# [13] Absorption of *trans*-Resveratrol in Rats

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## Introduction

Discrepancies between the *in vitro* biological activities of antioxidant polyphenols present in red wine, such as *trans*-resveratrol, (+)-catechin and quercetin, and the *in vivo* effects of red wine oral consumption or that of the individual polyphenols by animals and humans have been described.[1] This paradox is especially striking for *trans*-resveratrol. Despite its ability to inhibit the synthesis and secretion of apolipoprotein B and cholesterol esters[2,3] and to block platelet aggregation and thromboxane synthesis,[4,5] daily administration of 375 ml of red wine enriched in this compound to healthy human volunteers for 4 weeks did not alter any of these functions (platelet aggregation, eicosanoid synthesis, or lipoprotein metabolism)[6,7] *In vitro, trans*-resveratrol manifests significant estrogen-like

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activity,[8,9] but after oral administration it has been very difficult to demonstrate consistent *in vivo* estrogenic effects.[10] Other examples of this paradox have been outlined in another comprehensive review,[11] raising the issue of failure of absorption as a reason for the lack response.

In 1996, Bertelli and colleagues[12,13] reported the absorption of *trans*-resveratrol by rats after a very small intragastric dose (25 μg) and went on to describe the results of pharmacokinetic studies involving sequential assays on blood, urine, and various tissues. Peak concentrations in plasma (around 30 μg/liter) occurred at 60 min, according to data presented. These papers provide little methodological detail, and no explanation was proposed for the attainment of such a high plasma concentration relative to the dose administered. Three years later, it was reported anecdotally that the *trans*-resveratrol concentration of rat plasma 15 min after an oral dose of approximately 0.5 mg was 175 μg/liter.[14] No experimental details (e.g., number of animals) were given, nor were data for other time periods described, although the authors stated that blood was also drawn at 30 and 45 min after the dose.

For several years we have been grappling with the problem of measuring *trans*-resveratrol concentrations in the blood at levels occurring after oral administration, and have developed a suitable method for doing so.[1] Before this was finally achieved, we were able to perform a series of experiments using *trans*-resveratrol radiolabeled with [$^3$H] in a stable nonexchangeable position within the first benzene ring. These observations were amplified by use of the new method[1] and are described in this article.

## Materials and Methods

*trans*-Resveratrol, tritiated in the 4-position of the first benzene ring and containing 170 μCi/ml, is from Sibtech, Inc. (Newington, CT) by special requisition, with purity >99% guaranteed. Of the original methanolic solution, 350 μl is diluted to 1 ml with 30% (v/v) ethanol to yield a stock solution of 60 μCi/ml. For intragastric administration, 2 μl (120 nCi) of this solution is added to 1 ml of one

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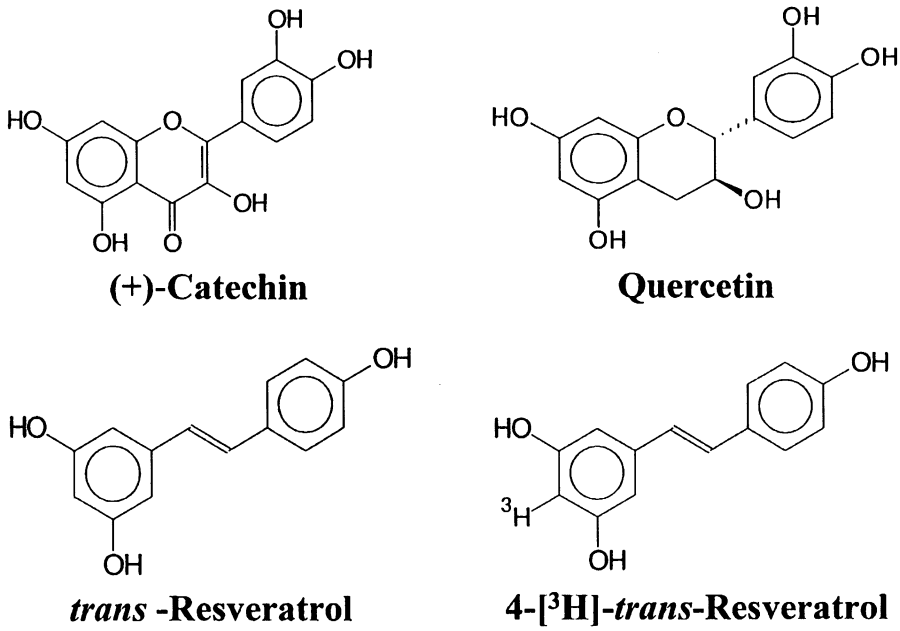


FIG. 1. Structures of unlabeled and [$^3$H]-labeled *trans*-resveratrol, (+)-catechin, and quercetin.

of the following three matrices: 10% (v/v) ethanol, V-8 homogenized vegetable cocktail (Campbell Foods), and white grape juice (Welch Food Co.) The matrices have values of pH 4.5–6.0. In some experiments, unlabeled *trans*-resveratrol, (+)-catechin, or quercetin (all from Sigma, St. Louis, MO) are coadministered together with the labeled *trans*-resveratrol. Their structures are illustrated in Fig. 1.

*Animal Procedures*

Male Wistar rats averaging 350 g are fed water and chow *ad libitum* and are acclimatized for 7 days. They are fasted overnight, lightly stunned with $CO_2$, and given one of the three matrix solutions with or without antioxidant polyphenols, as described earlier, by gavage: 1 ml initially and a further 1 ml of the matrix containing no additive by the same syringe to ensure washing out of all the material. The animals are placed individually in metabolic cages and observed carefully for the first 20 min to ensure that vomiting or regurgitation does not occur. On a few occasions the animal is rejected for this reason. Where appropriate, urine and feces are collected independently for a period of 24 hr. Two hours after gavage, chow and water are restored to the cages *ad libitum* except for short-term experiments where the animals are sacrificed within the first 2 hr to examine blood concentrations over this period. They are sacrificed by cardiac puncture and blood withdrawal; some is set aside for analysis as whole blood and the rest is centrifuged after clotting to produce serum.

Liver, kidneys, heart, and spleen are removed, washed in ice-cold isotonic saline, trimmed free of fat, finely chopped, and homogenized in a PRO-200 blender (Pro Scientific, CT) in 5 volumes of 95% (v/v) ethanol for three 1-min periods

separated by 30-sec intervals. The homogenate is centrifuged (1,500*g* for 15 min at 4°), the supernatant is transferred, and the residue is returned to the blender for two further extractions with the same volume of ethanol. The supernatants are pooled, and the volume is measured and recorded. Whole blood and blood serum are similarly extracted.

The bladder is carefully dissected, the contents are washed into the blender with 95% (v/v) ethanol, the bladder is finely chopped and added together with the 24-hr urine collection, and the mixture is extracted as described earlier. The final volume of the pooled supernatants is carefully measured. This material is referred to as urine.

The entire colon from the ileocecal junction to the anus is dissected free, briefly washed in isotonic saline, placed in a flat dish, and sliced longitudinally, and the contents are carefully scraped free with a spatula assisted by rinsing with 95% (v/v) ethanol. The contents are quantitatively transferred to the blender, together with the 24-hr fecal collection. The entire colon is finely chopped and added to the contents. Homogenization is performed following the procedure used for tissues, but with one additional extraction step. This material is referred to as stool.

Preliminary experiments demonstrated virtually complete recovery of radioactivity from all sources using the techniques just described; in no instance did a further extraction yield >1.5% of the value obtained with the standard procedures. During tissue preparation and extraction, all materials are kept on ice or at 4° and protected against light by metal foil. The receptacles used to collect the 24-hr urine and fecal samples are similarly protected against light and temperature. The full protocol is approved by the Animal Experimentation Committee of the University of Toronto.

### *Assays*

Radioactivity is determined as disintegrations per minute (dpm) after color quench corrections by adding 500 μl of the ethanol extract to 5 ml of scintillation fluid (Ready-Value, Beckman Instruments, Fullerton, CA) in a Beckman 600 scintillation counter. By reference to the total volume of extract, the total dpm attributable to each sample is calculated. Counts are measured over a 5-min period. Duplicates are run on each extract, and the results are averaged; if, as with very low values, the duplicates differed by >10%, two further replicates are analyzed and the results of all four assays are averaged.

## Results

### *Overall Absorption and Matrix Effect*

The percentage of tritiated *trans*-resveratrol absorbed over a 24-hr period is calculated according to the liquid matrix in which it was administered. Only trace

amounts (<1% of the dose administered) are detectable in the liver, kidneys, heart, or spleen, with the aggregate value for these tissues being <2%. Values for blood and plasma at that time are scarcely above background. Around 75% of the dose administered with each matrix is accounted for by adding the radioactivity of stool and urine. Taking as the measure of absorption the difference between the amount of radioactivity given and the amount recovered in the stool (24-hr feces plus colonic contents and colon), it appears that 77–80% of *trans*-resveratrol may be absorbed in the rat intestine, there being no differences among the three liquid matrices (Fig. 2). Clearly, all of the tritiated label present in the urine must have been absorbed and subsequently excreted, the values ranging from 49 to 61% with, again, no differences among the three matrices (Fig. 3). It therefore appears that, by any criteria, at least 50% of *trans*-resveratrol is absorbed by the rat and that alcohol up to 10% by volume does not enhance absorption. Therefore, the compound is no more bioavailable in wine than in aqueous beverages, lending credence to the notion that it can, if considered desirable, be administered as a food or beverage additive. At this time, we cannot explain the difference (approximately 25%) between the sum of stool plus urine radioactivity and the amount actually

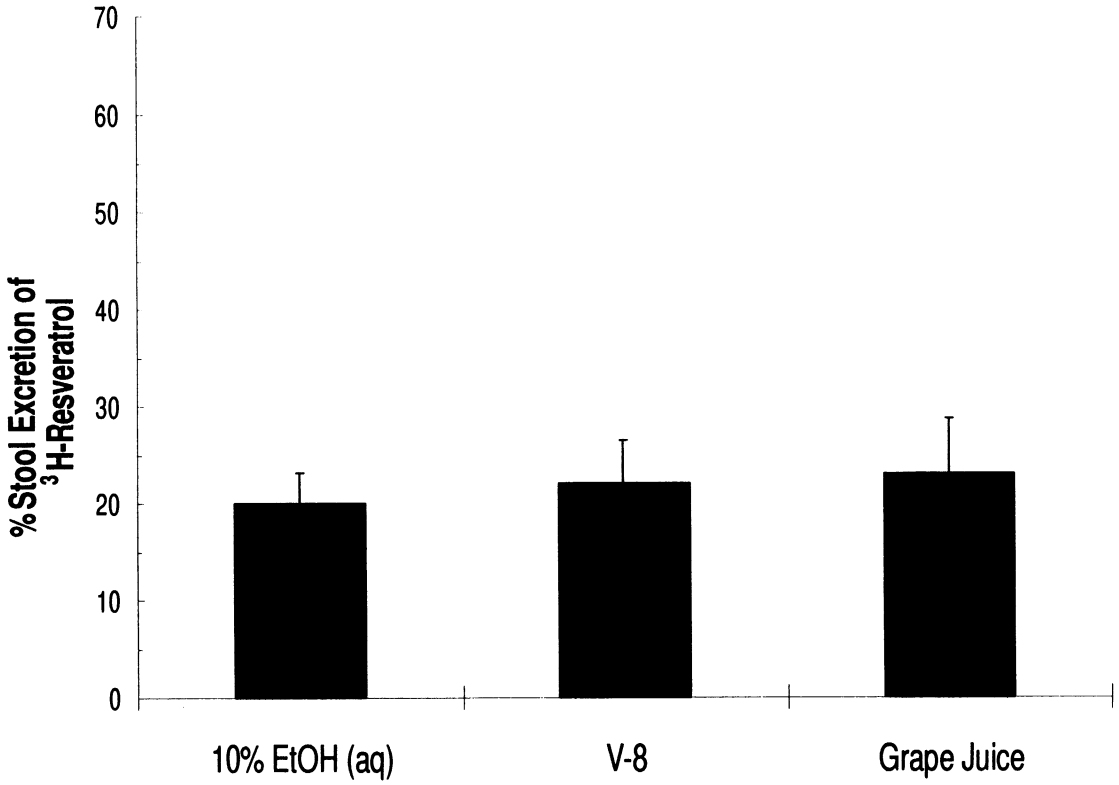


FIG. 2. Recovery of [$^3$H] label as percentage of dose given in the rat stool fraction (colon plus colon contents plus feces) 24 hr after intragastric administration of [$^3$H]-labeled *trans*-resveratrol in three different liquid matrices (mean ± SEM, $n = 8$). All assays are in duplicate.

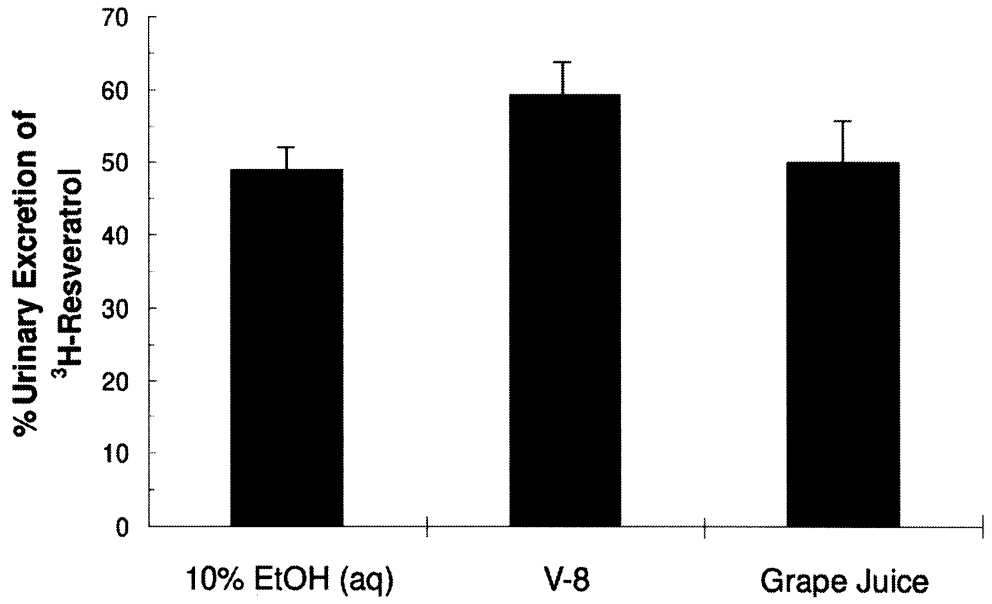


FIG. 3. Recovery of [$^3$H] label as percentage of dose given in the rat urine fraction (bladder plus contents plus urine) 24 hr after intragastric administration of [$^3$H]-labeled *trans*-resveratrol in three different liquid matrices (mean ± SEM, $n = 8$). All assays are in duplicate.

administered. Some could be accounted for by excretion via sweat and respiratory water as well as by metabolism to $CO_2$. Keeping in mind its high lipid solubility, *trans*-resveratrol might be deposited over a 24-hr period in adipose tissue and other tissues with high lipid content, such as the brain and nervous system. Skeletal muscle is another possible source that has not been examined. The amount of unaccounted radioisotope is not large (approximately 30 nCi), and the concentrations in such tissues, if any, would be difficult to measure accurately.

### *Competition Experiments*

The 24-hr urinary excretion of radioactivity was measured in rats in whom unlabeled *trans*-resveratrol, (+)-catechin, and quercetin were coadministered in a matrix of 10% (v/v) ethanol in concentrations ranging from 10 n*M* to 1 m*M*. No significant inhibition by these compounds was observed (Fig. 4). There would not appear to be competition among these three antioxidant polyphenols for a common absorptive mechanism, and the failure of unlabeled *trans*-resveratrol to reduce urinary excretion suggests that its absorption is not saturable over the range of concentrations used.

### *Time Course of* trans-*Resveratrol Absorption*

Values for the radioactivity in whole blood and serum of two rats after the administration of tritiated *trans*-resveratrol over the first 2-hr period are presented in Fig. 5. Significant radioactivity was present at 30 min after administration, but

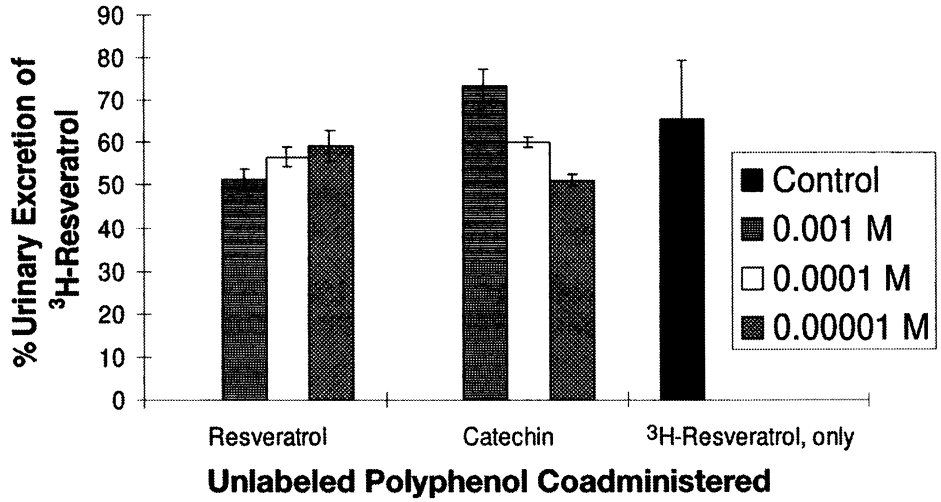


FIG. 4. Recovery of [$^3$H] label (120 nCi tritiated *trans*-resveratrol) as percentage of dose given together with one of three concentrations of *trans*-resveratrol, ( +)-catechin, or quercetin in 10% (v/v) ethanol by gavage in the rat urine fraction 24 hr after administration (mean ± SEM, $n = 8$). All assays are in duplicate.

remained around the same concentrations over the next 90 min. These observations are not consistent with the rapid rise and fall of *trans*-resveratrol in the blood of rats, peaking at 1 hr previously reported,[12] or as observed by us in human experiments.[1] However, it is invidious to compare experiments in which only the parent compound is analyzed with others in which this, as well as metabolites, is included in the estimate.

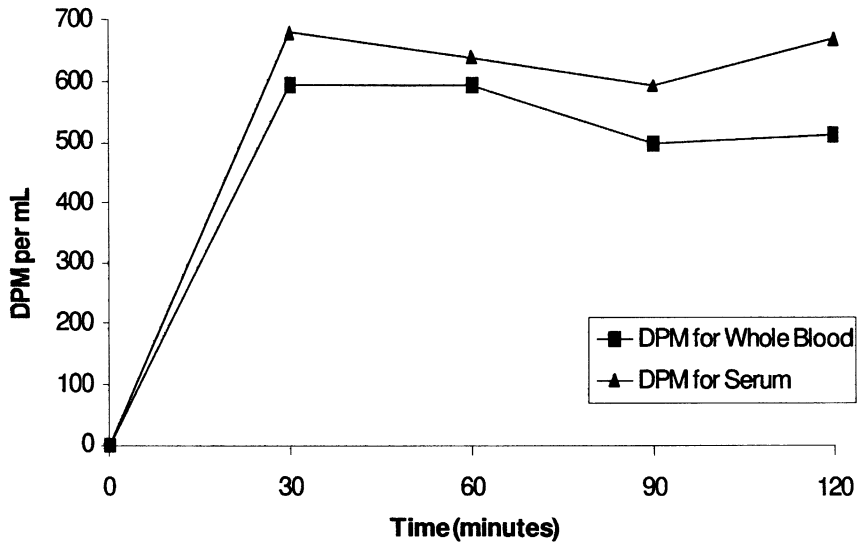


FIG. 5. Radioactivity (dpm per ml) of whole blood and blood serum in rats given 120 nCi of [$^3$H]-labeled *trans*-resveratrol at four intervals after intragastric administration in 10% (v/v) ethanol. Mean of results for two rats, all assays are in duplicate.

*Assays for Unlabeled* trans-*Resveratrol*

When the methods for assay of *trans*-resveratrol in serum and urine became available,[1] they were applied to its determination in all of the 24-hr urine samples (as well as in a few 24-hr serum samples) collected in experiments where cold and tritiated *trans*-resveratrol were administered simultaneously. These had been stored at −20°C for up to 4 months. The mean percentage of the dose administered measurable in the 24-hr urine ranged from 2.5% (10 n*M*) to 7.4% (100 n*M*) to 14.7% (1 m*M*). These results suggest that whereas the absorption of *trans*-resveratrol as indicated by the excretion of radioactivity is not affected by the dose administered over this range, the metabolic conversion of the parent compound is saturable so that as the dose is increased, a higher percentage is excreted unchanged in the urine. As expected, the *trans*-resveratrol concentrations of the 24-hr serum samples assayed ranged between the limit of detection (0.1 μg/liter) and the limit of quantitation (1 μg/liter) of the method.

A new set of experiments was conducted, in the first of which (A) rats were given 0.5, 1.5, and 2.5 mg of *trans*-resveratrol in 1 ml of 20% (v/v) ethanol as described earlier, and blood was withdrawn by cardiac puncture 60 min later. The mean concentrations in serum were 2.5 μg/liter after 0.5 mg, 3.6 μg/liter after 1.5 mg, and 5.7 μg/liter 2.5 mg; mean blood concentrations were, respectively, 2.2, 2.5, and 4.5 μg/liter. In the second set (B), the amount given was 5 mg and for two rats, blood was withdrawn at 15, 30, and 60 min. The results are illustrated in Fig. 6. High concentrations of *trans*-resveratrol were already evident in serum by 15 min after gavage, peaking at 30 min, and falling precipitously over the next 30 min. A similar time course was seen in whole blood, although the concentrations were consistently lower than those of serum.

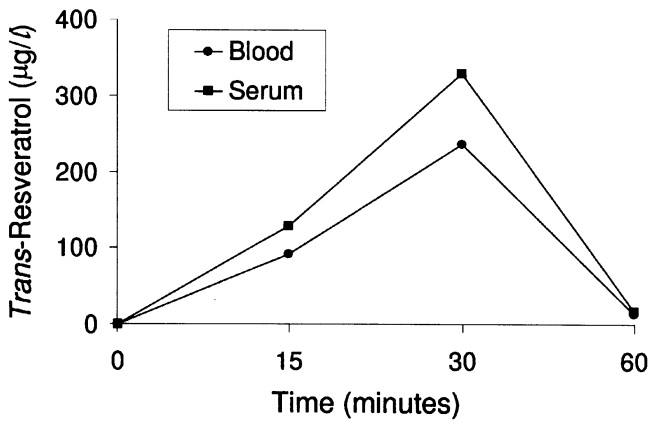


FIG. 6. *trans*-Resveratrol concentrations in whole blood and blood serum of rats at three intervals after intragastric administration in 20% (v/v) ethanol of 5 mg unlabeled compound. Data are means for two rats with all assays in duplicate.

## Discussion

These experiments extend the informations provided by previous investigations[12–14] in several directions. First, peak concentrations of *trans*-resveratrol occur in blood and serum very rapidly, around 15 min from the time of administration. The difference between this result and that of Bertelli *et al.*[12] may, at least in part, be attributable to the much greater dose used in the present work. Second, while the concentration of parent compound falls sharply after this peak, radioactive metabolites decline far more slowly. Third, taking account of excretion of the tritium label in feces and urine, 50–75% of orally administered *trans*-resveratrol appears to be absorbed.

This last estimate may be conservative. (+)-Catechin has been shown to undergo biliary excretion,[15] whereas quercetin, which does not seem to be excreted in rat bile, can be secreted by rat intestine after conjugation and absorption.[16] It is therefore conceivable that some of the stool radioactivity after gastric *trans*-resveratrol administration may represent excretion of part of the initial dose after absorption and metabolism.

Following its absorption, quercetin, which is highly hydrophobic, is converted rapidly to more water-soluble conjugates (glucuronide and sulfate) and glycosides.[17–19] Further, these conjugates are potent antioxidants, being fourfold more effective than Trolox in blocking the oxidation of low-density lipoproteins.[20] A similar metabolic pattern may prevail for *trans*-resveratrol. In support of this notion, we found that radioactivity was best extracted from all tissues and matrices by 95% (v/v) ethanol rather than by ethyl acetate, which was the most efficient solvent for extracting *trans*-resveratrol from these same preparations.[1] This suggests that the radioactivity was in large measure associated with metabolites more water soluble than the parent compound. It should also be noted that Casper *et al.*[21] found that when *trans*-resveratrol was administered to rats it was much more potent in blocking the function of the aryl hydrocarbon receptor (required for the conversion of proximal carcinogens to metabolites) than when incubated with cell

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cultures *in vitro*; they speculated that hepatic biotransformation generated one or more metabolites with much greater activity than the parent compound.

In certain red wines, substantial amounts of resveratrol glucosides (polydatins) are present, which, on hydrolysis with glucosidases, yield the free stilbene.[22] Since Hollman *et al.*[23] have shown that flavonoid glycosides are absorbed by humans much more effectively than free flavonoids,[23] it is reasonable to speculate that polydatins are likewise highly bioavailable and capable of supplementing the favorable biological effects attributable to *trans*-resveratrol.

On a final technical note, it is intriguing that when added to whole blood *in vitro*,[1] a much higher proportion of *trans*-resveratrol is associated with the cells than is the case when *in vivo* absorption into the blood takes place, as in the present work. This calls for considerable caution in extrapolating the results of *in vitro* distribution experiments to the whole organism.

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