Isopentenoid Synthesis in Isolated Embryonic Drosophila Cells

POSSIBLE REGULATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE ACTIVITY BY SHUNTED MEVALONATE CARBON*

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[1-14C]Mevalonate's rate of conversion to 14CO2 (a measure of total isopentenyl 1-pyrophosphate synthesis) was minimally 5-fold greater than that for neutral isopentenoid lipid synthesis (measured with either [5-3H]-, [3-14C]-, or [5-14C]mevalonate). However, [5-3H]mevalonate's rate of conversion into [3H]H2O (measure of shunted mevalonate carbon) was equivalent or greater than that measured for neutral isopentenoid lipid synthesis. [5-14C]Mevalonate radioactivity was incorporated into macromolecules and n-fatty acids. Kc cell extracts (100,000 × g supernatant fluid) readily oxidized alcohols with the following activity sequence: geraniol = nerol > farnesol = dimethylallyl alcohol > geranylgeraniol, isopentenyl alcohol, and allyl alcohol. Oxidation required NAD, and ethanol was not a substrate.

We conclude that (a) Kc cells shunted a significant fraction (≥40%) of their post-IPP carbon to prenols for oxidative catabolism and (b) that shunted mevalonate carbon may play a significant role in the mevalonate-mediated regulation of Kc cell HMG-CoA reductase activity.

HMG-CoA1 reductase (hydroxymethylglutaryl-CoA reductase (NADPH), EC 1.1.1.34) has been defined as the enzyme which catalyzed the rate-limiting reaction of sterol synthesis (1, 2). Unfortunately there is no evidence that the control coefficient (3–5) for HMG-CoA's rate of conversion to mevalonate is 1.0 or greater than any other reaction for the synthesis of isopentenoids. Furthermore, the studies of Bergstrom et al. (6) demonstrate that hepatic acetoacetyl-CoA synthetase and HMG-CoA synthase activities follow dietary and diurnal modifications similar to that for HMG-CoA reductase. Thus, HMG-CoA reductase's continued designation as the "rate-limiting enzyme for sterol (isopentenoid) synthesis should be used with caution (5). Regardless, reductase does serve as a reasonable reporter enzyme for monitoring changes in cellular demand for isopentenoid compounds (1, 2, 8, 9).

Two widely studied modifications of HMG-CoA reductase activity (amount) result from increased/decreased mevalonate (10–13) and/or cholesterol availability (1, 13). Sterologenic cells modulate HMG-CoA reductase activity in response to both of these perturbations (13). Consequently, it has been difficult to study the impact of sterol or mevalonate availability, independent of the other. Therefore, we have used (14, 15) an established Drosophila embryo cell line (Kc cells (16)) which has no apparent bulk sterol requirement nor the capacity to synthesize this isopentenoid (17) to investigate "sterol-independent" (13) regulation of HMG-CoA reductase activity.

We have proposed (18) that mevalonate-mediated modulation of Kc cell HMG-CoA reductase activity was due to changes in the pool size of a metabolite distal to isopentenyl 1-pyrophosphate (IPP). Potential metabolites would include prenyl 1-pyrophosphate esters, nonphosphorylated isopentenyl precursors, or end products (farnesol and ubiquinone for Kc cells (17)). However, it was possible that Kc cells "shunted" (19, 20) a significant fraction of post-IPP carbon at the level of C5–C10 prenyl 1-pyrophosphates for oxidative catabolism to acetyl-CoA (19, 20). If so, modulation in the amount of post-IPP shunting could have an independent immediate effect on carbon flow to isopentenoid end products (20, 21). Also the shunt pathway could generate direct negative effector(s) (post-IPP regulatory signal molecule(s)) which (a) reduce mevalonate carbon flux (22–24), (b) modulate HMG-CoA reductase's rate constant for reversible-irreversible activation (25–27), (c) alter reductase's gene expression (28–31), or (d) reflect a combination of these possibilities.

In this paper we report efforts to (a) determine whether mevalonate-mediated suppression of Kc cell HMG-CoA reductase activity correlated with apparent rates of IPP or isopentenoid lipid synthesis or the steady state profile for water-soluble isopentenoid phosphate esters and (b) define a metabolic balance sheet for Kc cell utilization of mevalonate carbon.

No simple correlation between HMG-CoA reductase suppression and mevalonate conversion to isopentenoid end products was obtained. However, we demonstrate that Kc cells

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1 The abbreviations used are: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HPLC, high performance liquid chromatography; IPP, isopentenyl 1-pyrophosphate; reductase, HMG-CoA reductase.
convert a significant (≥40%) fraction of their post-IPP carbon to non-isopentenoid end products.

The observations are discussed in terms of a need to define the post-IPP shunt pathway and to determine whether the latter plays a role in mevalonate-mediated suppression of HMG-CoA reductase activity.

MATERIALS AND METHODS

Biochemicals and Reagents

(3S)-3-Hydroxy-3-methyl-[5-14C]butyryl coenzyme A and (3R)-[1-3H]- and [3-'14C]-mevalonate; Research Products International Corp. (Mt. Prospect, IL), (3RS)-[5-14C]- and [4-5-14C]-mevalonate. Glucose 6-phosphate, glucose-6-phosphate dehydrogenase, NADP, NAD, and dihydrothioretol were purchased from Sigma. Geranylgeraniol was a generous gift from Dr. John Porter (Veterans Hospital, Madison, WI). All other reagents were purchased in the highest grade possible.

Maintenance of Cells

Kc cells were grown as described previously (14). Suspension and monolayer cultures were used for the experiments presented in this report. In order to save on valuable reagents, some experiments were performed with 10–20-fold concentrated cell suspensions (18). Specific manipulations and/or growth conditions are presented in figure and table legends.

Cellular Disruption

Kc cells were harvested and disrupted by sonic oscillation or nitrogen cavitation (14).

Enzyme Assays

HMG-CoA Reductase—Reductase activity was determined with (3S)-[5-14C]HMG-CoA (10–25 mCi/mol) as described previously (18). Alcohol Dehydrogenase—An aliquot (±0.1 ml) of Kc cell 100,000 g supernatant fraction (14) was added to a cuvette which contained 0.1 M glycylglycine buffer, pH 8.1 (2.3 ml), 0.1 ml of a NAD solution (28 mg/ml), and sufficient extraction buffer (13) to achieve 2.5 ml. Alcohol substrates (0.050 ml, dissolved in dimethyl sulfoxide), were added to initiate oxidation. The increase in NADH fluorescence was monitored with a Eppendorf 1101M photometer adapted for fluorometric analyses (Eppendorf fluorescence adapter 1030). None of the assayed samples quenched NADH fluorescence, and substrate independent increases in fluorescence (≤10%) were subtracted from the dehydrogenase activities reported. Relative fluorescence was converted to chemical units by comparison to a standard curve generated with known amounts of NADH.

Incorporation of Radioactive Precursors

Short Term—Kc cell suspensions (1–3 × 10^6 cells/ml) were centrifuged at 23 °C and resuspended to a density of 10–45 × 10^6 cells/ml in a 1:1 mixture of fresh-conditioned (spent media from centrifuged cells used for study) Echaller's media (16). Aliquots (2–5 ml) of the suspension were added to 10–25 ml Erlenmeyer flasks capped with serum stoppers (with/without plastic CO2 collection cups (Kontes Co., Vineland, NJ) which contained tuted 1-cm3 Whatman 3MM filter paper). Incubation (0–2 h) was initiated by the addition of radioactive substrate with shaking (gyratory shaker; 100 rpm). The protocol used to terminate an incubation was determined by the radioactive precursors to be analyzed (see below).

Long Term—Kc cells (1–2 × 10^6 cells/ml) were seeded in 25- or 75-cm^2 T-flasks which contained fresh medium and the radioactive precursors. At the appointed time cells plus media were removed, separated, and analyzed for radioactive compounds. Specific details are presented in figure and table legends.

Radioactive Product Analyses

14CO2—Only short-term incubation conditions were used to generate this [1-14C]mevalonate product. At the end of the incubation 14CO2 was liberated, trapped, and measured as [14C]bicarbonate (18).

3H,14C-Lipids—Total neutral Kc cell lipids were extracted and resolved by TLC (17) or HPLC (0.4 × 30-cm Waters Cyano column); heptane was used as the eluent (35). Lipid mass was monitored at 210 nm. Polynprolens were eluted from the TLC plate with chloroform:methanol (4:1, v/v) and further characterized by reverse phase HPLC on a 5-μm C18 column (0.4 × 25 cm); resolution was obtained with a 50-μl mixer 20-min gradient (50% water:50% acetonitrile to 100% acetonitrile). HPLC-resolved samples were collected as 1-ml fractions and their radioactivity determined by liquid scintillation spectrometry; Betafluor (National Diagnostics, Summnerfield, NJ) was used as the scintillant.

RESULTS

Isopentenoid Lipid Synthesis—Since we had proposed (18) that mevalonate mediated regulation of Kc cell HMG-CoA reductase activity was probably the consequence of increased formation of a compound distal to IPP, studies were initiated to determine whether the pattern of isopentenoid lipids synthesized from radioactive mevalonate was concentration dependent and, if so, did changes correlate with the extent of enzyme activity suppression. In Fig. 1 the distribution patterns for (3R)-[5-3H]mevalonate incorporated into isopentenoid lipids (2 h) at two different substrate concentrations were compared. The lipids were resolved by HPLC (see "Materials and Methods").

With 50 μM (3R)-[5-3H]mevalonate radioactivity was measured in lipids which migrated with retention times identical to ubiquinone, dolichol, and polyprenol standards; ubi-
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quione was the dominant lipid synthesized. However, a 20-fold increase in (3R)-[5-3H]mevalonate's concentration (1000 μM) caused a shift in the radioactivity distribution pattern toward polyprenols (---). This shift represented an absolute increase in polyprenol. That is, there was a 20-fold increase in the amount of [5-3H]mevalonate incorporated into total lipids plus a 28–50% increase in radioactive ubi-quinone. These data suggested that the polyprenol pool was expandable and we had approached ubiquinone's maximum rate of synthesis. All of the radioactivity in the polyprenol fraction was determined to be trans,trans-farnesol (70%) and geranylgeraniol (30%) (data not presented). Since our previous (17) long term studies had not indicated the synthesis of polyprenols, we determined whether the results in Fig. 1 were related to concentration and/or length of incubation. These experiments are summarized in Fig. 2. In contrast to the 2-h incorporation studies (Fig. 1), the pattern of radioactive isopentenoid lipids (120-h incubations) was not altered by increased mevalonate concentration (0.075 μM (---)) versus 1000 μM (——)). These results were consistent with either transient accumulation of polyprenols and/or differential rates of isotopic equilibration for [3H]mevalonate incorporated into isopentenoid lipids. To help distinguish between these two possibilities we compared the kinetics of [5-3H]mevalonate (500 μM) incorporation into farnesyl 1-pyrophosphate, polyprenols, ubiquinone, and dolichol. Our results are presented in Fig. 3.

Farnesyl 1-pyrophosphate and the polyprenols reached isotopic equilibrium within 30 min, whereas the ubiquinone and dolichol pools were not equilibrated during the 120-min incubation. Thus, the polyprenols are rapidly turning over intermediates whose pool sizes increase as a function of exogenous mevalonate concentration. However, the polyprenol's

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*---* Determined by reverse phase HPLC and gas-liquid chromatography/mass spectrometry analyses with the analytical facilities at Zeuscor Corp., Palo Alto, CA.

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![Fig. 1. Short term [3H]isopentenoid lipid pattern for Kc cells incubated with (3R)-[5-3H]mevalonate.](image)

![Fig. 2. Steady state [3H]isopentenoid lipid pattern for Kc cells.](image)

![Fig. 3. Kinetics of (3R)-[5-3H]mevalonate incorporation into isopentenoid precursors and products.](image)
Fig. 4. Comparison of HMG-CoA reductase suppression with the metabolism of [1-14C]- and [5-3H]mevalonate. Stock Kc cell cultures were centrifuged and resuspended in Echalier's medium to 5–10% of their initial volume. Depending on the parameter measured the following aliquots of concentrated cell suspension were used for the study: 14CO2 production, 2 ml; HMG-CoA reductase suppression, 3 ml; 3H-lipids, and [3H]isopentenoid phosphate esters, 5 ml. (3R)-[5-3H]Mevalonate (5000 cpm/nmol) was added to the appropriate flasks to obtain the final concentrations indicated. Non-radioactive (3R,3S)mevalonate was used for the HMG-CoA reductase suppression studies. All incubations were for 2 h (23 °C). HMG-CoA reductase activity, 14CO2, 3H-lipids, and [3H]isopentenoid phosphate esters were measured as described under “Materials and Methods.” The data presented are typical for one of three independent studies.

(3R)-[5-3H]Mevalonate's rate of incorporation into isopentenoid lipids approached saturation at a concentration of 5000 μM, whereas the amount of IPP formed (14CO2 production) and steady state content of water-soluble isopentenoid phosphate esters continued to increase. Furthermore, the sum of (3R)-[5-3H]mevalonate (nmol) incorporated into isopentenoid lipids and total water-soluble isopentenoid phosphate esters represented only a fraction (0.17–0.25) of the IPP (14CO2) generated in 2 h. Similar discrepancies between the rates of IPP and isopentenoid lipid synthesis have been reported for mammalian cells (20).

When we compared (by HPLC (18)) the steady state radioactive water-soluble isopentenoid phosphate esters patterns formed as a function of [5-3H]mevalonate concentration (0.5–2000 μM), only one trend surfaced (data not presented), continued expansion of each isopentenoid phosphate ester pool with a small bias toward mevalonate 5-phosphate accumulation at 2000 μM (3R)-mevalonate.

Reductase suppression did not appear to have an obvious causal relationship with either IPP flux or expansion of isopentenoid phosphate pools. The correlation between reductase suppression and the rate of total isopentenoid lipid synthesis is somewhat more suggestive (Fig. 4). However, the concentration of mevalonate required for 50% of the maximum rate of lipid synthesis is 4-fold greater than that needed to suppress reductase activity 50% in 2 h. These observations, coupled with the strong imbalance between IPP flux and lipid synthesis, suggested that meaningful progress towards linking a specific pathway flux with IPP metabolism with HMG-CoA reductase suppression would be unlikely in the absence of a total accounting for the amount of mevalonate carbon utilized. Therefore, we decided to investigate total mevalonate carbon utilization by Kc cells.

Mevalonate Utilization—Mevalonate carbon is converted to sterols, nonsterol isoprenoids, and shunted at the prenyl 1-pyrophosphate level to form acetyl-CoA (7, 8, 19). The use and limitations of variously radiolabeled mevalonate derivatives used to assess the relative contribution of these pathways to total mevalonate utilization have been discussed by Landau and Brunengraber (20). Since Kc cells do not synthesize squalene plus sterols, mevalonate’s conversion to these lipids is not an issue. However, mevalonate’s utilization for total lipid and IPP synthesis plus the shunt pathway had to be assessed. [5-3H]Mevalonate’s increased sensitivity and relative ease of use allowed us to monitor Kc cell nonsterol isoprenoid synthesis (Figs. 1 and 2) and shunt pathway (20). For the latter, α-tritium atoms in [3H]prenols would appear as [3H]H2O and/or in compounds reduced by NAD(P)H (20). We only measured [3H]H2O accumulation (see “Materials and Methods”). Therefore, our results reflect minimal values for mevalonate conversion to nonisopentenoid compounds. [1-14C]Mevalonate conversion to 14CO2 was used to assess total IPP formation (Fig. 4 (18, 20)).

The concentration-dependent rates of [5-3H]- and [1-14C] mevalonate incorporation into [3H]isopentenoid lipids, [3H]H2O and 14CO2, respectively are summarized in Fig. 5. [5-3H] Mevalonate’s incorporation into water exceeded lipid synthesis, and the sum of both processes approached the total amount of IPP (14CO2) produced. The remaining discrepancy (30–40%) in IPP carbon synthesized was probably distributed in the water-soluble isopentenoid phosphate esters (Fig. 3), nonisopentenoid products (20, 34), and any unmeasured metabolites reduced by NAD(P)H (20). Thus, a significant fraction of the “missing” mevalonate carbon (Fig. 4) appeared to be converted to prenyl alcohols (Figs. 1 and 3) and oxidized.

Although the discrepancy between IPP generation and lipid synthesis appeared to be largely met by prenyl alcohol catabolism (2-h studies), it was important to determine if this process was expressed continuously. The relative partitioning of (3R)-[5-3H]mevalonate radioactivity between isopentenoid lipids and [3H]H2O over a 72-h incubation is summarized in Fig. 6 (panels A and B). As observed in short-term studies (Fig. 5) a significant fraction of [5-3H]mevalonate’s radioactivity accumulated in [3H]H2O. Furthermore, the relative accumulation of 3H in lipids and water was mevalonate concentration dependent (compare panels 6A (0.075 μM) and 6B (1000 μM)). Thus, shunting of mevalonate carbon is a basal Kc cell process whose contribution to IPP utilization is linked to increased mevalonate concentration.

We assessed the distribution of radioactive activity retained by Kc cells incubated for 72 h in the presence of (3RS)-[5-14C] mevalonate (5000 μM). Our results are summarized in Table I. The fraction (80%) of [5-14C]mevalonate not incorporated into Kc cell’s neutral isopentenoid lipids was similar to that of

Fig. 5. Effect of mevalonate concentration on the rates of isopentenoid and nonisopentenoid product formation. A 600-μl culture of Kc cells was centrifuged and resuspended in 35 ml of Echalier’s medium. Equal aliquots (1 ml) were added to flasks which contained sufficient (3R)-[1-14C]mevalonate (350 cpm/nmol) to obtain the final concentration indicated. To each of another series of flasks was added 4 ml of the Kc cell suspension, (3R)-[5-3H]mevalonate (45 μCi/nmol), and unlabeled (3R,3S)-mevalonate (1 μM) to obtain the concentrations (R-isomer) indicated. All of the flasks were incubated for 2 h and analyzed for radioactive products as described under “Materials and Methods.” The Kc cell suspensions contained 5–6 mg of protein/ml.
predicted by the amount of \( ^{1}H \)H\_2O produced from [5-\text{H}] mevalonate (Fig. 6B). Most of the [\text{\textsuperscript{14}C}]mevalonate radioactivity in lipids accumulated as fatty acids. HPLC analysis (34) of the fatty acid fraction showed that the distribution of radioactivity and mass was identical (data not presented). Furthermore, the overall fatty acid composition was not dissimilar to what we reported previously (17). Thus, Kc cells converted a significant fraction of their mevalonate carbon to nonisopentenoid lipids. Similar observations have been made for vertebrates (20, 35) and the aphid Shizaphis graminum (36).

No effort was made to resolve the lipid solvent-extracted trichloroacetic acid-insoluble fraction. Therefore, we do not know the nature of the compounds labeled.

**Prenol Oxidation**—Since our physiological studies (Fig. 5 and Table I) suggested that Kc cells catabolized mevalonate to nonisopentenoid products, we decided to determine whether their cell-free extracts oxidized alcohols in general and prenols specifically. Drosophila have 10 electrophoretically different alcohol dehydrogenase activities, which arise from two different genes localized on separate chromosomes (37). One gene gives rise to seven alloenzyme forms (alcohol dehydrogenase), and the other (octanol dehydrogenase) accounts for the remaining three electrophoretically defined activities (37). Alcohol dehydrogenase is active with ethanol and other short chain alcohols, whereas octanol dehydrogenase catalyzes the oxidation of n-octyl alcohol and farnesol (37-39). Both alcohol dehydrogenase classes utilize NAD as the sole electron acceptor (37).

Kc cells were disrupted by sonic oscillation (14) and assayed for products as described under "Materials and Methods."
TABLE II

<table>
<thead>
<tr>
<th>Primary alcohol</th>
<th>Activity (pmol/min/mg protein)</th>
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<tbody>
<tr>
<td>Ethanol</td>
<td>ND</td>
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<tr>
<td>n-Propyl alcohol</td>
<td>ND</td>
</tr>
<tr>
<td>n-Butyl alcohol</td>
<td>ND</td>
</tr>
<tr>
<td>n-Octyl alcohol</td>
<td>800</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>ND</td>
</tr>
<tr>
<td>Isopentyl alcohol</td>
<td>ND</td>
</tr>
<tr>
<td>Allyl alcohol</td>
<td>ND</td>
</tr>
<tr>
<td>2-Buten-1-ol</td>
<td>ND</td>
</tr>
<tr>
<td>Dimethylallyl alcohol</td>
<td>600</td>
</tr>
<tr>
<td>3-Methyl-3-buten-1-ol</td>
<td>150</td>
</tr>
<tr>
<td>Geraniol</td>
<td>950</td>
</tr>
<tr>
<td>Nerol (cis-geraniol)</td>
<td>945</td>
</tr>
<tr>
<td>Citronelol</td>
<td>ND</td>
</tr>
<tr>
<td>trans,trans-Farnesol</td>
<td>600</td>
</tr>
</tbody>
</table>

Alcohol dehydrogenase substrate specificity was determined with the protocol described under "Materials and Methods." Stock (1 M) substrate solutions were prepared in dimethyl sulfoxide, and 0.05 ml of NADH. This lower limit of reliability, coupled with a 10-min assay, and the use of 0.6 mg of cell protein translated to a specific alcohol oxidation rate of 50 pmol/min/mg of protein. Therefore, rates less than this value were defined as not detectable (ND).

**DISCUSSION**

Vertebrates have been the primary experimental model to study mevalonate conversion to nonisopentenoid end products (20). Recently Nes et al. (36) have demonstrated that the aphid *Shizaphis graminum* converted [14C]mevalonate to n-alkyl alcohols (C6-C15). This observation represented the first demonstration that insects expressed elements of the trans-methylglutaconate pathway as proposed by Popjak (19). In this paper we report that an established *Drosophila* cell line (Kc cells) also divert mevalonate carbon to nonisopentenoid end products (Table II). Thus, mevalonate shunting is not limited to vertebrates.

Popjak’s (19, 20) trans-methylglutaconate shunt focuses on carbon diversion at the level of C5 and not C10/C15-prenyl-1-pyrophosphate esters. That is, dimethylallyl alcohol (hydrolyzed product from dimethylallyl 1-pyrophosphate) is oxidized by hepatic alcohol and aldehyde dehydrogenase to a C5 prenate (3-methylcrotonic acid) which, in turn, is metabolized by the mitochondrial leucine degradation pathway. Vertebrate catabolism of C5/C10 prenates was (is) not known. However, Seubert and associates (41–43) have defined a pathway for the complete catabolism of polypropenals by Psedomonas. C10/C15 prenals are oxidized to polypropenatos and degraded via a modified β-oxidation cycle (42, 43) to acetyl-CoA and 3-saethylycrotonyl-CoA. Schroepfer (8) has proposed that Seubert’s pathway (41–43) might be functional and relevant for vertebrate cells. Since catabolism of C10/C15-prenyl-1-pyro-phosphates yields identical end products (CO2, n-alkyl acids, n-alkyl alcohols, etc.), it will be difficult to distinguish between post-IPP shunting at the mono- or polyenol level. However, our data (Figs. 1 and 3) and that of other investigators (22, 35, 44–47) demonstrate that whole animals, isolated cells, and mitochondria-free cell extracts tend to "accumulate" [14C]- or [3H]mevalonate radioactivity in C5/C10/C15 prenals and/or prenolates rather than the corresponding C5 derivatives. Furthermore, there is insufficient information to ascribe the specificity of radioactivity in C5 prenals to their loss as isoprene (48) and/or a higher rate of metabolism relative to polypropenals. Therefore, polypropenol and polypropenolate synthesis appear to be favored over the C5 derivatives.

Kc cell’s alcohol dehydrogenase activity(ies) had an apparent substrate specificity for C6-C15-prenyl- and long chain n-alkahols (Table II). Ethanol was not oxidized (Fig. 7 and Table II) (40). Christophe and Popjak (44) demonstrated that horse and rat liver alcohol dehydrogenases oxidized a wide spectrum of n-alkahols and prenals. However, vertebrate alcohol dehydrogenase also exists in multiple isozymic forms (49). Therefore, it is possible that isozymes with substrate specificities similar to Drosophila alcohol dehydrogenase and octanol dehydrogenase were present in the preparations used by Christophe and Popjak (44).

Efforts to define a Kc cell activity which oxidized geranial to geranonic acid indicated that neither NAD nor NADP were the electron acceptors (data not presented). Furthermore, recent preliminary studies suggest that soluble Kc cell extracts express an oxidase activity which bleached dichloroindophenol (50) in the presence of geranial. Thus, the NAD-dependent alcohol and aldehyde dehydrogenase pathway proposed by Popjak (19, 44) does not appear to operate in Kc cells. A broad spectrum aldehyde oxidase has been previously described for Drosophila (40, 50, 51). Studies are in progress to characterize the Kc cell aldehyde oxidase activity.

Vertebrate cells divert minimally 40% of their IPP to nonisopentenoid compounds (Figs. 4–6 and Table I). The extent of post-IPP carbon shunting was related to exogenous mevalonate concentration (Figs. 5 and 6). Edmond and Popjak (35) have made similar observations with rats. Furthermore, dietary and hormonal perturbations have been shown to modulate the level of mevalonate shunting by vertebrates (52–55). Presently, we do not know if additional factors modulate Kc cell post-IPP carbon shunting.

We compared mevalonate-mediated suppression of Kc cell HMG-CoA reductase activity with changes in the apparent total and specific rates of isopentenoid lipid synthesis, total IPP formation, and isopentenoid phosphate ester steady state profile (Fig. 4). However, no simple direct relationship was obtained. Since no more than 40% of the IPP synthesized was converted to ubiquinone and dolichol (Fig. 6A), synthesis of these compounds was probably saturated under basal conditions. Furthermore, it is unlikely that mevalonate-mediated reductase suppression would be due to unique intermediates in ubiquinone and dolichol synthesis. Hence, we propose that the putative post-IPP regulatory synthesis molecule (18) is generated from shunted mevalonate carbon. In order to evaluate this possibility we need to know which nonisopentenoid intermediate(s) to monitor. Measurement of [3H]mevalonates conversion to [3H]H2O will not detect changes in a specific post-IPP intermediate. Thus, it will be necessary to define how post-IPP carbon is converted to acetyl-CoA. Studies are in progress with [14C]farnesyl-pyrophosphate and Kc cell extracts to delineate the shunt pathway.

Although we have focused on a potential linkage between post-IPP carbon shunting and HMG-CoA reductase suppression, it is possible that these processes represent independent parallel regulatory strategies (19, 20). The studies of Marinier et al. (54) with perfused rat livers provide experimental support for differential modulation of HMG-CoA reductase activity and post-IPP carbon shunting. That is, fasted or streptozan-induced diabetic rat’s decreased carbon flow to sterols appeared to result more from an increased level of shunted post-IPP carbon than enhanced HMG-CoA reductase activity suppression. Aguilerla et al. (55) have demonstrated that cholesterol feeding increases the shunting of plasma mevalonate in chickens. Therefore, the existence of regulatable post-IPP carbon shunting suggests that caution should be exercised in attributing changes in the rate of isopentenoid end product.
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synthesis exclusively to modulations of HMG-CoA reductase activity (content).

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44. Christophe, J., and Popjak, G. (1962) J. Lipid Res. 2, 244–257