Intermediates in the Conversion of Mevalonic Acid to Squalene by a Rat Liver Enzyme System*

Lloyd A. Witting and John W. Porter

From the Radioisotope Unit, Veterans Administration Hospital, and The Department of Physiological Chemistry, University of Wisconsin, Madison, Wisconsin

(Received for publication, February 27, 1959)

Since the discovery (3) isolation (4) and characterization (5) of mevalonic acid (3-methyl-3,5-dihydroxy valeric acid), a succession of reports has appeared on the incorporation of this substance into a great variety of isoprenoid compounds. These range from the mono- (6) and sesquiterpenes (7, 8) through the tri- (9-16), tetra- (17-20), and poly- (21) terpenes. In yeast mevalonic acid serves as the precursor for the triterpene squalene and ergosterol (9-12).

Most of the information available on intermediates in the conversion of mevalonic acid to squalene has been obtained with a yeast system. By the use of a soluble enzyme system Chaykin et al. (10) have shown the stepwise accumulation of 5-phosphomevalonic acid, 5-pyrophosphomevalonic acid, and isopenentenyl pyrophosphate (pyrophospho-3-methyl-but-3-ene-1-ol). Lynen et al. (8) have demonstrated the incorporation of chemically synthesized 5-phosphomevalonic acid (22) and isopenentenyl pyrophosphate into squalene by a similar yeast system. They have also characterized farnesyl pyrophosphate as an intermediate in this conversion. Partial purification of a mevalonic kinase from yeast has been reported by Tchen (23).

Much less information is available on the conversion of mevalonic acid to squalene in mammalian systems. Partial purification of a rat liver enzyme system for the conversion of mevalonic acid to squalene has been reported by Popjak et al. (13) and Markley and Gurin (14). The most exacting identification of an intermediate in the biosynthesis of squalene by the rat liver system has been achieved by Ogivie (7) who reported the characterization of 4-carboxy-farnesol. The reports on farnesenic (farnesolic) acid as an intermediate (24-25) are not convincing and are a contradiction of the data of Amudar et al. (20) which preclude this oxidation state. Furthermore, Wright (26) has found farnesenic acid to be an antimetabolite for Lactobacillus acidophilus which requires mevalonic acid as a growth factor. Presumably the rat liver enzyme system and the yeast enzyme system form squalene from mevalonic acid in an identical manner; but no common intermediates have been demonstrated.

The present report is concerned with the detection, separation, and partial characterization of nine compounds formed from 2-C<sup>14</sup>-mevalonic acid by the rat liver enzyme system. Several of these compounds appear to be identical to those synthesized by the yeast system. Others have not been previously reported. The enzyme system of Bucher and McGarrahan (27) as modified by Knauss et al. (15) and a still further modified system were used in these studies.

**EXPERIMENTAL**

**Materials**—Sources of many of the chemicals used in this study were reported in a previous paper (15). Other chemicals were obtained as follows: 1- and 2-C<sup>14</sup>-mevalonic acid were obtained from Isotopes Specialties Company. Adenosine triphosphate-P<sup>32</sup> (APPP<sup>32</sup>) was prepared by Dr. Robert Metzenberg by the method of Lowenstein (28). Collidine was obtained from the Aldrich Chemical Company, and kerosene was obtained from Fisher Scientific Company. Pritzeboe Brothers was the source of farnesol and geraniol.

**Methods**—The assay of reagents, preparation of the enzyme system and the incubation procedure were described in a previous paper (15). Fractionation of the radioactive compounds formed in the incubation mixture and identification of these components were achieved by the following methods.

**Separation of Components of Incubation Mixture**—Squalene, digitonin-precipitable sterols, and cholesterol were extracted, separated, and identified as previously reported. Petroleum ether soluble substances, other than non-saponifiable compounds, were extracted from the acidified incubation mixture (15) and then chromatographed in the kerosene-acetic acid system of Kaufmann and Nitsche (29).

A second method for the separation of the C<sup>14</sup>-labeled components of incubation mixtures was developed. In this method the protein was heat denatured (3 minutes at 70°), centrifuged, washed three times with ethanol-ethyl ether (1:1), and then discarded. Ethyl ether was removed by evaporation and the alcoholic extract was diluted with an equal volume of water. Non-saponifiable compounds and the terpenoid moieties of intermediates subsequent to isopenentenyl pyrophosphate were removed by three extractions with petroleum ether. Partition between 90% ethanol and petroleum ether brought compounds at or beyond the squalene level, in the biosynthesis of cholesterol, into the petroleum ether phase, as determined by means of subsequent chromatography. The extraction of the presqualene intermediates into the petroleum ether phase was accomplished when the alcoholic extract was diluted to 50% with water.

The deproteinized supernatant solution of the incubation mixture was diluted with an equal volume of alcohol and then non-unsaponifiable compounds were extracted with petroleum ether. Terpenoid compounds intermediate between mevalonic acid and squalene were extracted with petroleum ether after-acidification.
Conversion of Mevalonic Acid to Squalene

**Effect of removing cofactors and enzyme fractions upon incorporation of 3-Cl-14C-mevalonic acid into non-saponifiable and other petroleum ether soluble compounds**

The complete incubation system contained: ATP, 5.0 μmoles; MgCl₂, 30 μmoles; glucose-1-P, 22.5 μmoles; GSH, 30 μmoles; CoASH, 0.2 μmoles; DPN, 1.3 μmoles; TPN, 1.4 μmoles; 2-C¹⁴-mevalonic acid, 4.5 μmoles and 220,000 c.p.m. per μmole; phosphate buffer, pH 7.0, 500 μmoles; microsomes and ammonium sulfate precipitated soluble enzymes. 40 to 60% of saturation (total protein, 24 mg); and water to a volume of 5.0 ml. When Mn⁺⁺ was added in place of Mg⁺⁺, 30 μmoles were used. Incubation was made under oxygen at 38° for 3 hours. Terpenoid compounds were extracted with petroleum ether after the extraction of non-saponifiable compounds and the acidification of the residual solution to pH 1 to 2.

<table>
<thead>
<tr>
<th>Cofactor omitted</th>
<th>Non-saponifiable compounds</th>
<th>Other terpenoid compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>14.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Microsomes</td>
<td>1.1</td>
<td>16.6</td>
</tr>
<tr>
<td>Soluble enzymes</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>ATP</td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Mg⁺⁺</td>
<td>0.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Mg⁺⁺; Mn⁺⁺ added</td>
<td>6.4</td>
<td>2.8</td>
</tr>
<tr>
<td>Glucose-1-P</td>
<td>2.6</td>
<td>1.5</td>
</tr>
<tr>
<td>TPN</td>
<td>5.7</td>
<td>14.4</td>
</tr>
<tr>
<td>GSH</td>
<td>11.4</td>
<td>3.2</td>
</tr>
<tr>
<td>CoASH</td>
<td>14.6</td>
<td>6.0</td>
</tr>
</tbody>
</table>

of the alcoholic solution to pH 1 to 2. The terpenoid compounds from the heat denatured protein and those obtained on acidification of the supernatant solution were identical in chromatographic behavior in the kerosene-acetic acid system. These compounds were also identical chromatographically with the compounds obtained after saponification and acidification of an incubation mixture. These compounds were also chromatographed on silieic acid-Celite (1:1) columns.

The intact, water-soluble, acid-labile substances containing the higher terpenoid compounds were extracted from the incubation mixture with collidine. The extract was washed three times with water and the compounds were then chromatographed on paper in a system of n-aryl alcohol-collidine-water (1:1:1).

**Counting of Samples**—All samples were counted with either a Packard Tri-Carb liquid scintillation counter or a Nuclear gas flow counter. C⁴ and P³² were counted on Taps 3 and 1 respectively and quenching of count was determined through counts on internal standards. C⁴ and P³² counts were obtained on the flow counter without an aluminum absorber, whereas P³² counts were determined with the absorber.

**RESULTS**

**Requirement for Cofactors**—The requirements for cofactors and enzymes for the conversion of mevalonic acid to squalene and sterols by the fractionated rat liver enzyme system are shown in Table I. They include microsomes, that portion of the soluble enzyme system precipitating between 40 and 60% of saturation with ammonium sulfate, ATP, Mg⁺⁺, DPN, TPN, and glucose 1-phosphate. The latter compound could be replaced by DPNH and TPNH in experiments in which short incubation times (1 hour) were used. A 20% reduction in the conversion of mevalonic acid to non-saponifiable compounds was observed in the absence of glutathione and synthesis was unimpaired by the omission of CoA. The non-saponifiable compounds synthesized in one typical incubation were comprised of 23% of squalene, 57% of digitonin precipitable sterol, and 6% of cholesterol, determined as the dibromide after regeneration from the digitonide.

The synthesis of squalene was blocked by omission of microsomes, as reported by Popjak et al. (8). The small incorporation of mevalonic acid into squalene by the soluble enzyme system in this study and in the studies of Markley and Gurin (14) is considered insignificant, for it could have arisen through the sulfobilization of approximately 0.02 to 0.04 mg of microsomal enzyme per 10 mg of soluble supernatant enzyme. The accumulation of petroleum ether soluble C¹⁴-labeled compounds on omission of TPN or DPN or microsomes from the incubation mixture was observed (Table I).

More recent experiments than that of Table I have resulted in the conversion by the soluble enzyme system of 20 to 80 μmoles of mevalonic acid (per mg of protein) to petroleum ether soluble intermediates in 3 hours. One milligram of microsomal protein will convert the products formed from 200 to 400 μmoles of mevalonic acid to squalene and sterols during the same period. The soluble enzyme fraction retains stability (over 95%) on storage in a Dry-Ice chest for periods up to 10 months. Microsomes are somewhat less stable, but they may also be stored for considerable periods of time.

**Synthesis of C³² and P³²-Labeled Intermediates**—When 2-C¹⁴, mevalonic acid was incubated with all of the required cofactors and only the soluble enzyme system under either nitrogen or oxygen a number of intermediates in the biosynthesis of squalene accumulated. These intermediates were demonstrated through chromatography of the supernatant solution after the protein was denatured and washed. The combined washes and supernatant solution were chromatographed to determine the presence of intermediates in 3 hours. One milligram of microsomal protein will convert the products formed from 200 to 400 μmoles of mevalonic acid to squalene and sterols during the same period. The soluble enzyme fraction retains stability (over 95%) on storage in a Dry-Ice chest for periods up to 10 months. Microsomes are somewhat less stable, but they may also be stored for considerable periods of time.

**Table II**

**Identification of phosphorylated derivatives of mevalonic acid**

The complete incubation mixture contained ATP·P₃, 5.0 μmoles (APFP₃ 200,000 c.p.m./μmole); MgCl₂, 30 μmoles; GSH, 30 μmoles; 2-C¹⁴-mevalonic acid, 10 μmoles (100,000 c.p.m./μmole); phosphate buffer, pH 7.0, 500 μmoles; soluble enzyme, 21.3 μg of protein; and water to a final volume of 5.0 ml. Incubations were made under an atmosphere of nitrogen at 38° for 3 hours. Residual P³²-labeled ATP was removed by treatment with charcoal. P³²:C¹⁴ ratios were determined after chromatography in the system of Chaykin et al. (10), and elution from the paper was made with ethanol-ammonium hydroxide (8:1) in the case of pyrophosphate mevalonic acid, and with tert-butanol-water (4:1) in the case of the isopentenyl pyrophosphate.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rₖ values</th>
<th>P³²:C¹⁴ ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mevalonic phosphate . . .</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Mevalonic pyrophosphate .</td>
<td>0.03</td>
<td>0.34</td>
</tr>
<tr>
<td>Isopentenyl pyrophosphate</td>
<td>0.32</td>
<td>0.65</td>
</tr>
</tbody>
</table>

* The value is that reported by Tchen (9).
† The values are those reported by Chaykin et al. (10).
The water-soluble, collidine-extractable compounds synthesized from isopentenyl pyrophosphate or 2-C\textsuperscript{14}Mevalonic acid were synthesized from 2-C\textsuperscript{14}Mevalonic acid in the presence of P\textsuperscript{32} in Fig. 2 were detected in this extract. These compounds were soluble in petroleum ether and were readily resolved by subsequent chromatography in the kerosene-85% acetic acid system of Kaufmann and Nitsche (29) (Fig. 2).

**Synthesis of \textsuperscript{14}C-Labeled Intermediates beyond Isopentenyl Pyrophosphate**—When isopentenyl pyrophosphate was incubated with the soluble enzyme system, a nearly complete conversion to other intermediates in the biosynthesis of squalene was effected. These intermediates were extractable with collidine, and separable on chromatography, from residual traces of isopentenyl pyrophosphate. These compounds could also be synthesized from 2-\textsuperscript{14}C-Mevalonic acid in the presence of the soluble enzyme system, ATP, and Mg\textsuperscript{2+}. When a collidine extract of the latter incubation mixture was chromatographed on Whatman No. 1 paper in the system amyl alcohol-collidine-water (4:4:1) five poorly resolved peaks of radioactivity were found between \( R_f \) 0.60 to 0.85. Isopentenyl pyrophosphate remained at the origin. The compounds with the higher \( R_f \) values were not extracted with petroleum ether after elution from the paper and solution in 50% ethanol. Treatment of these compounds with alkaline phosphatase or water moccasin venom resulted in the liberation of petroleum ether soluble Compound No. 5, \( R_F = 0.75 \) (Fig. 2). A small quantity of Compound No. 4 was also liberated when the water moccasin venom was used. Acidification of the collidine extract to pH 1 to 2, after removal of isopentenyl pyrophosphate, resulted in the complete transfer of radioactivity into the petroleum ether phase. All of the compounds shown in Fig. 2 were detected in this extract.

The water-soluble, collidine extractable compounds synthesized from isopentenyl pyrophosphate or 2-\textsuperscript{14}C-Mevalonic acid were synthesized from 2-\textsuperscript{14}C-Mevalonic acid in the presence of P\textsuperscript{32}.

![Fig. 1. Chromatographic separation of \textsuperscript{14}C-labeled compounds synthesized in an incubation mixture complete as in Table I except for the omission of microsomes, TPN, and CoA. See the text for the components of the chromatographic system and a discussion of the identity of the \textsuperscript{14}C-labeled compounds. The numbers over the peaks are count rates at complete scale deflection of the count rate meter for each peak.](http://www.jbc.org/)

![Fig. 2. Chromatographic separation of the terpene moieties derived from water-soluble, acid-labile terpenoid compounds. The incubation mixture was the same as that of Table III. The terpenoid moieties were released from the water-soluble compounds by treatment with acid, and they were extracted into petroleum ether. Chromatographic separation was achieved on Whatman No. 4 filter paper impregnated with kerosene and developed with 85% acetic acid saturated with kerosene.](http://www.jbc.org/)
neither had dropped to zero at the end of 6 hours. Net synthesis of glucose-1-P and 1.4 pmoles of DPN. The terpenoid moiety continued until Compound 3 remained as the principal product. The terpenoid moieties were extracted with petroleum ether. Aliquots were chromatographed in the kerosene-85% acetic acid system to determine the components present. These were then eluted and counted. Over a period of 6 hours (Fig. 3) the concentrations of Compounds 6, 4, and 5 rose to a maximum, and the ethyl ether was evaporated. After acidification to pH 3, 5-di-nitrobenzoate derivative was obtained which crystallized in an ice bath from petroleum ether (b. p. 35 to 60°).

Characterization of Compound 6 as farnesol

The incubation mixture and the conditions of incubation were the same as those of Table III except for the addition of 22.5 μmoles of glucose-1-P and 1.4 μmoles of DPN. The terpenoid compounds were extracted into collidine and washed free of starting material and lower intermediates with water. The 3,5-di-nitrobenzoate derivative was made by the standard method. The product was crystallized as one of the terpenoid moieties arising from 2-C14 mevalonic acid through chromatography and preparation of the 3,5-di-nitrobenzoate derivative. In initial experiments, farnesol was found to have an Rp of 0.75 in the kerosene-85% acetic acid system. The petroleum ether soluble compound with an Rp of 0.75, and purified by chromatography on silicic acid-Celite columns (1:1), was added to carrier farnesol and the 3,5-dinitrobenzoate was formed. Very little radioactivity was found in the derivative. However, when nonradioactive farnesol was added before cleavage of the water soluble terpenoid compounds with acid a 3,5-dinitrobenzoate derivative was obtained which crystallized to constant specific radioactivity (Table IV). It is quite possible that the difference in results was caused by an acid-catalyzed oxotropic rearrangement (30) of the small quantity of C14-labeled farnesol to nerolidol when carrier farnesol was not added to the system before treatment with acid. Further investigation of this possibility is in progress.

The identity of the other compounds synthesized from 2-C14 mevalonic acid is under investigation. There is some indication that Compound 6 may be geraniol. Compounds 3 and 4 have Rp values which are nearly the same as that reported for 4-carboxy-farnesol in the ethanol-ammonia system by Ogilvie (7). However, incubation of 1-C14 mevalonic acid alone or C14-labeled NaHCO3 with nonradioactive mevalonic acid in the rat liver system has not led to the synthesis of C14-labeled Compounds 3 or 4.

Conversion of Intermediates to Squalene and Sterols—A mixture of petroleum ether soluble compounds, 3, 4, and 5 (25, 35, 40% respectively) (Fig. 2), was obtained from heat denatured protein of an incubation mixture through extraction with ethanol:ethyl ether (1:1). These three compounds, one of which was farnesol, were reintroduced into a complete incubation mixture or one lacking a single cofactor required for the conversion of mevalonic acid to sterols. Over 60% of the radioactivity introduced was converted to digitonin-precipitable sterols (Table V). Of this quantity 4% was in cholesterol, as determined by formation of the 3,5-dinitrobenzoate derivative. However, when nonradioactive farnesol was added to the system before treatment with acid. Further effect of TPNH on the conversion of the water-soluble terpenoid compounds to squalene and sterols is also demonstrable. Omission of TPN from an incubation mixture results in an accumulation of these compounds. Addition of TPNH to the incubation mixture results in a decrease in the concentration of these compounds and an increase in squalene and sterols (Fig. 4).

Discussion

The synthesis of squalene and sterols from mevalonic acid requires the presence of ATP, Mg2+, DPN, TPN, glucose 1-phosphate, microsomes, and that portion of the soluble protein from rat liver precipitating between 40 to 60% of saturation with ammonium sulfate. Glucose 1-phosphate is not a necessary cofactor if the reduced pyridine nucleotides are used. The requirements for glutathione and coenzyme A found when acetate is the substrate (13) or the absolute glutathione requirement reported by Popjak et al. (13) for a cruder enzyme system were not observed. Stabilization of the enzyme system was achieved through storage in a Dry-Ice chest.
When squalene formation was blocked by elimination of TPN, DPN or microsomes from the incubation mixture, nine intermediates were found to accumulate. From the chromato- graphic behavior and P-32:C-14 ratios of three of these compounds it was concluded that they were 5-phosphomevalonic acid, 5-pyro- phosphomevalonic acid and isopentenyl pyrophosphate. These compounds were originally characterized by Tchen (9) and Chaykin et al. (10) as compounds synthesized by the yeast enzyme system. Terpene biogenesis apparently, therefore, follows a common pathway in both yeast and rat liver enzyme systems.

Water-soluble, acid-labile, intermediates subsequent to iso- pentenyl pyrophosphate were isolated and purified. On treat- ment with alkaline phosphatase, water mocassin venom, or acidification of pH 1 to 2 one of these compounds liberated farnesol as established by chromatography and crystallization of the 3,5-dinitrobenzoate derivative to constant specific radio- activity. Authentic carrier farnesol was added before cleavage of the water-soluble derivative of farnesol to prevent the conversion of the tracer quantities of farnesol to a compound of a different structure. It is thought that an acid catalyzed oxo- tropic rearrangement of C-14-farnesol to nerolidol occurred in the absence of large quantities of carrier farnesol. Further investigations of this possibility are in progress.

Since Lynen (8) has isolated farnesyl pyrophosphate from the yeast system it is not surprising that a compound containing farnesol should be found in the rat liver system. The confusion that appears in Lynen’s work, namely that farnesyl pyrophosphate was first reported as the geraniol derivative (31) and then subsequently reported to be contaminated with geranyl geraniol (32) may possibly be explained by a lack of an adequate chroma- tographic system for the separation of the higher terpene derivatives. In the present work it is shown that after cleavage with acid the terpene alcohol moieties are readily separated on paper in the kerosene-55% acetic acid chromatographic system or on silicic acid-Celite (1:1) columns. Six unsaturated terpenoid materials have been shown to arise from this treatment of a colidine extract of the rat liver system. Some success has been achieved in the separation of the water-soluble compounds on paper chromatograms with the system amyl alcohol-collidine- water (4:4:1), but completely satisfactory resolution has not been obtained. However, where concentrations have been ob- tained, after repeated chromatography, a 1:1 relation has been found to exist between a given peak of water-soluble component and a petroleum ether soluble fragment.

An order of appearance of the compounds above isopentenyl pyrophosphate is suggested from the results of C-14 incorporation into the six water-soluble terpenoid compounds. This order is as follows:

\[
\text{Isopentenyl pyrophosphate} \rightarrow \text{Compound 6} \downarrow \text{Compound 4} \downarrow \text{Farnesol X} \downarrow \text{Compounds 1 and 2} \downarrow \text{Compound 3} \downarrow \text{Squalene}
\]

Whether all of these compounds are on the direct pathway to squalene is unknown, but at present there is no evidence to sug- gest that they are not.

The position of 4-carboxy-farnesol in squalene biogenesis is not immediately evident. It is quite possible however, that this compound may account for the recurring reports of farnesenic (farnesolic) acid in the literature (24, 25). Whether one of our compounds is 4-carboxy-farnesol is as yet unknown. However, we have been unable to demonstrate CO2 fixation from C-14- NaHCO3 or retention of radioactivity from 1-C-14-mevalonic acid in our postisopentenyl pyrophosphate intermediates. The occurrence of at least six intermediates between the C5 stage and squalene certainly suggests, however, that the reaction sequence for the synthesis of squalene is considerably more complicated than one would expect from the theories of Chaykin et al. (10) or Lynen et al. (8).

Although P-32 from ATP is readily detected in isopentenyl pyrophosphate and 5-pyrophosphomevalonic acid, we have as yet been unable to detect labeled phosphate in the higher, water- soluble, acid-labile, terpene derivatives. Furthermore, the

**TABLE V**

**Conversion of terpenoid compounds to digitonin-precipitable sterols**

<table>
<thead>
<tr>
<th>Cofactor omitted</th>
<th>Precipitable with digitonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>62</td>
</tr>
<tr>
<td>ATP</td>
<td>60</td>
</tr>
<tr>
<td>Mg++</td>
<td>64</td>
</tr>
<tr>
<td>TPN</td>
<td>16</td>
</tr>
<tr>
<td>DPN</td>
<td>28</td>
</tr>
<tr>
<td>Glucose-1-P</td>
<td>10</td>
</tr>
</tbody>
</table>

**FIG. 4.** Conversion of water-soluble, acid-labile terpenoid compounds to nonsaponifiable compounds in the presence of TPNH. Incubation mixtures were the same as those of Fig. 3; 5 µmoles of TPNH were added after 3 hours of incubation.
isolated petroleum ether soluble moieties were readily and almost completely converted to digitonin precipitable sterols, including cholesteral, without a requirement for ATP, when reintroduced into the otherwise complete enzyme system. These apparent peculiarities of the rat liver enzyme system as contrasted to the yeast enzyme system are now under investigation.

SUMMARY

1. An ammonium sulfate precipitated fraction of the water-soluble, supernatant enzyme system from rat liver has been found to form at least nine intermediates between mevalonic acid and squalene. Three of these have been identified as 5-phospho-mevalonic acid, 5-pyrophosphomevalonic acid, and isopentenyl pyrophosphate (pyrophospho-3-methyl-but-3-ene-1-ol). The other six are water-soluble, acid-labile derivatives of higher terpenoid compounds. Farnesol has been identified as a component of one of these compounds.

2. Studies of the time of appearance of C14 in the terpenoid compounds suggest the following order of synthesis: No. 6, No. 4, farnesyl x, No. 1 and 2, No. 3. The identity of the compound linked to the terpenoid moieties is unknown, but no evidence was obtained that it is a pyrophosphate compound containing phosphate derived from the terminal phosphate unit of adenosine triphosphate.

Acknowledgments—The authors gratefully acknowledge the technical assistance of Mr. Herbert Simonson and Mr. Michael Rice on a portion of the experimentation reported in this paper. They also acknowledge the gift of large quantities of rat liver by Dr. W. H. McShan of the Zoology Department, University of Wisconsin.

REFERENCES

Intermediates in the Conversion of Mevalonic Acid to Squalene by a Rat Liver Enzyme System
Lloyd A. Witting and John W. Porter


Access the most updated version of this article at http://www.jbc.org/content/234/11/2841.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/234/11/2841.citation.full.html#ref-list-1