Effect of Biphenylylbutyric Acid on the Cholesterol and Phospholipid Metabolism in the Rat

By R. E. Ranney, Ph.D., and Sally E. Weiss, B.S.

The effects of treatment of rats with biphenylylbutyric acid were assessed by measuring the amount of acetate-1-C14 incorporated into the cholesterol and phospholipid of several tissues. The drug inhibited the inclusion of the label into the plasma and hepatic lipid fractions. The treatment had no significant effect upon the lipid metabolism of either the testis or the adrenal. In the aorta, although the incorporation of the labeled acetate into cholesterol was unchanged, the amount of label found in the phospholipids was strikingly enhanced by the treatment. The unique nature of aortic lipid metabolism as evidenced by this work and previous reports is discussed.

In 1956 Steinberg and Fredrickson reviewed the hypothesis that the inhibition of endogenous cholesterol synthesis might effect a reduction in the plasma cholesterol concentration, and pointed out that a therapeutic approach based on this hypothesis might serve in the treatment of atherosclerosis. These authors evaluated phenylbutyric acid, a compound previously advanced as an inhibitor of endogenous cholesterol synthesis, and found that, although this material efficiently blocked cholesterol synthesis in rat liver slices in vitro and in liver tissue in vivo, no reduction in plasma cholesterol levels was induced in treated animals. These workers also tested this compound clinically and found that it did not affect serum cholesterol levels of hypercholesterolemic patients.

Recently Garattini et al. observed that α, 4-biphenylylbutyric acid inhibited the in vitro incorporation of sodium acetate-1-C14 into the cholesterol of rat liver slices to a much greater extent than did phenylbutyric acid. These investigators also found that when hepatic cholesterol synthesis in rats was elevated by Triton (WR 1339: p-isooctylpolyoxyethylenephenol) administration, biphenylylbutyric acid significantly inhibited the formation of C14 labeled cholesterol by the liver, and reduced plasma cholesterol levels toward normal. In 1957 Tavormina and Gibbs reported that the mechanism of action of biphenylylbutyric acid differed from that of phenylbutyric acid. The latter compound seemingly inhibited only acetate incorporation into cholesterol in a rat liver homogenate, whereas biphenylylbutyric acid diminished both acetate and mevalonate incorporation into the sterol product of the homogenate system. The enhanced potency, and the apparent difference in the mechanism of action of biphenylylbutyric acid as compared to phenylbutyric acid, indicated that this new compound might be a closer approach to a truly effective inhibitor of endogenous cholesterol synthesis and that further study of the effects of this compound was warranted.

Although reduction of plasma cholesterol levels through inhibition of hepatic cholesterol synthesis seems desirable as a therapeutic measure in the treatment of atherosclerosis, it must be emphasized that inhibition of sterol metabolism elsewhere in the body may result in endocrine dysfunction. Both the adrenal and the gonadal hormones are derived, at least in part, directly from cholesterol, and any profound diminution in the normal formation of this lipid in these organs might result in undesirable side effects. The purpose of the present report is to assess in vivo the effect of biphenylylbutyric acid upon the cholesterol and phospholipid metabolism of the adrenal, testis, liver and aorta.

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*Rats treated with another type of inhibitor of endogenous cholesterol synthesis, Δ4-cholestenone, have shown adrenal hypertrophy.
METHODS

In Vivo Test. In a preliminary experiment rats were treated subcutaneously with 50 mg./Kg. of biphenylylbutyric acid daily for 12 days. Measurement of the incorporation of acetate-1-C\textsubscript{14} into the cholesterol of liver and testis showed a decrease in the hepatic cholesterol specific activity, but the reduction of this value in the testis was equivocal. Because of undesirable tissue reactions at the sites of injection, and the ambiguous testicular response, a larger dose administered orally was chosen in the subsequent experiment.

Young adult male rats (Sprague-Dawley) weighing 250 to 300 Gm. were divided into 2 groups of 6 animals each. Biphenylylbutyric acid was administered orally at a daily dose of 100 mg./Kg. in 20 per cent propylene glycol to the test group and the controls received only the vehicle. After 12 days of treatment each animal received 1 to 2 mg. sodium acetate-1-C\textsubscript{14} in saline by intracardiac injection. Half of the animals in each group were killed 30 min. after the isotope administration, and the other half were killed at 120 min. Plasma, liver, aorta, adrenal and testis samples were taken for analysis. Lipid extraction of the tissues and plasma was carried out according to the chloroform-methanol method of Folch et al.\textsuperscript{8} The solvent was removed with gentle heating. The lipid fraction was dissolved in petroleum ether, and the cholesterol in this fraction was determined according to the method of Zlatkis, et al.\textsuperscript{9} The solvent was removed with gentle heat, and the residues were saponified in 30 per cent KOH. The nonsaponifiable lipid fraction was dissolved in petroleum ether, and the cholesterol in this fraction was determined according to the method of Zlatkis, et al.\textsuperscript{9} After chemical and isotope analyses for cholesterol had been completed, the washed chloroform-methanol extracts of each tissue were pooled, and the solvent was removed with gentle heating. The lipids were dissolved in aceton and the phospholipids precipitated with a saturated solution of magnesium chloride in absolute ethanol. The precipitate was washed twice with aceton, dissolved in methanol and ethyl ether, and the phosphorus in the extracts was measured by the method of King.\textsuperscript{10} The radioactivity measurements of all lipid C\textsuperscript{14} were made on direct mounts of the extracts, with an end-window geiger tube.

In Vitro Test. When the drug and labeled acetate were administered in vivo, hepatic cholesterol synthesis was inhibited while aortic cholesterol synthesis was augmented slightly. For a further study of this difference, the response of aorta and liver in vitro were compared. Two male rat thoracic aortas (170 ± 30 mg.) or liver slices (200 ± 10 mg.) were shaken in each flask for 3 hours at 37 C. in Krebs-Ringer bicarbonate buffer at pH 7.4. Each flask received 0.2 mg. (0.6 \mu c.) sodium acetate-1-C\textsuperscript{14}, and had a final volume of 2.2 ml. The gas phase was 5 per cent carbon dioxide in oxygen. Lipid extraction and radioactivity measurement were the same as those described above.

RESULTS

In Vivo Test. The treatment caused no overt toxic manifestations, since neither the body weight of the test rats nor their behavior

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time (min.)</th>
<th>Specific Activity\textsuperscript{a} (X10\textsuperscript{2} mg./Gm.)</th>
<th>Concentration\textsuperscript{b} (mg./Gm.)</th>
<th>Specific Activity\textsuperscript{c} (X10\textsuperscript{2} mg./Gm.)</th>
<th>Concentration\textsuperscript{d} (mg./Gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>30</td>
<td>34.5 ± 1.94</td>
<td>42.1 ± 8.54</td>
<td>.305</td>
<td>.346</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>32.1 ± 6.6</td>
<td>31.5 ± 4.8</td>
<td>.305</td>
<td>.346</td>
</tr>
<tr>
<td>Liver</td>
<td>30</td>
<td>22.8 ± 5.8</td>
<td>12.9 ± 2.8##</td>
<td>2.91</td>
<td>2.98</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>21.7 ± 4.3</td>
<td>13.9 ± 3.9</td>
<td>21.8</td>
<td>13.0</td>
</tr>
<tr>
<td>Aorta</td>
<td>30</td>
<td>23.5 ± 6.9</td>
<td>34.1 ± 6.8</td>
<td>6.76</td>
<td>6.37</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>22.2 ± 4.6</td>
<td>35.6 ± 8.4</td>
<td>1.81</td>
<td>2.17</td>
</tr>
<tr>
<td>Testis</td>
<td>30</td>
<td>31.0 ± 7.2</td>
<td>16.6 ± 3.8</td>
<td>2.82</td>
<td>3.32</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>30.2 ± 2.0</td>
<td>24.5 ± 4.0</td>
<td>25.2</td>
<td>17.4</td>
</tr>
<tr>
<td>Adrenal</td>
<td>30</td>
<td>6.30 ± 2.2</td>
<td>5.10 ± 1.4</td>
<td>25.8</td>
<td>27.1</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>5.29 ± 1.5</td>
<td>8.80 ± 4.0</td>
<td>9.32</td>
<td>10.4</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Specific activity is expressed as per cent administered C\textsubscript{14} found per mg. lipid.

\textsuperscript{b}Treated animals received 100 mg./Kg. biphenylylbutyric acid orally daily for 12 days.

\textsuperscript{c}Cholesterol and phospholipid concentrations are expressed as the average values from each group, irrespective of time of killing.

\textsuperscript{d}No values are given for plasma phospholipids since these extracts were lost through accident.

\textsuperscript{e}Mean and standard error.

\textsuperscript{f}p < .01.

\textsuperscript{g}##p < .05.

TABLE 1.—The Effect of Biphenylylbutyric Acid on Cholesterol and Phospholipid Metabolism in the Plasma, Liver, Aorta, Testis and Adrenal of the Rat in Vivo
differed measurably from the controls. Biphénylylbutyric acid administration did not modify the cholesterol concentration in the plasma or in any of the tissues analyzed (table 1). There was, however, a marked inhibition of the incorporation of acetate-1-C\textsuperscript{14} into the cholesterol of the 120 min. plasma sample and of the 30 min. liver sample. It should be pointed out that demonstration of statistically significant differences in the data presented is difficult because of the small number of animals sampled, this number being limited by the amount of the drug available to us.

In the adrenal and the aorta, treatment with biphénylylbutyric acid effected little or no inhibition of acetate inclusion into the sterol, and in fact it would appear that the synthesis of aortic cholesterol from this substrate was actually enhanced in the treated rats. When our data are compared with those of Steinberg and Fredrickson\textsuperscript{1} it is evident that biphénylylbutyric acid is much more effective in reducing acetate incorporation into cholesterol than is phenylbutyrate. These authors observed approximately a 24 per cent decrease in the hepatic cholesterol specific activity of rats treated with 150 to 200 mg./Kg. phenylbutyrate for 40 days, while in the present case biphénylylbutyric acid produced a reduction of liver cholesterol specific activity of nearly 40 per cent in animals treated with 100 mg./Kg. of the drug for 12 days.

The treatment with biphénylylbutyric acid did not modify the phospholipid concentrations in the tissues (table 1). The amount of C\textsuperscript{14} found in this lipid fraction, presumably in the fatty acid moiety, roughly paralleled that observed for cholesterol. There was a noticeable diminution in the phospholipid specific activity in the liver; in the testis any significant difference due to the treatment seems equivocal; and the drug had no apparent effect upon adrenal phospholipid metabolism. In the aorta there was a marked increase in the incorporation of acetate-1-C\textsuperscript{14} into phospholipid, the difference between the control and treated groups being much more marked than the difference in cholesterol specific activities.*

We have integrated graphically the specific activity-time curves drawn from the data of table 1 to indicate the per cent of the labeled acetate pool incorporated into cholesterol and phospholipid per unit time (table 2). When the data are expressed in this fashion it is more strikingly evident that there was inhibition of acetate incorporation into the lipids of the liver particularly, and to a lesser extent of the testis and adrenal. In the aorta, on the other hand, the apparent increase in lipid synthesis is emphasized.

Steinberg and Fredrickson\textsuperscript{1} have pointed out that, in examining the effects of agents that inhibit cholesterol synthesis, the labeled "free acetate" pool of the body may not represent the total acetate available for cholesterol synthesis. That is, acetyl-CoA derived from the metabolism of other tissue components need not be in equilibrium with the labeled acetate, if under the influence of the compound either the formation or utilization of coenzyme A were impaired.

If this were true in the present experiment then, with inhibition of utilization of free acetate by tissues such as liver, testis and adre-

### Table 2—The Rate of Incorporation of Acetate-1-C\textsuperscript{14} Into the Cholesterol and Phospholipid of the Tissues of Rats Treated with Biphénylylbutyric Acid

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Per cent C\textsuperscript{14} found in lipid/mg. lipid/hr. (X10\textsuperscript{1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Treated control** Control Treated control**</td>
</tr>
<tr>
<td>Plasma</td>
<td>47.4 33.5 71 - -</td>
</tr>
<tr>
<td>Liver</td>
<td>19.3 11.0 56 31.9 18.2 57</td>
</tr>
<tr>
<td>Aorta</td>
<td>20.3 30.6 181 10.0 74.5 745</td>
</tr>
<tr>
<td>Testis</td>
<td>26.5 17.5 68 37.1 26.5 71</td>
</tr>
<tr>
<td>Adrenal</td>
<td>5.20 4.20 72 15.4 11.9 77</td>
</tr>
</tbody>
</table>

*The acetone-soluble lipid which contains both triglyceride and cholesterol showed essentially the same results, i.e., a lowered specific activity for the extra-aortic tissues of the treated animals, and an augmented incorporation of C\textsuperscript{14} into this lipid fraction of the aorta of these animals.
neral, the specific activity of the acetate pool in the treated animals would be higher than that of the controls. For the purpose of argument we have made the assumption that all tissues other than aorta were affected by the drug in the same way as were liver, testis, and adrenal. In this case the inhibition of extra-aortic utilization of acetate for cholesterol synthesis could be estimated at approximately 35 per cent (table 2), and thus the activity of this pool available for cholesterol and phospholipid synthesis by the aorta would be 1.35 times that used in the calculations of the data exhibited in table 1. Recomputing of aortic cholesterol and phospholipid specific activities of the treated animals with this factor gives respective values of 22.6 X 10\(^{-4}\) (per cent C\(^{14}\) recovered as cholesterol/mg. cholesterol/hr.) and 55.0 X 10\(^{-4}\) (per cent C\(^{14}\) recovered as phospholipid/mg. phospholipid/hr.). Although this value for cholesterol approximates that of the control, the corrected figure for the rate of incorporation of acetate into aortic phospholipid is still much higher than the corresponding value for the untreated animals. Thus, if our assumptions were correct, it is evident that aortic cholesterol metabolism was either unchanged or slightly augmented by the treatment, but that the drug gave an apparent enhancement of phospholipid synthesis.

**DISCUSSION**

Although treatment with biphenylylbutyric acid induced pronounced inhibition of the incorporation of acetate into the cholesterol and phospholipid of the liver, such an effect in the testis was equivocal and in the adrenal it was negligible. Inasmuch as the dose used here was 10 to 20 times that used clinically,\(^{11}\) it is probable that with low doses of the drug any reduction in the formation of precursors of the steroid hormones would be minimal and could be disregarded.

It is evident that the response of aortic lipid metabolism to biphenylylbutyric acid is unique among the other tissues studied. There were no modifications of cholesterol or phospholipid concentration in the tissues investigated. Whereas liver showed consistent reductions in the incorporation of the label into these lipid fractions, aortic cholesterol synthesis seemed unchanged, and the formation of phospholipid by this tissue appeared enhanced.

Inhibition of the synthesis of cholesterol and phospholipid is not surprising, since biphenylylbutyric acid has been shown not only to depress cholesterol formation in liver homogenates\(^6\) but also to suppress coenzyme A formation or utilization.\(^3\) The difference we have observed between hepatic lipid metabolism and that of the aorta is not unlike the findings of Feller and Huff.\(^{12}\) They measured the effect of exogenous cholesterol upon hepatic and aortic cholesterogenesis, and observed that while cholesterol feeding inhibited in vitro incorporation of acetate into cholesterol in rabbit liver slices, there was no diminution in cholesterol formation in aortic strips taken from the cholesterol-fed animals.

Since in vitro biphenylylbutyric acid is an effective inhibitor of aortic as well as hepatic-

### Table 3

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>Per cent C(^{14}) recovered in nonsaponifiable fraction</th>
<th>Per cent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>Control</td>
<td>.345</td>
<td>0</td>
</tr>
<tr>
<td>Aorta</td>
<td>0.5 mM biphenylylbutyric acid</td>
<td>.025</td>
<td>93</td>
</tr>
<tr>
<td>Aorta</td>
<td>1.0 mM biphenylylbutyric acid</td>
<td>.015</td>
<td>96</td>
</tr>
<tr>
<td>Liver</td>
<td>Control</td>
<td>.487</td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>0.5 mM biphenylylbutyric acid</td>
<td>.229</td>
<td>53</td>
</tr>
</tbody>
</table>
cholesterol synthesis it is conceivable that in vivo the locus of lipid synthesis in the aorta was excluded from the body pool of this drug. The synthetic mechanisms of the other tissues, however, must have been in equilibrium with this body compartment in order to exhibit the degrees of inhibition seen in them. It is not unlikely that the data of Feller and Huff may be explained on the same basis, e.g., the augmented body pool of cholesterol, while affecting inhibition of hepatic cholesterol synthesis, may not have affected aortic synthesis because the metabolite had been excluded from the site of lipid synthesis.

It is difficult to interpret the enhanced incorporation of C14 into aortic phospholipid, but one explanation for this phenomenon may be that the drug also blocked the extra-aortic utilization of a phospholipid precursor other than acetate. Thus, if such a labeled metabolite was readily formed in extra-aortic tissue but could be utilized only by the arterial wall, a build-up of labeled phospholipid would occur in the aorta.

**Summary**

The incorporation of acetate-1 C14 into the tissue cholesterol and phospholipid of rats treated with biphenylylbutyric acid was studied at two different time intervals. The inclusion of the label into the cholesterol and phospholipid in liver was significantly inhibited; in the testis such incorporation was equivocal, and the effect upon adrenal lipids was negligible. Aortic cholesterol synthesis was unaffected by the treatment, and there seemed to have been an enhancement of the incorporation of the label into aortic phospholipid. The unique nature of aortic lipid metabolism is discussed.

**Summario in interlingua**

Le incorporation de acetato-1-C14 in le cholesterol e le phospholipido histic de rattos tractate con acido biphenylybutyric esseva studiate a duo differente periodos de tempore. Le inclusion del marca in le cholesterol e in le phospholipido hepatic esseva inhibite de maniera significative. In le testes iste incorporation esseva equivoc. In le caso de lipidos adrenal le efecto esseva negligibile. Le synthese de cholesterol aortic non esseva afficite per le tractamento. Il pareva occurrer un promotion correspondente del incorporation de' marca in le phospholipido del aorta. Le natura unica del metabolismo de lipido in le aorta es discutite.

**REFERENCES**

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