Metabolism of Quercetin and Tricin in the Male Rat[1] (36773)

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(Introduced by W. G. Martin)
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Mammalian degradation of quercetin and other flavonols has been extensively documented (1–5). It is clear that the simple phenols and phenolic acids which occur in the urine of flavonol-fed animals originate largely from bacterial degradation in the intestine (5, 6), although Douglas (7) has reported that quercetin was degraded to simpler phenols when it was incubated with rat kidney homogenates. It is also known that the mammal is able to methylate the degradation products of flavonols (1).

In spite of the fact that flavones are commonly present in higher plants and therefore in the mammalian diet, the ability or inability of the mammal to metabolize these flavonoids has not been widely studied. Das and Griffiths (8) reported that male guinea pigs secreted 4′-hydroxyflavone, and small amounts of 3′,4′-dihydroxyflavone and salicylic acid, in the urine when the animals were fed flavone, whether or not high doses of antibacterial compounds were administered to the animals. No additional metabolites of flavone were detected in the urine. Flavone itself was not reported to be excreted in the urine and the feces was not examined for the presence of this compound. Flavone has not yet been isolated from plant tissue. Bickoff, Livingston and Booth (9) stated that degradation products of tricin could not be found in the urine when this flavone was fed to rats. They assumed that tricin was not absorbed from the intestinal tract, but did not measure the fecal excretion for tricin.

The present paper compares the metabolism of tricin, a flavone commonly present in grasses, and quercetin. Specifically, it compares the amounts of flavonoid in the feces and extractable phenol in the urine, when rats are fed a control diet or one containing tricin or quercetin.

Materials and Methods. Animals and diet. Highly inbred male albino rats of the Wistar strain, each weighing approx 180 g, were housed in separate stainless steel metabolism cages and given water *ad libitum* throughout the experimental period. The control diet consisted of: cornstarch, 62.8 g; casein, 14.4 g; cellulose, 6.5 g; methionine, 0.3; salt mix USP XIV (Nutritional Biochemicals Corp., Cleveland, OH), 4.0 g; fish liver oil containing at least 2250 IU vitamin A and 600 IU vitamin D/g (Midland-Western, Inc., Reading, PA), 3.0 g; corn oil containing 5 mg α-tocopherol, 4.0 g; vitamin mix [Rao, Meta and Johnson (13)], 5.0 g. The rats were fed 20 g of this diet/day. After 1 wk the animals were placed in three groups with five rats per group. Group 1 continued to receive 20 g of the control ration each day. Groups 2 and 3 received 20 g of control ration supplemented with 5 mg of quercetin (Control No. 3081, Nutritional Biochemicals Corp., Cleveland, OH) and tricin (Dr. E. M. Bickoff, USDA, ARS, Western Utilization Research and Development Division, Albany, CA), respectively, each day for 10 days and then 20 g of control diet/day for an additional 5 days.

Urine. The urine was maintained at an acidic pH by the addition of 5 ml of 6 *M* H_2SO_4 to each receiving flask. The urine from each rat was pooled in three 5-day fractions. Five milliliters of the acidified urine of each fraction was extracted once with 10 ml

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of ethyl acetate and three times with 5 ml of ethyl acetate. The ethyl acetate extracts from each sample of urine were pooled and several of these samples were chromatographed in two dimensions on cellulose thin layer plates. The first solvent was the lower phase of chloroform–glacial acetic acid–water (3:1:1), and the second solvent was *n*-butanol–ethanol–ammonia 0.88 (1:1:1) (14). The phenols were located by spraying the developed chromatograms with diazotized sulfanilamide (14).

The phenols in each combined ethyl acetate extract were quantitatively assayed by the method of Swain and Hillis (15) using catechol as the standard.

Feces. The feces from each rat was also pooled for the three 5-day periods. Hair and small amounts of feed were removed from each sample and they were then powdered in a mortar and pestle and placed in cellulose extraction thimbles. The thimbles were placed in Soxhlet extraction apparatus and extracted for 24 hr with 200 ml of absolute methanol. The methanol extracts were diluted with methanol to give a constant ratio of 22 ml of methanol/g of fresh feces. A 20-ml aliquot of each methanol extract and 10 ml 2% aqueous Na_2CO_3 were mixed and extracted three times with 10 ml of chloroform. The chloroform layers were discarded. The aqueous layers were adjusted to pH 2.0 with 2 *M* HCl and extracted with three 5-ml portions of chloroform. The absorption of the sixfold diluted, pooled chloroform extracts was measured at 345 mμ and recorded.

Before mascerating the feces from three of the control animals, 3, 5 and 10 mg of pure tricin were added in duplicate. These samples were then ground and extracted exactly the same as the rest of the samples. A standard curve (Fig. 1) was constructed from the absorption at 345 mμ of the extracts of these six samples and was used in determining the levels of tricin in the extracts of the other feces samples.

Basic lead acetate was added to the chloroform extract of the feces from a tricin-fed animal until no additional precipitate formed. The precipitate was washed with

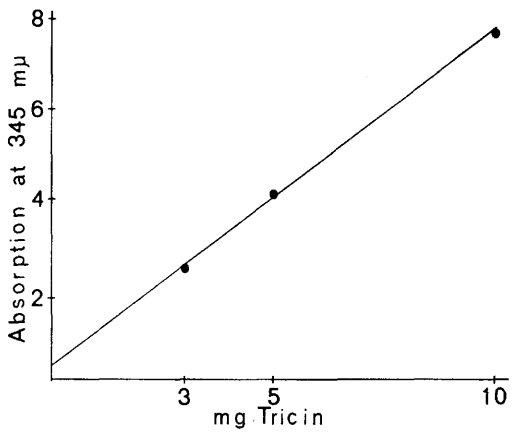

FIG. 1. Standard curve of the recovery of tricin added to the feces of rats fed the control diet. Each value is the average of two values and represents the quantity of tricin in the entire chloroform extract.

chloroform and regenerated in chloroform by bubbling hydrogen sulfide through the solution. After removing the excess hydrogen sulfide by boiling, the ultraviolet spectrum of this solution was compared with the spectrum obtained from a solution of authentic tricin in chloroform.

Results and Discussion. Several of the urine extracts were chromatographed on cellulose thin layer plates. It was demonstrated that the phenolic content of the urine of the tricin-fed rats was qualitatively the same as the phenolic content of the urine of the control animals. The developed chromatograms of the urine extracts of the quercetin-fed rats showed a greater total amount of phenol and several phenolic spots not present in the extracts of control urine. No attempt was made to identify or quantitate the individual phenols of these chromatograms because several literature reports have done this (1, 2, 4, 16) and because the goal of the present work was to determine if most of the ingested tricin could be recovered from the urine or feces. The quercetin-fed animals were included as a check of the methods.

The amount of phenols detected in the ethyl acetate extracts of the urine samples is shown in Table I. It is obvious that a substantially greater amount of phenols was extracted from the urine of the quercetin-fed

TABLE I. Phenol Extracted from the Urine and Feces of Tricin-Fed (T), Quercetin-Fed (Q) and Control (C) Rats.

Collection period (days)	Phenol (mg/collection period)[a]					
	Feces			Urine		
	T	Q	C	T	Q	C
1–5	10.9 ± 2.8[b]	0.8 ± 0.3	0.8 ± 0.5	54.8 ± 7.6	96.3 ± 15.2[b]	66.1 ± 5.2
6–10	10.9 ± 1.1[b]	0.4 ± 0.1	0.3 ± 0.1	53.8 ± 6.3	83.6 ± 8.4[b]	59.3 ± 10.0
11–15	1.4 ± 0.4	0.3 ± 0.1	0.2 ± 0.1	64.2 ± 7.7	73.4 ± 9.4	68.8 ± 3.9

[a] Each value is the mean ± SEM of five animals except the phenol from the feces of control rats for the second and third collection periods, which is the mean ± SEM of two rats.

[b] Significantly greater ($p < .01$) than the values for the corresponding animals in the same collection period (Duncan's new multiple range test).

rats than from the urine of either of the other groups of animals. It appears that with the quercetin-fed animals a greater quantity of phenols was extracted from the urine of the first collection period than from the second, although the animals were fed the same amount of quercetin during the two periods. Perhaps with prolonged feeding times a greater proportion of the phenolic compounds were methylated and thus not as easily detected by the method chosen. On the other hand, the rat or the microorganisms of the intestine may have utilized or stored a greater quantity of quercetin with prolonged feeding times.

It also appears that nearly the same amounts of phenol were extracted from the urine of the three groups of animals during the third collection period. This is not surprising since all the animals were fed control diet during this period.

The average amounts of ultraviolet-absorbing material present in the feces extracts are shown in Table I. As shown, little or no quercetin was excreted in the feces during the present study. Although quercetin does not absorb maximally at 345 mμ, it has sufficient absorption that one can be confident of the validity of this finding.

Extracts of feces from tricin-fed rats contained a significant quantity of ultraviolet absorbing material (Table I). Further purification of this material by precipitation with lead acetate and regeneration with hydrogen sulfide yielded a solution with an ultraviolet spectrum identical to authentic tricin.

Each tricin-fed rat received 5 mg of tricin/day during the first 10 days of this experiment. The data of Table I show that less than 50% of this tricin was recovered from the feces. This is especially significant in view of the fact that degradation products of tricin were not present in the urine extracts and were present in the feces only during the time that tricin was present in the diet.

Perhaps the microorganisms of the intestine of mammals are able to degrade flavonols (1–5), catechins (16) and isoflavones (17), but not flavones (9). This statement is difficult to believe, however, since in the work reported here much of the ingested tricin could not be accounted for. In this light it may be prudent to look again at the reported physiological activities of flavones (10) and the greater physiological activity of flavones compared to flavonols (authors, unpublished data).

Summary. Increased levels of phenols were detected in the urine of quercetin-fed, but not tricin-fed rats. Undegraded flavonoid was detected in the feces of tricin-fed, but not quercetin-fed rats. Less than 50% of the tricin ingested by the rats was located in the feces. Fecal excretion of tricin stopped abruptly when tricin was removed from the diet.

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1. Booth, A. N., Murray, C. W., Jones, F. T., and DeEds, F., J. Biol. Chem. 223, 251 (1956).

2. Kallianos, A. G., Petrakis, P. L., Shetlar, M. R.,

and Wender, S. H., Arch. Biochem. Biophys. 81, 430 (1959).

3. Masri, M. S., Booth, A. N., and DeEds, F., Arch. Biochem. Biophys. 85, 284 (1959).

4. Petrakis, P. L., Kallianos, A. G., Wender, S. H., and Shetlar, M. R., Arch. Biochem. Biophys. 85, 264 (1959).

5. Smith, G. E., and Griffiths, L. A., Biochem. J. 118, 53 (1970).

6. Nakagawa, Y., Shetlar, M. R., and Wender, S. H., Biochim. Biophys. Acta 97, 233 (1965).

7. Douglas, C. D., Fed. Proc., Fed. Amer. Soc. Exp. Biol. 17, 213 (1958).

8. Das, N. P., and Griffiths, L. A., Biochem. J. 98, 488 (1966).

9. Bickoff, E. M., Livingston, A. L., and Booth, A. N., J. Pharm. Sci. 53, 1411 (1964).

10. Williaman, J. J., J. Amer. Pharm. Ass., Sci. Ed. 44, 404 (1955).

11. Jeney, E., Acta Physiol. Acad. Sci. Hung. 34, 193 (1968).

12. Neville, P. F., and Luckey, T. D., J. Nutr. 101, 1217 (1971).

13. Rao, P. B. R., Meta, C. V., and Johnson, C. B., J. Nutr. 69, 387 (1959).

14. Scheline, R. R., Acta Pharmacol. Toxicol. 24, 275 (1960).

15. Swain, T., and Hillis, W. E., J. Sci. Food Agr. 10, 63 (1959).

16. Griffiths, L. A., Biochem. J. 92, 173 (1964).

17. Braden, A. W. H., Hart, N. K., and Lamberton, J. A., Aust. J. Agr. Res. 18, 335 (1967).

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