corn Oil and Endocrines on Tissue Cholesterol in Rats

By Cheng-Chun Lee, Ph.D., and Roy G. Herrmann, Ph.D.

A diet containing 65 per cent sucrose with added cholesterol resulted in hypercholesteremia and increased tissue cholesterol deposition in rats. Ten per cent corn oil with added cholesterol in Purina laboratory chow had no apparent effect on the serum total cholesterol but increased cholesterol deposition in tissues. Ovariectomy was without significant effect. Thyroidectomy or hypophysectomy aggravated the hypercholesteremia and the increased tissue cholesterol deposition induced by the sucrose diet. The effect of hypophysectomy was not due to the loss of thyrotrrophic hormone alone. Addition of vitamin D aggravated hypercholesteremia and increased liver cholesterol deposition in normal, thyroidectomized or hypophysectomized rats fed the sucrose diet. In ovariectomized rats, the tissue cholesterol deposition was also augmented. When corn oil diet was fed, the supplementary vitamin D increased the serum total cholesterol level of the thyroidectomized rat.

Pfleiderer observed in 1932 that the development of arterio- and atherosclerosis was stimulated by the combined administration of vitamin D and cholesterol. Others reported that vitamin D intensified the degree and increased the rate of development of hypercholesteremia in rabbits on a cholesterol diet. To study agents that may enhance the hypercholesteremic effect of sucrose diet, vitamin D was added.

It has been shown that estrogen administration to man produces a slight but significant lowering of serum cholesterol. Evidence has been presented which suggests that the hypercholesteremia of hypophysectomized dogs is due to an acute deficiency of thyroid hormone. On the other hand, it has also been observed that the hypercholesteremia of hypophysectomized dogs is abolished by hypophysectomy. In view of these reports, the effect of ovariectomy, thyroidectomy and hypophysectomy on the serum and tissue total cholesterol levels in rats fed the sucrose diet and the corn oil diet has been studied. Preliminary reports of this work have appeared.

METHODS

Female rats of the Holtzman strain weighing between 135 and 155 Gm. were used in all cases except the hypophysectomized female animals. Thyroidectomy and ovariectomy were performed one week prior to the start of the experiment. All rats were fed one of the following diets ad lib. for 8 weeks: (1) 2 per cent cholesterol, 10 per cent corn oil and 88 per cent Purina laboratory chow with and without 400 USP units of vitamin D per gram of diet (corn oil diet); (2) 2 per cent cholesterol, 98 per cent high carbohydrate test diet (table 1)† with and without 400 USP units of vitamin D per gram of diet (sucrose diet). All diets were kept under refrigeration and the food cups were filled every other day.

Daily food consumption was measured and weekly body weight recorded during the experi-

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From The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.

Received for publication November 24, 1958.
DIET AND TISSUE CHOLESTEROL

ment. At weekly intervals, blood samples were drawn by heart puncture under light ether anesthesia and serum total cholesterol was determined. At the termination of the experiment, all animals were sacrificed and the liver and aorta were removed for total cholesterol analysis. The liver was blotted on filter paper to remove excess blood. The adhering fat and the connective tissue of the entire aorta were removed by scraping with a pair of fine curved forceps. The total cholesterol of serum and tissue was determined by the ferric chloride method as adapted by Herrmann. The heart, kidney, adrenals and spleen were examined grossly and removed for microscopic examination. Fatty degeneration was found in the liver, which showed increased cholesterol deposition.

RESULTS

Effect of Diet. The normal rats fed a diet containing 10 per cent corn oil with added cholesterol did not show any significant change in serum total cholesterol concentrations during the entire course of the experiment (fig. 1). When normal rats were fed a diet containing 65 per cent sucrose, on the other hand, the serum total cholesterol levels were, at the end of the experiment, 2 to 3 times higher than the initial levels.

The liver total cholesterol of the normal control rats was 246 ± 5 mg./100 Gm. wet weight and that of the aorta was 177 ± 4 mg./100 Gm. wet weight (table 2). The normal rats fed the corn oil diet showed a significant increase in both liver and aorta total cholesterol: 2980 ± 695 and 253 ± 24 mg./100 Gm. wet weight, respectively. The sucrose diet also resulted in a significant increase in the tissue total cholesterol levels. Total cholesterol concentrations of 2331 ± 251 and 201 ± 5 mg./100 Gm. wet weight were found in the liver and the aorta, respectively.

Effect of Ovariectomy. Ovariectomy was found to have no significant effect on serum and liver total cholesterol levels as compared with normal rats fed the same sucrose diet (table 2). However, in the aorta a slight increase in total cholesterol concentration was observed.

Effect of Thyroidectomy. The serum total cholesterol of thyroidectomized rats fed the corn oil diet increased gradually during the first half of the experiment and was 50 per cent to more than 100 per cent higher than the initial levels throughout the experiment. These rats had significantly higher liver and aorta total cholesterol concentrations than the normal controls (table 2), i.e., 3080 ± 633 and 225 ± 11 mg./100 Gm. wet weight, respectively. However, these serum and tissue total cholesterol levels were not significantly different from those of the normal rats receiving the corn oil diet.

The serum total cholesterol level of thyroidectomized rats fed the sucrose diet increased rapidly during the first 3 weeks of the experiment and continued to increase for the remaining period of the experiment (fig. 2). At the termination of the experiment, a level of 578 mg. per cent was reached. The sucrose diet resulted in a liver total cholesterol level of 3931 ± 430 mg./100 Gm. wet weight in these rats (table 2). The aorta total cholesterol concentrations averaged 272 ± 18 mg./100 Gm. wet weight. These serum and tissue total cholesterol concentrations were significantly higher than those of the normal rats receiving the sucrose diet.

Effect of Hypophysectomy. Hypophysectomy had a pronounced effect on serum and tissue total cholesterol levels (table 2). The serum and liver total cholesterol concentra-

| Table 1.—Composition of High Carbohydrate Test Diet |
|----------------|------------------|
| Ingredients                | Per cent |
| Vitamin-free test casein, GBI | 18 |
| Sucrose                   | 66 |
| Vegetable oil (hydrogenated) | 8 |
| Yeast—liver powder        | 4 |
| Salt mix, USP XIV          | 4 |
| Vitamin supplements       | Gm./100 lbs. |
| Alpha-tocopherol           | 13,500 |
| Calcium pentothenate       | 0.585 |
| Choline chloride           | 112,000 |
| Linoleic acid              | 225,000 |
| Purinaxine                 | 0.360 |
| Riboflavin                 | 0.725 |
| Vitamin A                  | 5,000 USP U./lb. |
| Vitamin D                  | 150 USP U./lb. |

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tions of these rats fed either the sucrose diet or the corn oil diet were increased two- to threefold and the aorta total cholesterol was also higher than that of normal rats fed the corresponding diets. It is of interest that the corn oil diet produced a twofold increase in the deposition of cholesterol in the liver as compared with the sucrose diet.

Effect of Hypophysectomy-Thyroidectomy. Thyroidectomy-hypophysectomy in rats receiving the corn oil diet did not raise the serum and tissue total cholesterol beyond that induced by hypophysectomy alone (table 2). These rats, however, exhibited higher serum and tissue total cholesterol levels than did the thyroidectomized rats fed the same diet (fig. 3, table 2).

Effect of Vitamin D. In normal, thyroidectomized or hypophysectomized rats receiving the sucrose diet (table 2), addition of vitamin D aggravated the hypercholesteremia and increased cholesterol deposition in the liver. The total cholesterol concentration in the aorta was apparently not altered in these rats. In ovariecetomized rats, the supplementary vitamin D did not significantly affect the hypercholesteremia induced by the sucrose diet but it increased the cholesterol deposition in both the liver and the aorta.

Addition of vitamin D to corn oil diet had no significant effect on the serum total cholesterol concentrations of the normal or hypophysectomized rat, but aggravated the hypercholesteremia in the thyroidectomized rat. The cholesterol deposition in liver and aorta was without apparent change.

Discussion

The sucrose diet with added cholesterol produced hypercholesteremia and increased tissue total cholesterol deposition in rats under various experimental conditions. Portman et al. suggested that the altered gastrointestinal flora in rats fed a diet containing sucrose might be responsible for the increased serum cholesterol. A decreased excretion of total cholic acids in the bile and of total cholic acids and \( \beta \)-hydroxysterols in the feces was reported in rats fed such diets. Grant and Fahrenbach attributed the hypercholesteremic effect of sucrose in chicks to a greater uptake of dietary cholesterol. In the present experiments, the amount of sucrose diet con-
### Table 2.—Terminal Tissue Cholesterol Concentrations of Normal, Ovariectomized, Thyroidectomized and Hypophysectomized Rats

<table>
<thead>
<tr>
<th>Animal</th>
<th>Diet</th>
<th>No. of animals</th>
<th>Ave. food consumption (Gm./day/rat)</th>
<th>Ave. weight gain or loss (Gm./rat)</th>
<th>Tissue cholesterol concentration (mg. ± S.E./100 ml. or Gm. wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serum Liver Aorta</td>
</tr>
<tr>
<td>Normal</td>
<td>Purina chow</td>
<td>12</td>
<td></td>
<td></td>
<td>100 ± 5 246 ± 3 177 ± 4</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>6</td>
<td>10.4 + 58</td>
<td></td>
<td>233 ± 38 2351 ± 251 201 ± 5</td>
</tr>
<tr>
<td></td>
<td>Sucrose with vitamin D</td>
<td>6</td>
<td>11.9 + 58</td>
<td></td>
<td>332 ± 33 4136 ± 167 175 ± 5</td>
</tr>
<tr>
<td></td>
<td>Corn oil</td>
<td>6</td>
<td>11.6 + 61</td>
<td></td>
<td>111 ± 8 2980 ± 695 252 ± 24</td>
</tr>
<tr>
<td></td>
<td>Corn oil with vit. D</td>
<td>6</td>
<td>11.9 + 55</td>
<td></td>
<td>100 ± 7 2048 ± 378 235 ± 20</td>
</tr>
<tr>
<td>Ova X</td>
<td>Sucrose</td>
<td>7</td>
<td>10.7 + 72</td>
<td></td>
<td>202 ± 37 2113 ± 548 220 ± 6</td>
</tr>
<tr>
<td></td>
<td>Sucrose with vitamin D</td>
<td>6</td>
<td>12.4 + 106</td>
<td></td>
<td>192 ± 17 3868 ± 457 241 ± 10</td>
</tr>
<tr>
<td>Thy X</td>
<td>Sucrose</td>
<td>6</td>
<td>8.1 + 17</td>
<td></td>
<td>578 ± 85 3931 ± 430 272 ± 18</td>
</tr>
<tr>
<td></td>
<td>Sucrose with vitamin D</td>
<td>6</td>
<td>8.4 + 22</td>
<td></td>
<td>750 ± 74 4147 ± 447 283 ± 14</td>
</tr>
<tr>
<td></td>
<td>Corn oil</td>
<td>6</td>
<td>13.2 + 40</td>
<td></td>
<td>149 ± 28 3080 ± 633 225 ± 11</td>
</tr>
<tr>
<td></td>
<td>Corn oil with vit. D</td>
<td>6</td>
<td>12.0 + 20</td>
<td></td>
<td>199 ± 15 3984 ± 358 228 ± 7</td>
</tr>
<tr>
<td>Hyp X</td>
<td>Sucrose</td>
<td>5</td>
<td>5.6 − 16</td>
<td></td>
<td>557 ± 66 4269 ± 294 256 ± 24</td>
</tr>
<tr>
<td></td>
<td>Sucrose with vitamin D</td>
<td>6</td>
<td>6.1 − 19</td>
<td></td>
<td>716 ± 27 4817 ± 410 278 ± 10</td>
</tr>
<tr>
<td></td>
<td>Corn oil</td>
<td>6</td>
<td>8.3 + 4</td>
<td></td>
<td>536 ± 82 8385 ± 830 293 ± 18</td>
</tr>
<tr>
<td></td>
<td>Corn oil with vit. D</td>
<td>8</td>
<td>7.3 − 8</td>
<td></td>
<td>473 ± 69 8460 ± 540 201 ± 13</td>
</tr>
<tr>
<td>Hyp X-Thy X</td>
<td>Corn oil</td>
<td>7</td>
<td>8.1 + 1</td>
<td></td>
<td>552 ± 47 8606 ± 777 292 ± 8</td>
</tr>
</tbody>
</table>

Summed by the rats was about the same as, or even less than the amount of corn oil diet consumed. Apparently the hypercholesteremic effect of the sucrose diet cannot be the result of an increased dietary cholesterol uptake.

The corn oil diet had no apparent effect on the serum total cholesterol of normal rats. In thyroidectomized rats fed the same diet, the serum total cholesterol was significantly increased. Furthermore, thyroidectomy aggravated the hypercholesteremia and the cholesterol deposition in the liver induced by the sucrose diet. Several investigators have shown that hypothyroidism is associated with a rise of plasma cholesterol due to a relatively greater decrease in the rate of cholesterol elimination, despite a markedly depressed rate of hepatic cholesterol synthesis. These authors observed that hypothyroid rats eliminated about one half as much cholesterol in the bile as did the normal controls. Eriksson extended these findings and demonstrated that the formation of bile acids and their elimination in the bile was diminished in the hypothyroid state. Therefore, a changed rate in cholesterol and cholic acid elimination through the biliary system seems to be involved in the development of abnormal cholesterol metabolism induced by both the sucrose diet and by thyroidectomy in these studies.

Entenman et al. suggested that an acute deficiency of thyroxine per se was responsible for the elevation of blood lipids after ablation of the pituitary gland in dogs. On the other hand, Thompson and Long reported that the presence of the hypophysis was necessary for the development of hypercholesteremia in thyroidectomized dogs. In the present studies, both the thyroidectomized and the hypophysectomized rats receiving the sucrose diet developed about the same degree of serum and tissue total cholesterol levels. When corn oil diet was fed, the hypophysectomized and
the hypophysectomized-thyroidectomized rats developed higher serum and tissue total cholesterol levels than those of the thyroidectomized rats. It appears that the pituitary gland is not essential for the development of hypercholesteremia in thyroidectomized rats and that the loss of thyrotrophic hormone is not the only factor responsible for the increased cholesterol concentrations in the serum and tissues after removal of the pituitary gland.

It is of interest that the addition of vitamin D in the present study aggravated the hypercholesteremia in the normal, thyroidectomized or hypophysectomized rats fed the sucrose diet, whereas in these rats on the corn oil diet, supplementary vitamin D had no apparent effect. In the ovariectomized rat receiving the sucrose diet, vitamin D appeared to have no effect on serum total cholesterol but significantly increased cholesterol deposition in tissues. It may be pointed out that 25 units of vitamin D were required to yield a density-reading equivalent to one microgram of cholesterol by the analytical method employed. Therefore, storage of this vitamin in serum and tissues could not account for the increased cholesterol values.

SUMMARY

Without addition of cholic acid, the sucrose diet caused hypercholesteremia in rats, whereas the corn oil diet had no apparent effect on the serum total cholesterol concentration. Both the sucrose and the corn oil diet significantly increased the cholesterol deposition in the liver and in the aorta. In rats on the sucrose diet, ovariectomy did not significantly affect serum and liver total cholesterol levels as compared with levels in normal rats on the same diet. Thyroidectomy aggravated the hypercholesteremia and cholesterol deposition in the liver and the aorta induced by the sucrose diet. The serum and tissue total cholesterol concentrations of thyroidectomized rats receiving the corn oil diet did not differ significantly from those of normal rats receiving the same diet. Hypophysectomy augmented the hypercholesteremia and cholesterol deposition in the liver and the aorta induced by the sucrose diet. It also greatly increased the serum and tissue total cholesterol concentrations in rats receiving the corn oil diet. The high serum and tissue total cholesterol levels induced by hypophysectomy could not be explained by loss of thyrotrophic hormone alone.

When the sucrose diet was fed, the addition of vitamin D increased the serum and liver total cholesterol concentrations of normal, thyroidectomized or hypophysectomized rats. In ovariectomized rats, the serum total cholesterol concentration was not significantly affected but the cholesterol deposition in tissues was higher when vitamin D was added.

When the corn oil diet was fed, the supplementary vitamin D increased the serum total cholesterol level of thyroidectomized rats but had no significant effect on the serum and tissue total cholesterol concentrations of normal or hypophysectomized rats.

ACKNOWLEDGMENT

The authors are indebted to Dr. P. N. Harris for pathologic studies and to Messrs. R. H. Tust and R. O. Froman for assistance.

SUMMARIO IN INTERLINGUA

Sin addition de acido cholic, dietas a sucrosa causava hypercholesterolemia in rattos, du-rante que dietas a oleo de mais habeva nulle apparente effecto super le concentration de cholesterol total del sero. Sed ambes, le dieta a sucrosa e le dieta a oleo de mais, augmentava significativamente le deposition de cholesterol in le hepate e in le aorta. In le rattos recipiente dietas a sucrosa, ovariectomia non alterava significativamente le nivello de cholesterol total in sero e hepate, in comparation con le valores in rattos intacte que recipiva le mesme dieta. Thyroidectomy aggravava le hypercholesterolemia e le deposition de cholesterol induce in le hepate e in le aorta per dietas a sucrosa. Le concentrationes de cholesterol total in le sero e in le histos de thyroidectomisate rattos que recipiva dietas a oleo de mais non differeva significativamente ab le valores constatate in rattos intacte que recipiva le mesme dieta. Hypophysectomia
augmentava la hypercholesterolemia e le deposition de choles-
teric in hepate e aorta previemente induce per un dieta a sucrosa. Illo
etiam augmentava grandemente le concentrationes del cholesterol histic total in rattos que
recipeva le dieta a oleo de mais. Le alte ni-
vellos de cholesterol histic total que esseva in-
ducite per hypophysectomia non poteva esser
explicate esclusivemente per le perdita de
hormon thyrotrophic.

Sub le conditiones del dieta a sucrosa, le
addition de vitamina D augmentava le eon-
centrationes de cholesterol total in le sero e le
hepate de rattos normal, de rattos thyroidecto-
misate, e de rattos hypophysectomisate. In
rattos ovarioectomisate, le concentration de
cholesterol histic total in le sero non esseva afficite
significative, sed le deposition de choles-
terol in le histos cresceva post le addition de
vitamina D.

Sub le conditiones del dieta a oleo de mais,
supplementation con vitamina D augmentava
le nivello de cholesterol total in le sero de
rattos thyroidectomisate sed exerceva nulle
influentia significative super le concentrationes
de cholesterol total in sero e histos de rattos
normal o de rattos hypophysectomisate.

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Circ Res. 1959;7:354-359
doi: 10.1161/01.RES.7.3.354

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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