ABSTRACT:

Retinaldehyde (RAL), a key intermediate in retinoid metabolism, acts as a retinoic acid (RA) precursor, but is also reduced to retinol (ROH), which can subsequently be esterified to retinyl esters, the storage form of vitamin A. Limited information is available on the metabolism of geometric isomers of RAL such as the trans- and cis-RAL isomers. Such information would be very helpful for the assessment of the teratogenic potency of RAL isomers, as teratogenesis represents a major side effect of retinoid use in pharmacotherapy. In the present study we examined concentrations of retinoids in plasma, maternal tissues, and embryos of pregnant rats 2 h after a single oral dose (100 mg/kg body weight) of all-trans-, 13-cis-, or 9-cis-RAL on gestational day 13. The main findings of this study were the very similar patterns of retinoid metabolites (consisting of retinoids with mainly the all-trans-configuration) after administration of all-trans- and 13-cis-RAL, and the high concentrations of 9-cis-RA, 9,13-dicis-RA, and 9-cis-retinoyl-β-D-glucuronide after dosing with 9-cis-RAL. In addition, all-trans-RA as a RAL metabolite reached the embryos to a much greater extent than any of its cis-isomers. The results are discussed in view of in vitro data on enzymes involved in the biotransformation of RAL isomers.

Vitamin A is essential for a variety of physiological processes including vision, reproduction, embryogenesis, cell growth, and differentiation (Blomhoff, 1994). Endogenous vitamin A in mammals is derived from the intake of vitamin A alcohol (ROH) and its esters (retinyl esters) from animal food or of carotenoids from ingested plants. Provitamin A carotenoids like β-carotene can be cleaved in the small intestine, thus yielding retinaldehyde (RAL), which can be converted into ROH as well as into retinoic acid (RA). In addition, RAL represents an intermediate of the oxidation of ROH (also resulting from retinyl esters) to retinoid acid (Blomhoff, 1994; Napoli, 1996). Besides the important physiological role of vitamin A, several natural and synthetic vitamin A derivatives (collectively called retinoids) are used in therapy or in prevention of some dermatological and oncological disorders (Vahlquist, 1994; Hong and Itri, 1994). However, the use of retinoid drugs is limited by teratogenicity as a major side effect (Nau et al., 1994). Numerous studies reviewed by Agnish and Kochhar, 1993 and Nau et al., 1994) have examined the pharmacokinetics of various retinoids in pregnant animals and the embryonic exposure to retinoids to assess the role of metabolism, the extent of transfer to the embryo, and the proximate/ultimate teratogen in retinoid-induced teratogenesis.

Recently, all-trans-RAL has attracted much attention as a topical agent in humans (Saurat et al., 1994). Application of all-trans-RAL is considered to yield a controlled rate delivery of retinoid metabolites to cells and is now successfully used in dermatology. Furthermore, topical use of 9-cis-RAL has been investigated in this regard in a mouse model (Didierjean et al., 1998). These RAL isomers represent precursors of the corresponding RA isomers. All-trans-RA and 9-cis-RA are considered key molecules in retinoid physiology and pharmacology because they are ligands of nuclear retinoid receptors. Retinoid receptors act as transcription factors in the regulation of a large number of genes (Giguère, 1994; Chambon, 1996). All-trans-RA is a high-affinity ligand of retinoic acid receptors (RAR), whereas 9-cis-RA is bound with high affinity by both RAR and retinoid X receptors (Allenby et al., 1993). Several subtypes and isofoms exist for both RAR and retinoid X receptors, and their patterns of expression are highly regulated in the developing embryo, both temporally and spatially (Giguère, 1994; Mangelsdorf et al., 1994).

Although the metabolism and transplacental pharmacokinetics of RA isomers have been thoroughly examined in pregnant animals (Creech Kraft et al., 1989; Collins et al., 1992; Eckhoff et al., 1994; Tzimas et al., 1994a, b; Collins et al., 1994; Kochhar et al., 1995; Tzimas et al., 1996; Eckhoff and Willhite, 1997, reviewed by Agnish and Kochhar, 1993 and Nau et al., 1994), little information on these
issues exists for the isomers of RAL. Therefore, we present the results of a study on the in vivo metabolism of a single high oral dose of 13-cis-, 9-cis-, and all-trans-RAL (Fig. 1) in pregnant rats on gestational day (GD) 13. Preliminary results of the investigation on the metabolism of 9-cis-RAL have been reported previously (Sass et al., 1994; Tzimas et al., 1994).

Materials and Methods

Chemicals. All-trans-, 9-cis-, and 13-cis-isomers of retinaldehyde as well as bovine serum albumin (BSA) were purchased from Sigma (München, Germany). Isomers of both retinoic acid and 4-oxo-retinoic acid were kindly provided by Hoffmann-La Roche (Basel, Switzerland and Nutley, NJ). All-trans-retinoyl-β-D-glucuronide (all-trans-RAG) as a reference compound was a gift from Dr. A. B. Barua and Dr. J. A. Olson (Iowa State University, Ames, IA) or synthesized in our laboratory. Retinol, retinyl palmitate, and Tween 20 were obtained from Serva (Heidelberg, Germany). Organic solvents of highest purity [high-performance liquid chromatography (HPLC) grade] were purchased from Merck (Darmstadt, Germany). Isomers of both retinoic acid and 4-oxo-retinoic acid were kindly provided by Hoffmann-La Roche (Basel, Switzerland and Nutley, NJ). All-trans-retinoyl-β-D-glucuronide (all-trans-RAG) as a reference compound was a gift from Dr. A. B. Barua and Dr. J. A. Olson (Iowa State University, Ames, IA) or synthesized in our laboratory. Retinol, retinyl palmitate, and Tween 20 were obtained from Serva (Heidelberg, Germany). Organic solvents of highest purity [high-performance liquid chromatography (HPLC) grade] were purchased from Merck (Darmstadt, Germany). Water was purified with a Milli-Q water purification system (Millipore Corp., Eschborn, Germany).

Laboratory Precautions. All work with retinoids was performed under dim amber light. Retinoids and their solutions were stored at –20°C.

Animals. Wistar rats (Hsd/Win/WU; Winkelmann, Borchern, Germany) were kept under specific pathogen-free conditions and a 12-h standard light/dark cycle. They received a standard pellet diet (Altromin 1324; Altromin, Lage, Germany) and tap water ad libitum. The animals were mated during a 12-h period in the morning. The following 24 h were considered GD 0 (Chahoud et al., 1997). On GD 13, three groups of rats received

Administration of RAL Isomers. On GD 13, three groups of rats received a single intragastric dose of 100 mg of either 9-cis-RAL, all-trans-RAL, or 13-cis-RAL per kg body weight. The dosing volume was 5 ml/kg; the vehicle for the administration of RAL consisted of acetone, Tween 20, and water (2.5:50:47.5, by volume). Blood samples were collected 1 and 2 h after treatment. At the 1-h time point, blood was collected under light ether anesthesia from the retro-orbital sinus using heparinized capillaries. Two hours after RAL administration, blood was drawn from the posterior vena cava into a heparinized syringe, again under ether anesthesia. Plasma was separated by 10-min centrifugation of the blood at 4°C and 1500g. After bleeding at 2 h, the animals were sacrificed by cervical dislocation, and embryos, yolk sacs, placenta as well as maternal tissues liver, kidney, lung, spleen, and thymus were immediately removed, put into preweighed vials, and frozen after determination of the sample weight. Plasma and tissue samples were stored at –20°C until analysis.

HPLC Analysis. Plasma, embryo, and yolk sac samples were extracted with a 3-fold volume of isopropanol, followed by solid-phase extraction according to the method described by Collins et al. (1992). Very small yolk sac samples were filtered with water to yield 100 mg before addition of 300 μl of isopropanol. Other organs were homogenized in a Potter-Elvehjem glass-Teflon homogenizer after addition of an equal (for liver, a 9-fold) volume of ice-cold water (for liver, an aqueous solution of 0.9% w/v NaCl). If necessary, tissues were also slashed using a pair of scissors. To a 200-μl aliquot of a homogenized sample, 600 μl of isopropanol was added. Further processing was performed according to Collins et al. (1992).

After solid-phase extraction, retinoid concentrations were determined by reversed-phase HPLC using an already described multilinear gradient formed of eluent A (60 mM ammonium acetate/methanol, 50:50) and eluent B (methanol/iso-propanol, 50:50) (Collins et al., 1992), called HPLC system I. Calibration was performed using solutions of bovine serum albumin spiked with defined concentrations of reference compounds. Quantification of 9,13-dicis-RA was based on the absorbance of 13-cis-RA at 340 nm. All RAG isomers were quantified on the basis of the calibration factors of all-trans-RAG. Part of the samples were analyzed with the HPLC method II using a binary gradient with eluent A consisting of 60 mM ammonium acetate/methanol (50:50) and methanol as eluent B (Tzimas et al., 1994b). Some samples were examined with the HPLC method III (eluent A: water + 0.2% trifluoroacetic acid; eluent B: acetonitrile + 0.2% trifluoroacetic acid), which has been developed especially for separation of isomers of retinoyl-β-D-glucuronide and retinoic acid and allows the separation of 13-cis-RA and 9,13-dicis-RA (Sass and Nau, 1994). UV detection was performed at 340 and 356 nm by use of a 2-channel SPD 10 A(V) detector (Shimadzu, Kyoto, Japan).

Results

Administration of either all-trans-RAL or 13-cis-RAL yielded comparable patterns of metabolites, and the concentrations of all-trans-RA, all-trans-RAG, and all-trans-4-oxo-RA clearly exceeded those of the corresponding 13-cis-isomers (Fig. 2). This phenomenon was not only observed in plasma, but also in all maternal tissues examined. For example, the concentrations of all RAG isomers were very similar after treatment with either all-trans-RAL or 13-cis-RAL (Table 1). In contrast, 9-cis-RAG was the predominant retinoyl glucuronide in rat plasma and tissues after administration of 9-cis-RAL, as reported previously (Sass et al., 1994). 9-cis-RAG was not detected after administration of either all-trans-RAL or 13-cis-RAL. Table 2 shows concentrations of RA isomers in maternal plasma, embryos, and selected maternal organs. All-trans-RA was the main RA isomer in plasma, embryos, kidney, and thymus 2 h after treatment with

![Fig. 1. Structural formulae of retinaldehyde (RAL) isomers.](image1)

![Fig. 2. HPLC chromatograms of rat plasma samples (sample volume, 12.5 μl) obtained 2 h after intragastric administration of 100 mg of 13-cis-RAL (A) or 100 mg of all-trans-RAL (B) per kg to pregnant rats on GD 13. HPLC method I, detection at 356 nm. ROH: 16:0/18:1. retinyl palmitate/retinyl oleate; 18:0, retinyl stearate.](image2)
13-cis-RAL or all-trans-RAL. These patterns were tentatively found in other maternal tissues as well; however, interfering background signals prevented the presentation of valid concentration values. After administration of 9-cis-RAL, 9-cis-RAL was the predominant RA iso- 
mer in maternal tissues and 9,13-dicis-RAL in maternal plasma. The identification of 9,13-dicis-RAL (which was not detected after administration of 13-cis- or all-trans-RAL) as a major plasma retinoid after application of 9-cis-RAL or 9-cis-RAL to mice and rats has been described previously (Tzimas et al., 1994; Kojima et al., 1994; Horst et al., 1995).

Examination of the ratios of embryo versus maternal plasma concentrations (E/M ratios) of RA isomers reveals very high E/M ratios (>2.5) for all-trans-RAL, irrespective of the RAL isoform administered (Table 3). In contrast, the E/M ratios of 13-cis-RAL after administration of all-trans- or 13-cis-RAL were less than one tenth of those of all-trans-RAL. The E/M ratios of 9-cis-RAL were higher after dosing with all-trans- and 13-cis-RAL than after dosing with 9-cis-RAL. The E/M ratio of 9,13-dicis-RAL as a 9-cis-RAL metabolite was extremely low, as this retinoid was hardly detectable in rat embryos. Finally, none of the RAG isomers was detected in rat embryos, in spite of their considerable maternal plasma and tissue concentrations.

A metabolite of 9-cis-RAL eluting slightly in front of 13-cis-RAL and all-trans-RAL was tentatively identified as 9-cis,13,14-dihydro-RAL based on retention time and absorption characteristics. This compound has been recently discovered as a metabolite of 9-cis-RAL in rats (Shirley et al., 1996). Due to lack of reference standard we could not quantify this metabolite. After treatment with 13-cis- or all-trans-RAL, peaks were detected in plasma at 290 nm, which might also represent 13,14-dihydro-RA metabolites (data not shown). However, the concentrations of metabolites indicated by those peaks were much lower than those of putative 13,14-dihydro-RA found after treatment with 9-cis-RAL and hence did not enable further characterization.

Reduction to ROH and subsequent esterification represented the main metabolic pathway of all three RAL isomers.

**Discussion**

The most important findings of this study can be summarized as follows: 1) the pattern of polar retinoid metabolites in rat plasma and tissues was very similar after a high dose of 100 mg/kg of all-trans- or 13-cis-RAL on GD 13, 2) a different metabolite profile was observed for administration of 9-cis-RAL as compared with the other isomers, and 3) differences became evident in the transplacental distribution of the RA isomers produced from RAL isomers. All-trans-RAL as a RAL metabolite reached the embryos to a much greater extent than any of its cis-isomers.

Plasma and tissue concentrations of all-trans-RAL, all-trans-RAG, all-trans-4-oxo-RAL and the corresponding 13-cis-isomers were very similar after administration of either all-trans- or 13-cis-RAL. Our results indicate that pronounced isomerization to all-trans-retinoids took place after administration of 13-cis-RAL. Although a remarkable degree of isomerization of 13-cis-RAL has already been described (McCormick et al., 1983; Creech Kraft et al., 1989), a nearly congruent pattern of metabolites of 13-cis- and all-trans-retinoids, as we have found after treatment with 13-cis-RAL and all-trans-RAL, was
TABLE 2
Concentrations of RA isomers in plasma, embryos, kidney, and thymus 2 h (in plasma also 1 h) after intragastric administration of 100 mg of 13-cis-, 9 cis-, or all-trans-RAL per kg body weight to rats on GD 13

<table>
<thead>
<tr>
<th>Compartment</th>
<th>RAL Isomer Administered</th>
<th>13-cis-RA, ng/ml or ng/g</th>
<th>9,13-dicis-RA, ng/ml or ng/g</th>
<th>9-cis-RA, ng/ml or ng/g</th>
<th>all-trans-RA, ng/ml or ng/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma, 1 h</td>
<td>13-cis-</td>
<td>58.0</td>
<td>n.d.</td>
<td>13.4</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>9-cis-</td>
<td>292</td>
<td>189</td>
<td>28.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>all-trans-</td>
<td>65.0 ± 11.5</td>
<td>n.d.</td>
<td>22.8 ± 3.8</td>
<td>325 ± 91.2</td>
</tr>
<tr>
<td>Plasma, 2 h</td>
<td>13-cis-</td>
<td>59.4 ± 1.3</td>
<td>n.d.</td>
<td>11.8 ± 1.8</td>
<td>82.2 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>9-cis-</td>
<td>353 ± 50.5</td>
<td>131 ± 31.4</td>
<td>11.0 ± 4.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>all-trans-</td>
<td>72.1 ± 39.8</td>
<td>n.d.</td>
<td>19.3 ± 12.3</td>
<td>164 ± 83.5</td>
</tr>
<tr>
<td>Embryos, 2 h</td>
<td>13-cis-</td>
<td>9.7</td>
<td>n.d.</td>
<td>5.9</td>
<td>253</td>
</tr>
<tr>
<td></td>
<td>9-cis-</td>
<td>3.7 ± 0.4</td>
<td>16.7 ± 3.7</td>
<td>25.1 ± 6.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>all-trans-</td>
<td>11.8 ± 2.3</td>
<td>n.d.</td>
<td>6.6 ± 1.7</td>
<td>345 ± 84.1</td>
</tr>
<tr>
<td>Thymus</td>
<td>13-cis-</td>
<td>16.3 ± 5.9</td>
<td>n.d.</td>
<td>n.d.</td>
<td>233 ± 157</td>
</tr>
<tr>
<td></td>
<td>9-cis-</td>
<td>34.9 ± 5.2</td>
<td>94.7 ± 24.7</td>
<td>21.4 ± 1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>all-trans-</td>
<td>16.6 ± 2.0</td>
<td>n.d.</td>
<td>4.0 ± 3.4</td>
<td>253 ± 65.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>13-cis-</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>262 ± 332</td>
</tr>
<tr>
<td></td>
<td>9-cis-</td>
<td>trace</td>
<td>82.0 ± 16.7</td>
<td>224 ± 38.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>all-trans-</td>
<td>34.5 ± 18.8</td>
<td>n.d.</td>
<td>18.9 ± 10.6</td>
<td>381 ± 136</td>
</tr>
</tbody>
</table>

n.d. = not detectable. Mean ± S.D. (n = 3–4; n = 2 for 1-h-plasma after administration of 13-cis-RAL and 9-cis-RAL, and embryos after dosing with 13-cis-RAL).

TABLE 3
E/M ratios 2 h after intragastric administration of 100 mg of 13-cis-, 9 cis-, or all-trans-RAL per kg to rats on GD 13

<table>
<thead>
<tr>
<th>Sample</th>
<th>RAL Isomer Administered</th>
<th>13-cis-RA, E/M ratio</th>
<th>9,13-dicis-RA, E/M ratio</th>
<th>9-cis-RA, E/M ratio</th>
<th>all-trans-RA, E/M ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>E/M ratio, 2 h</td>
<td>13-cis-</td>
<td>0.16</td>
<td>0.011 ± 0.002</td>
<td>0.13 ± 0.03</td>
<td>2.76 ± 1.65</td>
</tr>
<tr>
<td></td>
<td>9-cis-</td>
<td>0.21 ± 0.13</td>
<td></td>
<td>0.57 ± 0.55</td>
<td>2.56 ± 1.33</td>
</tr>
</tbody>
</table>

Mean ± S.D. (n = 2–4).

never described after treatment with RA isomers. Our data are in agreement with early findings of Ames et al. (1955), who have suggested isomerization of 13-cis-RAL to all-trans-RAL to explain the "biopotency" of 13-cis-RAL. However, it cannot be concluded whether isomerization occurred at the RAL level and/or at the level of more polar metabolites. Although the HPLC analyses performed in the present study did not allow separation of RAL isomers, several recent in vitro studies may be interpreted in favor of isomerization on the RAL level. For example, El Akawi and Napoli (1994) have found that all-trans-RAL and 9-cis-RAL, but not 13-cis-RAL, are substrates of a rat liver cytosolic retinal dehydrogenase, which converts RALs to the corresponding RAs. In addition, Labrecque et al. (1995) have identified an aldehyde dehydrogenase in rat kidney with high affinity for 9-cis-RAL and all-trans-RAL, whereas catalysis for 13-cis-RAL was barely detectable. Finally, both the microsomal retinal monooxygenase system and cytosolic retinal oxidase from rabbit liver also have higher affinity for all-trans-RAL and 9-cis-RAL than for 13-cis-RAL (Tomita et al., 1993; Tsujita et al., 1994). Corresponding enzymes in the rat may also accomplish catalysis of RAL oxidation in vivo. Thus, there are several enzymes which favorably catalyze the in vitro oxidation of all-trans-RAL and 9-cis-RAL to the corresponding acids. It may be presumed that these enzymes would oxidize 13-cis-RAL only after conversion to all-trans-RAL. The tendency toward slightly lower RA levels after treatment with 13-cis-RAL, if compared to all-trans-RAL, may reflect a delay in metabolism due to the necessity of isomerization. However, the nature of the in vivo isomerization of 13-cis-RAL (e.g., biological site, subcellular localization, enzymatic, or nonenzymatic) remains to be determined.

This study has shown a different pattern of retinoid metabolites observed after administration of all-trans- or 13-cis-RAL, 9-cis-RAL, 9,13-dicis-RAR and 9-cis-RAG were the major polar retinoids in maternal plasma and tissues, whereas all-trans- and 13-cis-isomers of those retinoids were present at much lower concentrations (Tables 1 and 2). In addition, 9-cis-13,14-dihydro-RA was tentatively identified in rat plasma and tissues after dosing with 9-cis-RAL, thus corroborating results of a recent study with 9-cis-RAL administration to rats (Shirley et al., 1996). In contrast, the corresponding isomers of this novel retinoid metabolite were not identified after administration of all-trans- or 13-cis-RAL.

An interesting aspect of 9-cis-RAL metabolism is that plasma concentrations of 9,13-dicis-RA were considerably higher than tissue concentrations. Therefore, the high ratio of plasma levels of 9,13-dicis-RA versus 9-cis-RA may result not only from extensive isomerization but also from differences in the tissue distribution of 9-cis-RA and 9,13-dicis-RA. The varying concentrations of the retinoyl-glucuronides in maternal tissues appear to reflect essentially the differences in their UDP glucuronyltransferase activities (Dutton, 1980). The liver is not only the most important organ for retinoid storage, but also a main site of glucuronidation of retinoids (Blaner and Olson, 1994).

Furthermore, the present study has shown that 9-cis-RAL and 9,13-dicis-RA (as metabolites of 9-cis-RAL) as well as 13-cis-RA (as a metabolite of 13-cis-RAL and all-trans-RAL) reach the rat embryo to a limited extent only, as compared to all-trans-RAL. This is suggested by the E/M ratios 2 h after dosing, which were highest for all-trans-RA, much lower for 13-cis-RA and 9-cis-RA, and even lower for 9,13-dicis-RA. Although these E/M ratios at a single time point do not provide a reliable marker of placental transfer, they are compatible with differences in the extent of placental transfer of 13-cis-RA, all-trans-RA, and 9-cis-RA, as demonstrated on the basis of embry-
onic and maternal plasma pharmacokinetics after administration of the corresponding acids (Creech Kraft et al., 1989; Collins et al., 1992; Eckhoff et al., 1994; Tzimas et al., 1994a,b; Collins et al., 1994; Kochhar et al., 1995; Tzimas et al., 1996; Eckhoff and Willhite, 1997). The reasons for the low extent of placental transfer of RA isomers other than all-trans-RA are not known, but may be related to the fact that all cis-isomers of RA have a much lower affinity to embryonic cellular retinoic acid-binding protein than all-trans-RA (Allenby et al., 1993; Horst et al., 1995). Cellular retinoic acid-binding protein may facilitate embryonic uptake of all-trans-RA (Nau, 1990). A remarkable aspect of our findings is the higher E7 ratio of 9-cis-RA after dosing with 13-cis-RAL or all-trans-RAL than with 9-cis-RAL (Table 3). This may result from isomerization of all-trans-RA (the main RA isomer after dosing with 13-cis- and all-trans-RAL) to 9-cis-RA taking place in the embryo. Interestingly, a recent study has provided evidence for conversions of 9-cis-RA to all-trans-RA catalyzed by an isomerase activity in rat conceptus homogenate (Chen and Juchau, 1998).

Finally, it can be discussed whether the embryonic concentrations of the potent teratogen all-trans-RA found after dosing with all-trans-RAL and 13-cis-RAL would indicate teratogenic potency of these retinoids in the rat. Although such predictions cannot be based on concentration measurements at only one time-point after drug administration, the fact that the concentrations of all-trans-RA in rat embryos 2 h after dosing with all-trans and 13-cis-RAL (Table 2) were severalfold higher than the concentrations of all-trans-RA found after administration of a teratogenic dose of this compound during organogenesis (Collins et al., 1994) strongly indicates that the 100 mg/kg oral doses of all-trans-RAL and 13-cis-RL will induce teratogenic effects in the rat if administered during a sensitive stage of embryonic development. Future experiments could combine pharmacokinetic analyses with teratology studies to find out in which way differences in metabolite patterns of the three RL isomers and the embryonic retinoid exposure are associated with differences in their teratogenic potencies.

In conclusion, our work has shown distinct differences in the metabolism of all-trans-RAL and 13-cis-RAL versus 9-cis-RAL and has confirmed differences in the placental transfer of RA isomers. Further studies on the biotransformation of RL isomers are needed for understanding of the metabolic fate of these RA precursors.

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References


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