Inhibitory Effect of "Isocholesterol" on the Absorption of Cholesterol

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The presence on carbon-3 of a free hydroxyl group that may be esterified is a common characteristic of the known sterol inhibitors of cholesterol absorption. The 30-carbon sterols found in wool fat possess such a group, but differ from the previously demonstrated inhibitors of cholesterol absorption in that they do not form insoluble precipitates with digitonin. To determine whether these substances would influence cholesterol absorption, "isocholesterol," a mixture of 30-carbon wool fat sterols, has been fed to the albino rat alone and in combination with cholesterol.

A number of sterols have been observed to interfere with the intestinal absorption of cholesterol. The sitosterols, widely occurring plant sterols, have been the most extensively studied. Peterson found that in chicks the addition of either mixed soybean sterols or of \( \beta \)-sitosterol to a cholesterol enriched diet prevented the anticipated hypercholesterolemia. The hypocholesterolemic effect of the sitosterols has subsequently been demonstrated in mammals, in studies utilizing the rabbit, the rat and man. Other sterols which have been reported to exert an inhibitory effect on cholesterol accumulation in cholesterol fed animals are stigmasterol, ergosterol and dihydrocholesterol.

These compounds share with cholesterol the general properties common to sterols (fig. 1). They all have 4 carbocyclic rings with a side chain attached to the fourth ring in position 17. They have a single secondary alcohol group on the first ring in position 3 which may be esterified. The 30-carbon sterols occurring in wool fat, lanosterol, dihydrolanosterol, agnostero1 and dihydroagnosterol also possess a tetracyclic nucleus with a free hydroxyl group on carbon-3 and a side chain on carbon-17 (fig. 1). The wool fat sterols differ, however, from cholesterol and the sterols which have been demonstrated to interfere with cholesterol absorption in that they have 3 additional methyl groups attached to the cyclic nucleus, two at position 4 and one at position 14.

In view of the differences in structure of these 30-carbon sterols it became of interest to determine their effect, if any, on cholesterol absorption. Difficulty in separating the 4 C-30 sterols of wool fat necessitated the use in this study of the mixture which has been termed "isocholesterol." Lanosterol has been reported to be the predominant component of the mixture with lesser amounts of dihydrolanosterol, agnostero1 and dihydroagnostero1.

Methods

Four experimental diets were prepared as follows: Basic diet, Purina rabbit pellets plus 5 per cent cottonseed oil; High cholesterol, basic diet plus 1 per cent cholesterol, the cholesterol being dissolved in the warmed cottonseed oil prior to its addition to the rabbit pellets; "Isocholesterol," basic diet plus 5 per cent "isocholesterol," the pellets being impregnated with the isocholesterol dissolved in chloroform, and the chloroform removed by evaporation prior to the addition of the cottonseed oil; Cholesterol-Isocholesterol, basic diet plus 1 per cent cholesterol and 5 per cent isocholesterol. The pellets again were impregnated with the isocholesterol dissolved in chloroform and the chloroform evaporated prior to the addition of the cholesterol in cottonseed oil.

Male white rats (Holtzman) of approximately 320 Gm. were employed. In the initial study 16 animals were divided into 4 groups of 4 animals each, the groups having similar mean weights. They were housed in mesh-bottomed cages and maintained in an airconditioned laboratory at approximately 25C. Each of the 4 diets was fed to one of the experimental groups, being offered ad libitum (except as

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INHIBITORY EFFECT OF "ISOCHESTEROL" ON CHOLESTEROL ABSORPTION

Fig. 1. Structural formulas of cholesterol and sterol inhibitors of cholesterol absorption. The previously reported inhibitors of cholesterol absorption (on the left) differ from cholesterol only in the carbon-17 side chain and number and position of double bonds in the cyclic nucleus. The 30-carbon sterols found in wool fat (on the right) have the further difference of 3 additional methyl groups attached to the nucleus, and are not precipitated by digitonin.

noted below) for a period of 13 days. Tap water was provided at all times. At the end of the 13 day period of the special diets the animals were anesthetized with intraperitoneal amobarbital sodium, blood obtained by cardiotomy and the liver removed. The livers were blotted dry, weighed, and digested over night in a 20 per cent solution of potassium hydroxide in alcohol. Cholesterol in serum and liver was determined by the method of Abell and associates.11

In the second study 18 animals were subjected to radiation destruction of the thyroid by the intraperitoneal injection of 875 mc. of I131.4 Two weeks later they were divided into 3 groups of 6 animals each. Each group received one of the following experimental diets: basic diet, high cholesterol diet, and cholesterol-isocholesterol. At the end of 13 days of the special diet the animals were sacrificed and serum and liver cholesterol concentrations determined.

RESULTS AND DISCUSSION

The results of the first experiment, employing euthyroid animals, are given in table 1. The addition of 1 per cent cholesterol to the diet for a period of 13 days resulted in a slight increase in serum cholesterol, which is not statistically significant at the 1 per cent level. There was, however, a pronounced increase in liver cholesterol. The livers in the cholesterol fed rats were grossly fatty, having a yellowish color, increased extractable total lipid, and the cells at the periphery of the lobules were loaded with stainable lipid.

The serum and liver cholesterol levels in the animals fed 5 per cent isocholesterol did not differ significantly from those of the animals fed basic diet alone. Schonheimer in 1930 was unable to detect any intestinal absorption of isocholesterol.12 The recent report of Clayton and Block that lanosterol, the major component of isocholesterol, may serve as a precursor of cholesterol in liver homogenates suggests the possibility that any absorbed isocholesterol might be converted into cholesterol in the liver.13 If this does occur, however, it is not in sufficient amount to result in an increase in liver or serum cholesterol.

In the remaining group of euthyroid animals, the addition of 5 per cent isocholesterol to the 1 per cent cholesterol diet prevented the accumulation of cholesterol in serum and liver which otherwise occurred on the high cholesterol diet. The mean serum and liver concentrations of cholesterol in this group do not differ significantly from those of the group fed basic diet alone. It will be noted that this group lost weight during the period on the experi-

Table 1.—Effects of the Addition of Cholesterol and Isocholesterol Singly and in Combination to the Diet of Euthyroid Rats

<table>
<thead>
<tr>
<th>Diet</th>
<th>Number</th>
<th>Weight change (%)</th>
<th>Serum cholesterol (mg/100 ml.)</th>
<th>Liver cholesterol (mg/100 Gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic diet...</td>
<td>4</td>
<td>+11 ± 3</td>
<td>72 ± 5</td>
<td>303 ± 6</td>
</tr>
<tr>
<td>1 per cent cholesterol..</td>
<td>4</td>
<td>+14 ± 3</td>
<td>88 ± 8</td>
<td>1323 ± 285</td>
</tr>
<tr>
<td>5 per cent isocholesterol...</td>
<td>4</td>
<td>+12 ± 4</td>
<td>82 ± 6</td>
<td>324 ± 26</td>
</tr>
<tr>
<td>1 per cent cholesterol and 5 per cent isocholesterol...</td>
<td>4</td>
<td>−9 ± 5</td>
<td>68 ± 5</td>
<td>342 ± 32</td>
</tr>
</tbody>
</table>

* Values given are the mean and standard deviation (estimated using N — 1).
ment of diet, in contrast to the other three groups, all of which gained weight. The limited supply of isocholesterol available at the time necessitated limiting the amount of food offered to 40 Gm./rat/day. The feeding containers were usually empty in the morning, and it is possible that the weight loss resulted from insufficient food. To determine to what extent this limitation of food might have contributed to the failure of serum and liver cholesterol to increase, a second experiment was conducted when additional isocholesterol became available.

In the second experiment, in an attempt to increase the differences in serum and liver cholesterol resulting from changes in the amount of cholesterol absorbed, animals were rendered hypothyroid by the administration of $^{131}$I.

The results are given in table 2. This time there were no significant differences between the three groups with regard to weight change, and the differences in serum and liver cholesterol can be attributed to the sterols added to the diet. The mean weight loss of 7 to 10 per cent during the period on the experimental diet is the same as that previously observed in animals subjected to $^{131}$I destruction of the thyroid gland and is presumably the result of the hypothyroid state.

Again, as in the euthyroid animals, the addition of 1 per cent cholesterol to the diet resulted in a significant increase in liver cholesterol, and the further addition of 5 per cent isocholesterol had an inhibitory effect on this accumulation of cholesterol in the liver. The changes in serum cholesterol were in the same direction as those in the liver but with the small groups of animals employed are not statistically significant.

From these observations it would appear that isocholesterol, while not itself absorbed in detectable amounts, exerts a marked inhibitory effect on the absorption of cholesterol. Which of the components of isocholesterol are responsible for this effect cannot be determined from the present study.

The four components, lanosterol, agnosterol, and their dihydro derivatives differ only in number and position of double bonds, and all possess a tetracyclic nucleus with a free hydroxyl group on carbon-3. In this respect they resemble the other sterols (sitosterol, stigmasterol, ergosterol, dihydrocholesterol) which have been found to inhibit the absorption of cholesterol. The results of this study are thus compatible with the hypothesis that interference with cholesterol absorption by other sterols is dependent upon a free hydroxyl group on carbon-3 which may be esterified. It has been suggested that they may act by competing with cholesterol for esterification or in some other way preventing the esterification of cholesterol, a step in the transport mechanism by which it is absorbed.

The sterols previously reported as interfering with cholesterol absorption are all precipitable by digitonin. The constituents of isocholesterol are not so precipitated, and it may thus be concluded that the configuration which leads to formation of a difficultly soluble digitonid is not essential to interference with cholesterol absorption.

**SUMMARY**

The effects on serum and liver cholesterol of the administration of “isocholesterol,” a mixture of 30-carbon sterols occurring in wool fat, to the rat has been studied.

The addition of 5 per cent isocholesterol to a low cholesterol diet for 13 days had no significant effect on liver and serum cholesterol levels. The addition of 5 per cent isocholesterol to a 1 per cent cholesterol diet did, however, exert a marked inhibitory effect on the

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**Table 2.—Effects of the Addition of Cholesterol and Cholesterol plus Isocholesterol to the Diet of Hypothyroid Rats**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Number</th>
<th>Weight change (%)</th>
<th>Serum cholesterol (mg./100 ml.)</th>
<th>Liver cholesterol (mg./100 Gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic diet</td>
<td>6</td>
<td>$-8 \pm 4$</td>
<td>$85 \pm 13$</td>
<td>$262 \pm 27$</td>
</tr>
<tr>
<td>1 per cent cholesterol</td>
<td>6</td>
<td>$-8 \pm 2$</td>
<td>$116 \pm 24$</td>
<td>$1606 \pm 320$</td>
</tr>
<tr>
<td>1 per cent cholesterol and 5 per cent isocholesterol</td>
<td>6</td>
<td>$-10 \pm 2$</td>
<td>$98 \pm 6$</td>
<td>$355 \pm 66$</td>
</tr>
</tbody>
</table>

* Values given are the mean and standard deviation (estimated using $N - \bar{\mu}$).
accumulation of cholesterol in the liver which otherwise occurs on such a high cholesterol diet. It also resulted in a lower level of serum cholesterol, although the difference was statistically significant in only one of two experiments performed.

It is concluded that one or more of the constituents of isocholesterol interferes with the intestinal absorption of cholesterol. This finding is compatible with the hypothesis that interference by other sterols with the absorption of cholesterol is dependent upon a free hydroxyl group on carbon-3. This effect is not dependent, however, upon the configuration which leads to formation of a difficultly soluble digitonid.

SUMMARY IN INTERLINGUA

Esseva studiate in rattos le effectos exercite super le cholesterol del sero e del hepate per le administration de "isocholesterol," un mixitura de steroles 30-carbonic que occurre in grassia de lana.

Le addition de 5 pro cento de isocholesterol a un dieta a basse contento de cholesterol, mantenite durante 13 dies, non habeva un effecto significative super le nivellos de cholesterol in hepate e sero. Tamen, le addition de 5 pro cento de isocholesterol a un dieta continent 1 pro cento de cholesterol exerceva un marcate effecto inhibitori super le accumulation de cholesterol in le hepate che occurre normalmente con dietas a si alte contentos de cholesterol. Illo etiam resultava in un basse nivello de cholesterol del sero, ben que le differentia esseva significative in solmente un de duo experimentos executate.

Es formulate le conclusion che un o plures del constituentes de isocholesterol obstrue le absorption intestinal de cholesterol. Iste constatation es compatible con le hypothese che le obstruction del absorption de cholesterol per altere steroles depende del presentia de un gruppo hydroxylic libere a carbon-3. Sed le effecto non depende del configuration que resulta in le formation de un digitonido de solubilitate difficile.

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