Failure of Alpha-Phenylbutyrate and Beta-Phenylvalerate in Treatment of Hypercholesterolemia

By Donald S. Fredrickson, M.D., and Daniel Steinberg, M.D., Ph.D.

Sodium alpha-phenylbutyrate and sodium beta-phenyl-n-valerate have been administered to a carefully controlled group of hypercholesterolemic patients. Prior reports that these compounds are effective in the treatment of hypercholesterolemia are not confirmed. Two patients developed toxic reactions requiring cessation of treatment. The ineffectiveness of these drugs, despite their inhibitory effect on the synthesis of cholesterol from acetate, is discussed in terms of recent biochemical information concerning their site of action.

Recently a number of studies have appeared describing the therapeutic use of certain aromatic acids and their derivatives in hypercholesterolemia.1-2 Cottet and co-workers were first to report significant lowering of serum cholesterol following administration of the sodium salt or the amide of alpha-phenylbutyric acid,1* and the sodium salt of beta-phenylvaleric acid.4 (Also called phenylethylpropionic acid, sodium salt.)

Following these reports we initiated a series of studies in an attempt to establish the mechanism of action of these compounds. It was found that alpha-phenylbutyrate or beta-phenylvalerate strongly inhibited the rate of conversion of acetate-1-C14 to cholesterol and fatty acids both in vitro and in vivo.5 On the other hand, little or no effect of phenylbutyrate administration on the serum cholesterol in rats could be demonstrated.6

In the present paper we report our observations on the effects of sodium phenylvalerate and sodium phenylbutyrate in a small but carefully controlled series of 14 patients. Dietary intake, especially that of cholesterol and other fats, was maintained as constant as possible, including the use of liquid formula diets in 6 patients for periods up to 3 months.

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* In the European studies these compounds are referred to as phenylethylacetic acid, sodium salt, or phenylethylacetamide (the latter compound is a product of Theraplix, Inc., Paris, under the designation 412STH or Hyposterol).

Methods

A total of 14 adult patients were selected for study. Two patients were normcholesterolemic; the remaining 12 patients all had hypercholesterolemia of long standing. Ten patients had clear sera and normal or slightly elevated serum triglycerides. Half of these patients had demonstrable tendon xanthomas. Two patients had slightly turbid sera and definitely elevated triglyceride values. One of the 2 had tuberous xanthomas. Three patients were given a therapeutic trial with 2 different compounds. Six of the patients were hospitalized throughout the control and treatment periods. The remaining patients were followed weekly in the outpatient clinic. All patients were ambulatory. Many had clinical evidence of coronary artery disease, but no signs or symptoms of congestive heart failure. All patients were clinically euthyroid and non-diabetic.

Three types of diet were employed. Six patients were fed constant liquid formula diets, prepared after the method of Ahrens, Dole, and Blankenhorn.7 The composition of a typical diet is indicated in Table 1. The fat content of the diet, supplied as butter fat, provided 35 percent of the calories, about average for the American diet.8 The cholesterol content of 0.5 Gm. per day was chosen as representative of a normal average intake of this steroid.9 The remaining patients were instructed to remain on their usual diet, which in every case they had been following for at least 2 years. This consisted of either a “regular” diet, exercising free selection of foods but maintaining constant weight, or a “low-fat” diet, consisting of 40 to 50 Gm. of fat daily of mixed animal and vegetable origin. Inpatients were weighed daily; outpatients, weekly. With the exception of 1 patient (J.P.), who lost 2.9 Kg. during the study, all patients maintained constant weights to within ±1 Kg.

Blood samples were drawn in the morning in the fasting state. Serum cholesterol was determined by the method of Schoenheimer and Sperry,10 lipid phosphorus by the method of Fiske and SubbaRow,11 and triglycerides by the method of Bragdon.12 Lipoproteins were separated in the preparative.
TABLE 1.—Composition of a Typical Liquid Formula Diet

<table>
<thead>
<tr>
<th>Component</th>
<th>Gram/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>85.1</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>292.6</td>
</tr>
<tr>
<td>Fat</td>
<td>84.8</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.5</td>
</tr>
</tbody>
</table>

In this case, providing approximately 2,300 calories per day to a 76 Kg. patient. The ingredients were: butter (unsalted) 99 Gm., Lesofac (milk protein) 165 Gm., Dextrose 228 Gm., egg yolk 9.1 Gm., sodium chloride 8 Gm., water 2,220 ml. The emulsified diet was given in 4 feedings. One multivitamin capsule and ferrous gluconate, 0.6 Gm. per day, were the only supplements.

ultracentrifuge by Dr. Richard Havel, according to the method of Havel, Eder, and Bragdon. The results for the group as a whole indicate no effect of the drug on the mean serum cholesterol level.

The results in individual patients may be seen in tables 2 and 4. In table 2, the mean value during the treatment period is compared with the mean obtained during the control period. It can be seen that in 3 patients the mean level was 2.2, 6.3, and 13.3 per cent lower during treatment. However, the fact that in the remaining 6 patients the mean treatment value was higher by 4.6 to 15.9 per cent, suggested that the small apparent decreases were due to chance. To evaluate better the significance of the data a simple statistical analysis was performed. In table 4 the mean values during the control period are presented together with the standard deviation of the mean in milligrams per cent, and the results are analyzed in 3 different ways. The mean control level was compared with the cholesterol level at the time treatment was discontinued, the mean of the last 3 cholesterol determinations during treatment, and the mean of the cholesterol determinations during the fourth, fifth, and sixth weeks of therapy. A fall was considered signif-
TABLE 2.—Results of Treatment with Beta-Phenylvalerate

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Diet</th>
<th>Dose (mg./Kg.)</th>
<th>Control period</th>
<th>Treatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of days</td>
<td>No. of cholesterol determinations</td>
<td>Mean serum cholesterol (mg.%).</td>
</tr>
<tr>
<td>E.H.</td>
<td>regular</td>
<td>70</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>R.G.</td>
<td>regular</td>
<td>81</td>
<td>28</td>
<td>5</td>
</tr>
<tr>
<td>S.H.</td>
<td>low fat</td>
<td>100</td>
<td>68</td>
<td>9</td>
</tr>
<tr>
<td>M.B.</td>
<td>low fat</td>
<td>80</td>
<td>42</td>
<td>6</td>
</tr>
<tr>
<td>J.P.</td>
<td>constant</td>
<td>69-120</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>I.C.</td>
<td>constant</td>
<td>44-86</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>E.W.</td>
<td>constant</td>
<td>89</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>N.G.</td>
<td>constant</td>
<td>77</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>E.D.</td>
<td>constant</td>
<td>71</td>
<td>23</td>
<td>7</td>
</tr>
</tbody>
</table>

TABLE 3.—Results of Treatment with Alpha-Phenylbutyrate

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Diet</th>
<th>Dose (mg./Kg.)</th>
<th>Control period</th>
<th>Treatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of days</td>
<td>No. of cholesterol determinations</td>
<td>Mean serum cholesterol (mg.%).</td>
</tr>
<tr>
<td>M.G.</td>
<td>regular</td>
<td>36-46</td>
<td>56</td>
<td>8</td>
</tr>
<tr>
<td>R.G.</td>
<td>regular</td>
<td>61</td>
<td>28</td>
<td>5</td>
</tr>
<tr>
<td>N.G.</td>
<td>low fat</td>
<td>31-46</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>E.W.</td>
<td>low fat</td>
<td>17-34</td>
<td>26</td>
<td>5</td>
</tr>
<tr>
<td>M.L.</td>
<td>low fat</td>
<td>64</td>
<td>33</td>
<td>5</td>
</tr>
</tbody>
</table>

TABLE 4.—Analysis of Changes in Serum Cholesterol Level During Treatment

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Mean Control Value (mg. % ± S.D.)</th>
<th>Final Value (mg. %)</th>
<th>Mean of last 3 values (mg. %)</th>
<th>Mean value during 4th-6th week of treatment (mg. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-phenylvalerate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.H.</td>
<td>407 ± 17</td>
<td>406</td>
<td>396 - 11</td>
<td>395 - 12</td>
</tr>
<tr>
<td>R.G.</td>
<td>375 ± 30</td>
<td>411</td>
<td>399 + 24</td>
<td>398 + 24</td>
</tr>
<tr>
<td>S.H.</td>
<td>385 ± 16</td>
<td>458</td>
<td>437 + 52</td>
<td>440 + 55</td>
</tr>
<tr>
<td>M.B.</td>
<td>333 ± 18</td>
<td>321</td>
<td>308 - 12</td>
<td>308 - 12</td>
</tr>
<tr>
<td>J.P.</td>
<td>272 ± 16</td>
<td>390</td>
<td>354 + 52</td>
<td>278 + 52</td>
</tr>
<tr>
<td>I.C.</td>
<td>498 ± 33</td>
<td>578</td>
<td>545 + 47</td>
<td>515 + 17</td>
</tr>
<tr>
<td>E.W.</td>
<td>407 ± 29</td>
<td>478</td>
<td>471 + 47</td>
<td>449 + 42</td>
</tr>
<tr>
<td>N.G.</td>
<td>428 ± 10</td>
<td>373</td>
<td>375 - 53*</td>
<td>375 - 53*</td>
</tr>
<tr>
<td>E.D.</td>
<td>174 ± 8</td>
<td>198</td>
<td>191 + 17</td>
<td>194 + 20</td>
</tr>
<tr>
<td>Alpha-phenylbutyrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.G.</td>
<td>383 ± 22</td>
<td>351</td>
<td>341 - 42</td>
<td>341 - 42</td>
</tr>
<tr>
<td>R.G.</td>
<td>375 ± 30</td>
<td>381</td>
<td>391 + 16</td>
<td>391 + 16</td>
</tr>
<tr>
<td>N.G.</td>
<td>445 ± 15</td>
<td>421</td>
<td>410 - 35*</td>
<td>410 - 35*</td>
</tr>
<tr>
<td>E.W.</td>
<td>504 ± 13</td>
<td>533</td>
<td>540 + 36</td>
<td>524 + 20</td>
</tr>
<tr>
<td>M.L.</td>
<td>585 ± 62</td>
<td>543</td>
<td>512 - 73</td>
<td>512 - 73</td>
</tr>
</tbody>
</table>

* Indicates a fall greater than 2 standard deviations.

significant only if it exceeded twice the standard deviation of the mean during the control period.

On the basis of these criteria it will be noted that in only 1 patient (N.G.) was there a significant fall during treatment with β-phenylvalerate. This is true no matter which of the 3 methods of analysis is applied. The fall, however, was only between 12 and 13 per cent. The apparently significant rise occurring in several patients may only reflect the relatively small number of control determinations or it may represent a true upward trend during therapy.

It is of interest that the patient showing the greatest rise during treatment (S.H.), received placebos for 6 weeks before treatment was begun. Just prior to this, she was observed for 4 weeks without placebos. In the control period with placebos, the mean cholesterol changed less than 5 per cent from the mean value in the control period without placebos.

Five hypercholesterolemic patients were given sodium α-phenylbutyrate in doses ranging from 17 to 64 mg. per Kg. body weight for periods up to 67 days (table 3). One of the 5 (E.W.) had tendon xanthomata. All were observed as outpatients and were eating "regular" or "low-fat" diets.

The data from these patients were analyzed as described above for the β-phenylvalerate studies. The mean of all control values in the phenylbutyrate series was 109 per cent of the "zero time value" and the mean of all treatment values was 101 per cent of the "zero time values."

As shown in table 3, the mean value during treatment in 3 of the patients was 9.2, 10.7, and 11.4 per cent lower than the mean control value. On the other hand, it was higher than the mean control value by 2.9 and 5.4 per cent in the other 2 patients.

The statistical analysis in table 4 shows that there was a significant fall in blood cholesterol level in only 1 patient (N.G.). The mean of the
last 3 determinations, made during the fourth, fifth, and sixth weeks in this case, was only 7.9 per cent below the mean control level, but since the standard deviation for the control values was only ±3.4 per cent the fall is significant by the criterion chosen.

One normocholesterolemic man (C.G.) was given sodium α-phenylbutyramide for 41 days at a dose of 53 mg. per Kg. Several weeks after the drug was started, the patient complained of occasional lightheadedness. When the dose was increased to 87 mg. per Kg., this complaint, associated with diarrhea, became more severe. The drug was stopped and the symptoms promptly disappeared. No significant change occurred in his plasma cholesterol during the 51 days of therapy.

Total lipid analyses were done on all patients at the beginning and the end of therapy with all 3 compounds. Lipoprotein fractionations were also performed in the first 3 patients given phenylvalerate. No significant change occurred in the total lipid or lipoprotein patterns during therapy.

As previously mentioned, 2 patients developed side reactions requiring cessation of phenylvalerate administration. In both cases this manifestation was a generalized pruritic maculopapular skin eruption. In the first case, that of a 20-year-old hypercholesterolemic woman, the rash developed after 18 days of therapy on a dose of 82 mg. per Kg. She had, without our knowledge, also undergone an intravenous pyelogram at another clinic 2 days before development of the skin reaction. The second case, a man, aged 60 with xanthoma tuberosum, received 74 mg. per Kg. per day for 5 days when an identical rash appeared. The drug was stopped and the rash promptly disappeared. After several trials of a single 0.35 Gm. dose, the full dosage was resumed 18 days later. After 9 days the rash reappeared, disappearing again after the drug was stopped. The patient was receiving the constant formula diet with no medication other than vitamins and iron. It appeared justifiable to assume that the skin rash occurred as a result of phenylvalerate treatment. No other side effects developed in any patient with the exception of the aforementioned single patient given phenylbutyramide. The only observed laboratory change that occurred in all patients was the appearance of a "1+" reducing test for sugar in the urine during the period of therapy. This promptly disappeared at cessation of the drugs and was assumed to represent an excretion product, possibly a glucuronide. No changes were observed in the hemogram, phenolsulfonphthalein excretion, or liver function tests.

**DISCUSSION**

Only 1 patient in this study showed a significant fall in serum cholesterol, and this decrease was only 13 per cent from the mean control value. While the series was small, each patient appeared to be adequately controlled as indicated by the relatively stable levels of serum cholesterol during the control period. The data in table 2 include observations of the serum cholesterol in the 9 hypercholesterolemic patients during 12 separate control periods including 3 to 11 cholesterol determinations over periods of 16 to 68 days. All fluctuations were assumed to be random in the calculation of a standard deviation of the mean for each control period. The standard deviation of the mean ranged from 2.3 to 10.6 per cent with an average value of 5.5 per cent.

It is important to mention here that most of the patients in this study had hypercholesterolemia associated with normal serum triglycerides and clear sera, by far the commonest form of hypercholesterolemia seen in this clinic. Since none of these patients responded appreciably, even with treatment periods up to 3 months, our studies make it appear highly unlikely that these compounds will have general clinical value. Cottet and associates, using phenylbutyrate in doses of 2.4 to 3.2 Gm. per day, reported falls in cholesterol in 80 per cent of their patients. In all patients who responded, the fall occurred by the sixth week of therapy. The percentage decrease was greatest, averaging 40 per cent, in the 6 patients having initial cholesterols above 350 mg. per cent. In a second group of 52 patients, with initial cholesterols of 250 to 350 mg. per cent, there was an average fall of close to 30 per cent with treatment. Our patients given phenylbutyrate all fell into the first category of Cottet, i.e., initial blood
cholesterol levels greater than 350 mg. per cent, but showed no comparable response. Malmers has also reported no definite effect of phenylbutyrate in the treatment of hypercholesterolemia.\textsuperscript{14}

Cottet and co-workers found that \(\beta\)-phenylvalerate, in doses of 4.2 to 5.6 Gm. per day, was at least as effective as phenylbutyrate.\textsuperscript{4} Again we failed to observe any significant effect of this compound, with the possible exception of 1 patient who showed only a minimal response.

There is now sufficient biochemical information concerning the mechanism of action of these compounds to provide an explanation for their apparent failure to affect the serum cholesterol level. As we have previously reported, \(\alpha\)-phenylbutyrate and \(\beta\)-phenylvalerate strongly inhibited the rate of acetate-1-\(\text{C}^{14}\) incorporation into cholesterol by rat liver.\textsuperscript{5} This effect alone would appear to offer a mechanism for lowering cholesterol synthesis and eventually producing hypocholesterolemia. On the other hand, acetate incorporation into fatty acids and acetoacetate was also inhibited.\textsuperscript{5} This suggested a site of action at one of the earliest stages in the metabolism of acetate and not exclusively on the pathway to cholesterol synthesis. Subsequent studies in this laboratory have shown that the site or sites of action of phenylbutyrate include, in fact, the very first reaction by which acetate enters the biosynthetic pathway, i.e., the aceto-coenzyme A kinase reaction by which free acetate is activated to acetyl-coenzyme A.

In over all metabolism, glucose and fatty acids are probably the main sources of carbon for endogenous cholesterol synthesis. Both can give rise to acetyl-coenzyme A without degradation to the level of free acetate. Consequently, inhibition of acetate activation need not have any effect on the rate of cholesterol synthesis from these major precursors. From the results of current studies of the effect of these compounds on the incorporation of \(\text{C}^{14}\)-glucose into cholesterol it will be possible to evaluate further the validity of this explanation for the failure to obtain effects on serum cholesterol levels despite the effective inhibition of \(\text{C}^{14}\)-acetate incorporation into cholesterol. The failure of phenylbutyrate and related compounds to produce hypocholesterolemia in these studies may be considered supporting evidence that the contribution of free acetate to the pool of cholesterol precursors in the whole animal is a minor one.

While the above interpretations are compatible with all of the present findings, alternative explanations cannot be excluded. However, it is reasonable to suggest that the search for chemical agents designed to inhibit cholesterol synthesis should be focused on later steps in the biosynthetic pathway, possibly at the level of the known 5-carbon intermediates, or of compounds containing the cyclopentanophenanthrene nucleus.

**Summary**

Twelve hypercholesterolemic patients and 1 normocholesterolemic patient were given sodium alpha-phenylbutyrate and sodium beta-n-phenyl-valerate in doses of 17–120 mg. per Kg. for periods of 21 to 84 days. Dietary intake was carefully controlled, including the use of liquid formula diets in 6 patients.

No significant depression in serum cholesterol was observed during treatment with the exception of a single patient whose cholesterol level fell less than 15 per cent.

Two patients developed toxic reactions necessitating withdrawal of sodium phenylvalerate. No effect on serum cholesterol was observed in a single normocholesterolemic patient given sodium \(\alpha\)-phenylbutyramide.

Reasons for the failure of these compounds to produce hypocholesterolemia despite their demonstrated inhibitory effect on the incorporation of acetate into cholesterol are discussed in terms of more recent biochemical information concerning their sites of action.

**Acknowledgment**

The authors express their appreciation to Miss Dorothea Chu for technical assistance and to the Department of Nutrition, Miss Edith Jones, Director, for invaluable help in preparing the constant diets.

**Summario in Interlingua**

Dece-duo patientes hypercholesterolemic e 1 patiente normocholesterolémic recipecva alpha-phenylbutyrato e beta-n-phenylvalerato de
natrium in doses of 17 to 120 mg per kg during periods of 21 to 84 days. Le ingestion dietari
del pacientes eseva cautamente regulate. Sex
patientes recipeva dietas liquide a formula fixe.
Nulle depression significative del cholesterol
seral eseva observate in le curso del tracta-
mento, con le exception of un sol paciente in
qui le nivello de cholesterol descendeva per
minus que 15 pro cento.
Duo patientes disvelopava reactiones toxic
que necessitava le cessation del uso de phenyl-
valerato. Nulle effecto super le nivello seral
de cholesterol eseva notate in le caso del
patiente normocholesterolemic qui recipeva
alpha-phénylbutyramido de natrium.
Le rationes que explica le facto que iste
compositos non produce hypocholesterolemia
ben que illos exerce demonstratemente un
effecto inhibitori super le incorporation de
acetato in cholesterol es discutite super le
base de plus recente informationes biochimic
in re le sitos de lor action.

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In a study of 10 patients with systemic lupus erythematosus followed for an average period of
4 months, prednisone and prednisolone had been found to be potent suppressive agents. They are
capable of diminishing the fever, chills, malaise, anorexia, arthritis, rash, mucous membrane lesions,
cough, pleuritic pericardial pain, chest wall tenderness, pleural and pericardial friction rub, pul-
monary rales, abdominal pain and tenderness, headache, convulsive seizures, leukopenia, elevated
sedimentation rate, and C-reactive protein. These steroids were found to be approximately 4
times as potent as cortisone and hydrocortisone and did not cause salt or water reduction or hypo-
potassemia.

Kitchell
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