THE METABOLIC FATE OF RUTIN AND QUERCETIN
IN THE ANIMAL BODY

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The finding of 3,4-dihydroxyphenylacetic acid (DHPAA) in the urine of rabbits after the oral ingestion of rutin or its aglycone quercetin has been reported from this Laboratory (1). In this same report it was stated that in each case after the administration of the flavonoid an examination of paper chromatograms of the ether extracts of the urine revealed the excretion of at least four metabolites (one of which was DHPAA). Two more of these four substances have now been identified (2) and a more detailed report is presented herein.

One of these two compounds, m-hydroxyphenylacetic acid (mHPAA), was isolated and identified in this Laboratory over a year ago, but publication was withheld pending identification of the second compound, 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid). In the meantime, at least two other laboratories have reported the finding of mHPAA in control urines (3, 4). Also included in the present report are the results obtained from three species besides the rabbit, namely rats, guinea pigs, and humans. The metabolic fate of a flavonoid such as quercetin in animals other than the rabbit was considered especially important in view of the results obtained by Clark and MacKay with rats and humans (5).

EXPERIMENTAL

All urines were collected in receiving flasks containing sufficient hydrochloric acid to maintain an acid pH in order to retard oxidation of labile phenolic excretory products. The acid urines were extracted for 5 to 6 hours with ether in a continuous liquid-liquid extractor, and the ether extracts were evaporated to dryness under vacuum and nitrogen. Chromatograms were prepared by dissolving the ether residues in a few ml. of acetone, air-drying a fraction of the acetone solution (0.02 to 0.10 ml.) on a sheet of Whatman No. 1 filter paper, and migrating the fraction in two dimensions. The solvent for the ascending migration in the first direction (16 hours) was the lower phase of a mixture of chloroform, acetic acid, and water (2:1:1 by volume). Aqueous potassium chloride (20 per cent) was the solvent for the ascending migration in the second direction (2 to 3
The solvent fronts were allowed to advance 30 to 35 cm. in each direction. Detection of the location of the various hydroxyaromatic substances on the air-dried papers was accomplished by spraying with a solution of freshly prepared diazotized sulfanilic acid followed by 20 per cent sodium carbonate as described by Bray et al. (6).

The mHPAA was isolated from the ether extracts of the urine of rabbits which had received quercetin (0.5 gm. per kilo of body weight) via stomach tube. The basal diet was a commercial pellet preparation of natural feedstuffs. Solvent partition between ether and petroleum ether (Skellysolve F, b.p. 30-60°) was used to isolate mHPAA. Unfortunately, it was contaminated with small amounts of DHPAA and m-hydroxybenzoic acid (mHBA). Purification was achieved by preparation of the tribromo derivative, which was used to confirm the identity of the urinary metabolite as mHPAA.

Homovanillic acid was isolated from the ether extracts of urine of rats fed quercetin (2 per cent) in a diet consisting mainly of natural feedstuffs. After evaporation of the ether, the solids were extracted with hot xylene. Plates of impure homovanillic acid (HVA) were obtained as the solution cooled. These were purified by partitioning between water and chloroform, by taking advantage of the fact that homovanillic acid is more soluble in chloroform than is DHPAA.

An approximation of the quantities of the three metabolites of quercetin in urine was accomplished by migrating known quantities of the pure compounds (2 to 10 γ) and making comparisons of the spot size and color on paper chromatograms developed after migrating appropriate dilutions of the urine of rabbits which had received quercetin.

Since rutin and its aglycone quercetin yield the same metabolic products, quercetin was used in most of the experiments because more flavonoid could be administered per unit weight of material. The possibility of conjugated degradation products of quercetin being excreted in the urine was investigated by hydrolyzing the ether-extracted urine with 10 per cent hydrochloric acid for 3 hours, followed by ether extraction and development of chromatograms of the ether extracts.

**Results**

A typical schematic diagram of a chromatogram of an ether extract of rabbit urine is shown in Fig. 1. Before quercetin was given, the two most prominent spots visualized after spraying were a yellow area (Spot 4) due to mHBA and a yellowish orange spot indicating the presence of mHPAA and p-hydroxyphenylacetic acid (pHPAA) which migrated to the same area (Spot 2) under these conditions. However, pure mHPAA and pHPAA yield yellow and red dyes, respectively, after spraying with diazo-
tized sulfanilic acid. In the case of control rabbit urine, mHPAA predominated and hence a yellowish orange spot on the chromatogram resulted.

When urine from rabbits receiving quercetin was examined chromatographically, there were at least three striking changes in evidence compared to the control. In the first place, a black area (initially red after development of the color) was located at Spot 1 in Fig. 1 and has already been identified and reported as being DHPAA (1). Secondly, there was a marked increase not only in the size of Spot 2 but an intensification of the yellow color characteristic of mHPAA. Thirdly, a new area (Spot 3) was noted which was red in color after spraying with diazotized sulfanilic acid and has been identified as HVA.

Paper chromatographic analyses of the urines of rats, rabbits, guinea pigs, and humans receiving oral dosages of quercetin revealed that in all four of these species a striking similarity existed in the metabolic fate of this flavonoid. In every case there was unmistakable evidence of DHPAA, mHPAA, and HVA being excreted.

Evidence has also been obtained for the urinary excretion of a substance,
or substances, that fluoresce intensely yellow in ultraviolet light and are only slightly soluble in ether (the major portion being retained in the aqueous phase after ether extraction). Work is currently in progress to isolate this material.

In addition to the two-dimensional paper chromatographic criterion for the identity of mHPAA, the x-ray diffraction pattern of the tribromo derivative of the compound isolated from urine after quercetin administration has been found to be identical with that of the tribromo derivative of authentic mHPAA (Fig. 2). A third criterion establishing the identity of this substance isolated from urine was the set of optical and crystallographic data which were as follows: Some of the crystals of the sublimed tribromo derivative of mHPAA were plates or prisms having an end angle of 135°. Their birefringence was high and their extinction was sharp and symmetrical if the crystal was properly oriented. Such symmetrically extinguishing side views gave the refractive index γ for crosswise vibrations and α' for lengthwise vibrations. End views (which did not extinguish sharply) showed a rhomb-shaped silhouette whose acute angle was about 46°. The refractive indices shown by end views were γ for the direction which bisects the acute angle and β' for the other direction on those crystals oriented to give symmetrical extinction. Edge views (which had a rectangular outline) showed oblique extinction with the vibration direction α making an angle of 20° with the length of the crystal. The other index obtainable from this view was β. Acute bisectrix interference figures were sometimes obtainable from suitably tilted end views. They showed that the optical character is negative with 2γ = medium (2γ calculated = 66°) (2V calculated = 36°), dispersion (v > v) slight. The refractive indices are as follows: \( \alpha = 1.534, \alpha' = 1.558, \beta = 1.77, \gamma = 1.80. \)

Fig. 2. X-ray diffraction patterns. Left side, tribromo derivative of compound isolated from urine. Right side, tribromo derivative of synthetic mHPAA.
Likewise, in the case of homovanillic acid, it was found that the chromatographic evidence including $R_F$ values in two dimensions plus the quality of the color with diazotized sulfanilic acid was identical when compared with an authentic sample of homovanillic acid. The crystallographic data have also been found to be identical and will be reported in detail in a subsequent paper.

Having established the identity of three substances found in urine after giving an animal quercetin, and taking into account their structural relationships to quercetin and to each other, we concluded that DHPAA was probably an intermediate metabolite of quercetin and that some of the DHPAA can serve as a precursor of mHPAA by dehydroxylation and as a precursor of homovanillic acid by methylation. DHPAA was prepared by demethylation of the corresponding dimethoxy derivative and given orally to both rats and rabbits. As predicted, chromatograms were the same as those after the administration of quercetin. In other words, the administration of DHPAA resulted in the excretion of unchanged DHPAA, and there was unmistakable evidence of an increase in mHPAA as well as a definite area corresponding to homovanillic acid. Based on these results the proposed pathway of quercetin degradation in the animal body is shown in Fig. 3.

Another point which was briefly investigated was the question as to whether quercetin degradation takes place in the intestinal tract or in the
animal tissues after absorption from the gut. When quercetin was administered by intraperitoneal injection, in order to eliminate possible effects due to digestive enzymes or intestinal bacteria, unmistakable chromatographic evidence of homovanillic acid was found in the urine, as was the case when it was given orally. At the present time we have no explanation for the failure to find DHPAA or an increase in mHPAA. An analysis (chromatographic) of the intestinal contents of rats receiving quercetin in the diet was negative in regard to the presence of quercetin metabolites found in the urine.

Paper chromatograms of ether extracts of hydrolyzed urines failed to reveal any additional metabolites of quercetin which might have been conjugated.

Having identified three metabolites of quercetin in the urine, we attempted to estimate the amount of these compounds being excreted when a given dosage of quercetin was administered. When 2 gm. of quercetin were given to a rabbit, the values were 150 to 200 mg. of DHPAA, 20 to 40 mg. of homovanillic acid, and 25 to 45 mg. of mHPAA (corrected for a normal excretion of 10 to 20 mg. per 24 hours).

**DISCUSSION**

As an outgrowth of this work, a most interesting finding was the ability of the animal either to methylate a “meta” hydroxyl group or to remove a ‘‘para” hydroxyl group from a phenolic acid (DHPAA). This will be treated in more detail in a subsequent paper on the metabolism of 3,4-dihydroxyphenylalanine.

To date, all of the metabolic products which have been identified appear to have originated from that portion of the quercetin molecule containing the catechol nucleus (Fig. 3). The opposite side of the quercetin molecule has not as yet been accounted for. The possibility of using radioactive carbon to investigate this phase of the problem presents itself.

These results indicate that quercetin is the precursor of three urinary metabolites, two of which may be derived from DHPAA. Then it follows that a considerable amount of the orally administered quercetin must have been absorbed from the gastrointestinal tract. In view of the widespread occurrence of various flavonoids in the plant kingdom (7), it is not unreasonable to suspect that numerous flavonoids are continuously being ingested by animals, including man, and probably account for some of the many phenolic acids found in normal urines. Bray and Thorpe (8) have reviewed the results reported by various workers on the excretion of phenolic acids in the urine of rabbits on natural diets. They mention the possible role of the diet as a source of precursors of the thirty or more phenolic excretory products found in the urine, many of which have been
identified. However, they did not specifically implicate plant pigments including flavonoids as possible precursors of these urinary metabolites. These workers included pHPAA but not mHPAA in their list of urinary metabolites. Perhaps our use of a different basal diet than the one they used accounts for what might appear to be conflicting results. The use of purified diets in subsequent investigations should also aid in establishing a relationship, if any, between dietary constituents and urinary metabolites.

In a recent review by Willaman (9) on the biological effects of flavonoids, a summary of the data revealed that no less than thirty-three different types of physiological and biochemical activities have been reported for one or another of thirty different flavonoids. A question as yet unanswered is whether the degradation products of quercetin can exert the same physiological effects as those attributed to quercetin itself.

SUMMARY

Evidence has been presented which permits the identification of two additional metabolites in the urine after the animals were given rutin or quercetin orally, namely m-hydroxyphenylacetic acid and 3-methoxy-4-hydroxyphenylacetic acid. 3,4-Dihydroxyphenylacetic acid, also a metabolite of quercetin, has been shown to be the precursor of the two metabolites first mentioned.

The identification of these urinary metabolites of a flavonoid such as quercetin is offered as evidence of intestinal absorption having taken place.

The urinary excretion of m-hydroxyphenylacetic acid after feeding quercetin implicates flavonoids in the diet as being at least one example of a dietary precursor of a m-hydroxyaromatic acid in urine.

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