In Vivo Incorporation of Acetate-1-C\textsuperscript{14} into Cholesterol and Fatty Acids Following Testosterone Propionate Administration

By Robert L. Raiford, M.S., and Harry Y. C. Wong, Ph.D.

With the technical assistance of Rose Shim and Frances Farrow

With the development of a wide variety of hypocholesterolemic agents, the hypercholesterolemia commonly associated with coronary and aortic atherosclerosis has assumed a position of paramount importance in the quest for some reliable means of preventing atherosclerosis. Though hypercholesterolemia in itself has not been indicated as the primary cause of atherosclerosis, indications are that its incidence is considerably higher in those cases where abnormally elevated cholesterol values occur.

Studies from our laboratories\textsuperscript{1,2} and others\textsuperscript{3-7} have shown that the administration of small doses of testosterone propionate reduces the hypercholesterolemia and the severity and incidence of aortic and coronary atherosclerosis of cholesterol-fed animals. It appeared proper then that considerable emphasis should be centered on the mechanism(s) by which this drug depresses abnormal cholesterol levels.

This study was undertaken to determine the possible effects of testosterone propionate therapy on the rate of incorporation of acetate-1-C\textsuperscript{14} into liver, intestine, and plasma cholesterol, and fatty acids in the intact animal, and if possible to relate these changes to the observed lowering of the plasma cholesterol level.

**Method**

Thirty-two 30-week-old Hy-line cockerels were divided into four groups of eight animals each.

Groups I and II were maintained on a commercial or plain mash (P.M.) diet, while the remaining groups (III and IV) were fed a diet consisting of a mixture of 2 per cent cholesterol, 5 per cent cottonseed oil, and plain mash (A.D. or atherogenic diet). Beginning two weeks later, groups I and III received intramuscular injections of 1 ml. peanut oil for three weeks, five times per week. Groups II and IV received intramuscular injections of 1.25 mg. of testosterone propionate (T.P.) in peanut oil during the same period.

At the end of the third week, each was given an intraperitoneal injection of sodium acetate-1-C\textsuperscript{14} (50 \mu c./Kg. body weight) dissolved in physiological saline. Following administration of the isotope, blood samples were drawn from the alar wing at 20, 40, 60, and 80 minutes. The liver and small intestines were removed, immediately frozen, and stored in the frozen state.

The procedures for separation of the cholesterol and fatty acids of tissue and plasma closely follow those of Nishida et al.\textsuperscript{8} However, inasmuch as the procedures were changed slightly in numerous places, they will be given here in part for the sake of clarity and convenience.

Three Gm. of tissue from the A.D. birds and 6 Gm. from the P.M. birds were homogenized in hot ethanol, transferred to a 100-ml. flask, and ether added in sufficient quantity to make a 3:1 ratio of ethanol to ether. Twenty-four hours later, the extract was filtered and diluted to volume in a 100-ml. volumetric. One ml. of the liver or intestinal extract was removed for the determination of total cholesterol, according to the method of Bloor.\textsuperscript{9}

For the separation of the digitonide, 15 ml. of the liver, intestinal, or plasma extract was placed into a 15-ml. centrifuge tube and evaporated in a water bath under a current of air. To the dry residue, 2 ml. of 1:1 acetone-alcohol, one drop of 10 per cent acetic acid, and 12 ml. of 1 per cent digitonin in 80 per cent ethanol were added with stirring. The digitonide was allowed to precipitate overnight and the mixture centrifuged. After discarding the supernatant, the precipitate was washed twice with 80 per cent ethanol, once with ether, and dissolved in 10 ml. of warm methanol.

\textsuperscript{*}Digitonin-precipitable sterols.
Table 1
Plasma and Liver Cholesterol Levels and the Incorporation of Acetate into Tissue Cholesterol and Fatty Acids

<table>
<thead>
<tr>
<th>Treatment diet</th>
<th>Body weight</th>
<th>Plasma mg. %</th>
<th>Liver mg./100 Gm. tissue</th>
<th>Intestine mg./100 Gm. tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.M. + peanut oil group I</td>
<td>1,270 ± 67.5</td>
<td>104 ± 11.8</td>
<td>759 ± 11.8</td>
<td>334 ± 11.7</td>
</tr>
<tr>
<td>P.M. + T.P. group II</td>
<td>1,225 ± 57.5</td>
<td>111 ± 6.6</td>
<td>659 ± 32.3</td>
<td>315 ± 15.5</td>
</tr>
<tr>
<td>A.D. + peanut oil group III</td>
<td>1,239 ± 58.7</td>
<td>945 ± 75</td>
<td>3,942 ± 192.2</td>
<td>1,166 ± 110.2</td>
</tr>
<tr>
<td>A.D. + T.P. group IV</td>
<td>1,336 ± 71.2</td>
<td>667 ± 152</td>
<td>3,551 ± 313.8</td>
<td>981 ± 69.8</td>
</tr>
</tbody>
</table>

*Lieberman-Burchard positive sterols.
†Plain mash.
§Standard error.

Aliquots of this were then plated on aluminum planchetts and counted.

The procedure used for the tissue fatty acids was exactly the same as that given by Nishida. The procedure for plasma differs only in that 3:1 ethanol-ether was substituted for the 1:1 alcohol-acetone, and the cholesterol was determined by the method of Bloor.

Results

The administration of 1.25 mg. of testosterone propionate daily resulted in an increased incorporation of acetate into cholesterol and fatty acids in both the P.M. and A.D. cockerels (table 1). Animals on P.M. plus T.P. showed an 83 per cent increase in the incorporation of acetate to plasma cholesterol and an 85 per cent increase in plasma fatty acid synthesis over that of the plain-mash controls. Cockerels fed on A.D., and treated with T.P., exhibited a significant increase in conversion to plasma cholesterol and fatty acids also, 15 per cent and 51 per cent, respectively.

In the liver, similar results were obtained. However, the incorporation of the acetate to cholesterol in the P.M. plus T.P. cockerels was slightly, but not significantly, less than that of the P.M. The fatty acid incorporation in the P.M. and T.P., however, showed a significant 50 per cent increase over the non-treated animals. The atherogenic-diet birds converted only 56 per cent and 52 per cent as much acetate to liver cholesterol and liver fatty acids, respectively, as the atherogenic-diet animals treated with T.P. (fig. 1).

Values for the intestinal conversion of acetate to cholesterol and fatty acids indicated the same trend as exhibited in the liver conversion in the P.M. groups (table 1). The P.M. animals incorporated 87 per cent as much acetate to cholesterol as the P.M. and T.P. group (fig. 2). Fatty acid incorporation was 62 per cent that of the T.P.-treated animals (fig. 3). The atherogenic-fed groups, however, exhibited a reversal of the trend demonstrated in plasma and liver towards increased incorporation by T.P.-treated animals. Instead, the A.D. plus T.P. groups incorporated only 52 per cent and 55 per cent as much acetate to intestinal cholesterol and fatty acids, respectively, as the A.D. birds (fig. 4).

Discussion

Numerous factors have been shown to control cholesterol synthesis from acetate.
### Cholesterol digonide

<table>
<thead>
<tr>
<th>Plasma counts/100 ml.</th>
<th>Liver counts/100 Gm. tissue</th>
<th>Intestinal counts/100 Gm. tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,469 ± 150</td>
<td>440,722 ± 115</td>
<td>41,389 ± 217</td>
</tr>
<tr>
<td>5,431 ± 312</td>
<td>491,889 ± 201</td>
<td>47,488 ± 196</td>
</tr>
<tr>
<td>2,750 ± 130</td>
<td>8,334 ± 103</td>
<td>45,833 ± 212</td>
</tr>
<tr>
<td>3,250 ± 85</td>
<td>15,001 ± 178</td>
<td>23,889 ± 206</td>
</tr>
</tbody>
</table>

### Fatty acids

<table>
<thead>
<tr>
<th>Plasma counts/100 ml.</th>
<th>Liver counts/100 Gm. tissue</th>
<th>Intestinal counts/100 Gm. tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,808 ± 60</td>
<td>576,250 ± 147</td>
<td>37,292 ± 123</td>
</tr>
<tr>
<td>12,086 ± 190</td>
<td>1,163,458 ± 425</td>
<td>60,287 ± 135</td>
</tr>
<tr>
<td>2,063 ± 160</td>
<td>97,917 ± 175</td>
<td>41,263 ± 120</td>
</tr>
<tr>
<td>4,188 ± 96</td>
<td>189,167 ± 312</td>
<td>22,500 ± 156</td>
</tr>
</tbody>
</table>

§Testosterone propionate.
||Atherogenic diet.

Toukis et al. have shown that cholesterogenesis is subject to regulation by the intake of cholesterol. Frantz et al. and Cox et al. have reported a homeostatic regulation for hepatic cholesterol synthesis. Cholesterogenesis is also markedly affected by the endoerines, one example being the rate of incorporation of acetate into cholesterol in liver from diabetic animals.

Testosterone has been shown to have an anabolic effect with nitrogen retention and a resultant gain in weight. This phenomenon, however, has not been observed in any of our experiments to date. The dose level required to produce these effects is usually larger for the intact animal than in animals subjected to adrenalectomy and other procedures. Thus, our failure to observe this effect may be due to the dose level used and the fact that the cockerels were not subjected to any prior experimental procedures. It has also been reported by Noble that the digitonide from connective and liver tissue (in vitro) of male animals is higher in radioactivity than that of the female following the administration of labeled acetate. Other reports on in vitro studies, however, have recorded a decreased incorporation of acetate into fatty acids in liver tissue and no alteration of acetate incorporation into cholesterol following T.P. administration.

The data from this study appear to support the results obtained by Noble, inasmuch as in many cases the incorporation of acetate into cholesterol and fatty acids has been increased by as much as 80 per cent in the animals treated with T.P. This was found to be true irrespective of diet (P.M. or A.D.), except in the intestinal conversion of the atherogenic-fed cockerels, wherein its administration causes a decreased incorporation.

The cholesterol synthesis in the atherogenic birds was much less than that of the P.M. animals, as might be expected due to the relatively large amounts of exogenous cholesterol consumed. In spite of the decreased incorporation, however, the administration of T.P. resulted in a readily discernible increase in the incorporation of acetate to cholesterol. The increased incorporation noted in the atherogenic birds may well be due to both homeostatic regulation and a direct influence of the testosterone propionate on synthesis. It is fully apparent, however, that the marked
Incorporation of acetate-1-C\textsuperscript{14} into liver fatty acids.

Changes induced are not caused mainly by homeostatic control, inasmuch as plasma, liver, and intestinal cholesterol levels were practically identical in both of the P.M. groups. Yet, the conversion of acetate to cholesterol and fatty acids in the T.P. animals usually exceeded that of the nontreated P.M. animals by at least 50 per cent. Neither could these changes be due to differences in food consumption and body weight (table 1). The method of Bloor which was used for the total cholesterol determinations has been shown to compare favorably with that of Sperry and Webb and Schoenheimer-Sperry.\textsuperscript{17} Thus, considerable confidence may be placed in the accuracy of these values.

The most probable explanation for the drop in plasma cholesterol is a redistribution of cholesterol among the tissues and/or an increased catabolism of cholesterol in response to displacement of the normal acetate-cholesterol-bile acid conversion sequence. This view is supported by Caltabiano\textsuperscript{18} who has postulated that the action of testosterone propionate is probably mediated by means of an activation of the intrahepatic cholesterol metabolism and perhaps by partial inhibition of the adrenal cortex and hypophysis.

Summary
The administration of testosterone propionate for three weeks resulted in an increased
incorporation of sodium acetate-$^{14}$C into cholesterol and fatty acids. This increased conversion was evident in both the plain-mash and the atherogenic-diet cockerels. Thus, the hypcholesterolemic effect of testosterone propionate is not the result of a decreased synthesis of cholesterol and/or fatty acids. In addition, a trend towards lowering of the plasma and tissue cholesterol levels of the atherogenic-diet animals is indicated.

References

Books Received

Books received by Circulation Research are hereby acknowledged. Those of special interest to investigators in basic aspects of the circulation will be reviewed as space permits.

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