Studies on Flavonoid Metabolism

BILIARY AND URINARY EXCRETION OF METABOLITES OF (+)-[U-¹⁴C]CATECHIN

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1. The biliary and urinary excretion of (+)-[U-¹⁴C]catechin was studied in normal male rats after a single injection of the flavonoid. 2. In rats large amounts of radioactivity (33.6-44.3% of the dose in 24 h) were excreted in the bile as two glucuronide conjugates [one of which was a (+)-catechin conjugate] and three other unconjugated metabolites. 3. Excretion of radioactivity in the urine when the bile duct was not cannulated amounted to 44.5% of the dose. 4. In both the urine and bile the new metabolites showed maximum excretion in the $\frac{1}{2}$ -1 $\frac{1}{2}$ h after intravenous injection of [¹⁴C]catechin. 5. The metabolites *m*-hydroxyphenylpropionic acid, *p*-hydroxyphenylpropionic acid, δ -(3-hydroxyphenyl)- γ -valerolactone and δ -(3,4-dihydroxyphenyl)- γ -valerolactone originate from the action of the intestinal micro-organisms on the biliary-excreted metabolites of (+)-catechin. These phenolic acid and lactone metabolites are then reabsorbed and excreted in the urine. 6. It is proposed that, depending on the route of administration of (+)-catechin, there exists an alternative pathway, involving biliary excretion, for the metabolism of (+)-catechin.

It is generally recognized that one of the routes for excretion of foreign compounds injected into the rat may be through the bile (Brodie & Axelrod, 1948; Woods, 1954; Calvey, Somani & Wright, 1970; Pearson & Martin, 1966; Taylor, 1970; Karim & Taylor, 1970; Sandberg & Slaunwhite, 1957). However, so far there have been few reports on the biliary excretion of flavonoid compounds in the rat. Many reports have been published on the oral administration of different flavonoids to experimental animals and identification of their excretory metabolites in the urine (Booth, Murray, Jones & DeEds, 1956; Booth, Jones & DeEds, 1958*a,b*; Watanabe, 1959; Griffiths, 1964; Das & Griffiths, 1966). Work in this laboratory has shown that oral administration of (+)-catechin to the rat and guinea pig results in appreciable absorption of the compound from the gastrointestinal tract and that the compound is metabolized, the degradation products appearing in the urine in the free and conjugated forms (Griffiths, 1964; Das & Griffiths, 1968, 1969).

The present paper describes the pattern of biliary and urinary excretion of metabolites of (+)-[U-¹⁴C]catechin after intravenous administration of the flavonoid to the rat.

MATERIALS AND METHODS

Animals. Male rats (200-250 g) maintained on a standard diet as described by Griffiths (1964) were used.

Test substances. (+)-[U-¹⁴C]Catechin (sp. radioactivity 17.8 mCi/mol) was obtained from young shoots of *Unacria gambir* Roxb. to which ¹⁴CO₂ had been administered (Das & Griffiths, 1967).

Administration of test substance. Normal male rats that had been starved for 24 h were anaesthetized with pentobarbital sodium salt (3%, w/v, in water; 1.7 ml/kg body wt., injected intraperitoneally). The abdomen was opened by a mid-line incision, and a polyethylene catheter (size FG 2; external diameter 0.63 mm: W. Warne, Barking, Essex, U.K.) was inserted into the common bile duct approx. 1 cm above its junction with the duodenum. An incision was made at the proximal end of the tail and the right lateral tail vein was cannulated with a polyethylene catheter (size FG 2; external diameter 0.63).

(+)-[U-¹⁴C]Catechin (25 mg) dissolved in 2 ml of 0.9% NaCl was administered to each rat through the tail vein over a 1 $\frac{1}{2}$ min period. The animal was then infused with 5% (w/v) glucose in 0.9% NaCl at the rate of 5-9 ml/h for 24 h with a Braun continuous infusion apparatus (model Unita I; B. Braun Apparatebau, Melsungen, W. Germany). The animal was placed in a restraining cage for the entire duration of the experiment. Pentobarbital sodium salt (0.02%) was added to the perfusing fluid whenever the

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Table 1. R_F values and colour reactions of (+)-catechin metabolites excretedSolvents *A*, *B* and *C* refer to butan-1-ol-acetic acid-water (4:1:5, by vol.), butan-1-ol-

Metabolite no.	R_F			Colour			
	Solvent <i>A</i>	Solvent <i>B</i>	Solvent <i>C</i>	With Barton's reagent	With diazotized <i>p</i> -nitroaniline	With diazotized sulphanilic acid	With ammoniacal AgNO_3
1	0.79	0.83	0.72	Blue	Brown	Orange-brown	Greyish brown
2	0.67	0.74	0.42	Blue	Brown	Orange-brown	Greyish brown
3	0.31	0.22	0.35	Blue	Orange-brown	Orange-brown	Grey
4	0.2	0.03	0.07	Green	Purple	Orange-brown	Brown
5	0.40	0.13	0.70	Blue	Greyish brown	Orange-brown	Grey
6	0.28	0.07	0.70	Blue	Reddish grey	Orange-brown	Grey
7	0.16	0.04	0.27	Blue	Reddish purple	Orange-brown	Brownish grey
Standard (+)-catechin	0.66	0.73	0.42	Blue	Brown	Orange-brown	Greyish brown

animal became restless. At the end of the experiment the animal was killed with anaesthetic ether.

Collection of bile. Samples of bile were collected at $\frac{1}{2}$ h intervals over a period of 5 h after the intravenous injection of (+)-[U- ^{14}C]catechin. Collections from after 5 h to 25 h were carried out at 1 h or 2 h intervals.

Collection of urine. The collection of urine was carried out by cannulating the left ureter (with a size FG catheter) of each rat; the right ureter was simultaneously ligated. Urine samples were collected over the same period as the bile collections.

Measurement of radioactivity in bile and urine. Radioactivity in bile and urine was assayed by liquid-scintillation spectrometry. Samples of bile or urine (0.1 ml) were directly added to scintillation fluid (10 ml) in low-potassium glass vials. The composition of the scintillation fluid was as described by Das & Griffiths (1967). The radioactivity of the samples was usually counted at an efficiency of 69–78% in a Packard Tri-Carb model 3320-01 liquid-scintillation spectrometer. Counting efficiency was determined by using the automatic external standard provided in the spectrometer.

Paper chromatography. Whatman no. 1 paper was used for routine qualitative chromatography, but for the isolation of labelled metabolites by band chromatography Whatman 3MM paper was used. For qualitative analysis samples of bile or urine (30 μl) were applied as discrete spots to one end of a strip of Whatman no. 1 paper (10 cm $\times$ 45 cm). Labelled components in bile were resolved by ascending chromatography in butan-1-ol-acetic acid-water (4:1:5, by vol.) for about 18 h at room temperature. After development, the radiochromatograms were dried and viewed under u.v. light in the absence and presence of NH_3 vapour. The radiochromatograms were then sprayed with different reagents.

Radioassay. Radioactive spots on chromatograms were

located by radioautographs on X-ray film (Kodirex; Kodak Co. Ltd; Alexandra Road, Singapore) with exposure for 3–4 weeks.

Spray reagents. The reagent described by Barton, Evans & Gardner (1952), diazotized *p*-nitroaniline and diazotized sulphanilic acid (Smith, 1960) were used for the detection of phenolic compounds. The reagents described by Roux & Maihs (1960) were used for the identification of any flavonoids associated with condensed tannins: $\text{FeNH}_4(\text{SO}_4)_2$; vanillin-toluene-*p*-sulphonic acid; bis-diazotized benzidine; ammoniacal AgNO_3 . The colour reactions of these reagents with the different metabolites are presented in Table 1.

Enzymic hydrolysis of conjugates in the bile and urine. The enzymic hydrolysis of urine samples for glucuronide conjugates or sulphate conjugates was performed as described by Das & Griffiths (1968). For the bile samples the pH was adjusted to 5 with 0.1 M-HCl and to each 1 ml sample was added 3000 Fishman units of β -glucuronidase (EC 3.2.1.31) (type B-3 from bovine liver; Sigma Chemical Co., St Louis, Mo., U.S.A.). Similarly, to each 1 ml bile sample (pH 5) was added 10 units of arylsulphatase (EC 3.1.6.1) (type III from limpets; Sigma Chemical Co.). Control incubations of bile or urine were carried out in which the pH was adjusted to 5 with 0.1 M-HCl but no enzymes were added. All the tubes were incubated for 24 h at 37°C in a Dubnoff metabolic shaking incubator (Precision Scientific, W. Cortland St., Chicago, Ill., U.S.A.). At the end of the incubation each sample was taken and portions (30 μl) were used for qualitative ascending chromatography.

Detection of glucuronic acid. The glucuronic acids liberated by the action of β -glucuronidase (EC 3.2.1.31) on glucuronide conjugates were detected on paper chromatography by spraying with 0.2% naphtharesorcinol reagent (Elliot, Tao & Williams, 1965).

after intravenous injection of 25 mg of (+)-[U-¹⁴C]catechin into the rat

water (1:1, v/v) and sodium formate-formic acid-water (10:1:200, w/v/v) respectively.

	With bis-diazotized benzidine	With vanillin-toluene- <i>p</i> -sulphonic acid	With toluene- <i>p</i> -sulphonic acid	Fluorescence			
				Visible light	Visible light +NH ₃ vapour	U.v. light	U.v. light +NH ₃ vapour
Claret maroon	Claret maroon	Reddish violet	Light brown	Brown stain after 24 h	Light-green stain	Nil	Light blue
Claret maroon	Claret maroon	Reddish violet	Light brown	Brown stain after 24 h	—	Light blue	Blue
Claret maroon	Claret maroon	Pink	Light brown	—	—	Bright purple	Green
Claret maroon	Claret maroon	Nil	Nil	Yellowish-green stain	Bright yellowish stain	Bright blue	Green
Claret maroon	Claret maroon	Light purple	Light brown	—	—	Light purple	Purple
Claret maroon	Claret maroon	Light purple	Light brown	—	—	Absorbed	Absorbed
Claret maroon	Claret maroon	Purple	Light brown	—	—	—	—
Claret maroon	Claret maroon	Reddish violet	Light brown	Brown stain after 24 h	—	Light blue	Blue

Fig. 1. Excretion of radioactivity in bile of rats after intravenous injection of (+)-[U-¹⁴C]catechin (sp. radioactivity 17.8 mCi/mol): ○, 25 mg; ●, 50 mg. Results are expressed as the mean values of two experiments.

Ether extraction of urine. After removal of 30 μ l portions for ascending chromatographic analysis from the periodic urine collections, the $\frac{1}{2}$ –24 h urine collections were pooled and the pH was adjusted to 2 with conc. HCl. The acidified urine was continuously extracted with diethyl ether for 24 h. The ether-extracted urine was further acidified with conc. HCl (1 ml of acid/20 ml of urine) and refluxed in a boiling-water bath for 3 h. The hydrolysed urine was cooled and re-extracted with a fresh portion of ether for 24 h. The ether extracts of the urine before and after hydrolysis were separately evaporated to dryness

under reduced pressure in a rotary evaporator (W. Büchi, Flawil, Switzerland). The residues were taken up in 1 ml of acetone, and 0.05 ml portions were applied to Whatman no. 1 paper and subjected to two-dimensional chromatography as described by Das & Griffiths (1966).

RESULTS

Excretion of radioactivity in bile

The common bile ducts of rats were cannulated. Two rats were injected intravenously with 25 mg each and the other two with 50 mg each of (+)-[U-¹⁴C]catechin (sp. radioactivity 17.8 mCi/mol). The total excretion of radioactivity over the 24 h period of bile collection was in the range 33.6–44.3% of the dose, depending on the amount administered. Administration of 50 mg of (+)-[U-¹⁴C]catechin gave a total of 1832000 d.p.m. (33.6% of dose) of radioactivity in the 24 h bile collection and when the administered dose was decreased to 25 mg the total radioactivity in the 24 h bile collection amounted to 1208000 d.p.m. (44.3% of dose). However, the periodic pattern of excretion of radioactivity in bile for both sets of experiments is similar (Fig. 1). The excretion of radioactivity was maximum within the first, second and third $\frac{1}{2}$ h collections after intravenous administration of the labelled flavonoid and decreased to a very low value during 4–24 h of collection.

Chromatography of unhydrolysed bile. Portions (30 μ l) of the bile collections obtained before and after the intravenous administration of (+)-[U-¹⁴C]-

catechin were submitted separately to ascending chromatographic analysis. The solvents A–D used by Das & Griffiths (1969) did not give a good separation of the metabolites, but butan-1-ol-acetic acid–water (4:1:5, by vol.) gave very good separation of the metabolites. The positions of the radioactive metabolites were then determined both by radioautography and by subsequent spraying with the diazotized *p*-nitroaniline or ferric chloride–potassium ferrocyanide reagents. Five metabolites namely 3, 4, 5, 6 and 7, that were absent from the control bile (i.e. before intravenous injection of the (+)-[U-¹⁴C]catechin) were detected (Table 1). These spots do not correspond to any of the positions occupied by the previously characterized phenolic metabolites of (+)-[U-¹⁴C]catechin (Das & Griffiths, 1968, 1969).

Chromatography of hydrolysed bile. Each sample of bile was subjected to enzymic hydrolysis as described in the Materials and Methods section. The action of arylsulphatase on all the bile samples did not produce extra spots on the chromatograms as compared with the controls. However, β -glucuronidase gave rise to two new spots (1 and 2) with high R_f values, and spots 5 and 6 disappeared (Table 1). The two spots, namely 1 and 2, were absent from the controls. Metabolite 2 was identified as (+)-[U-¹⁴C]catechin by using the methods described by Das & Griffiths (1967).

Subsequent isolation of metabolites 3–7 separately by preparative band chromatography and treatment of each with β -glucuronidase showed that metabolites 5 and 6 were glucuronide conjugates of metabolites 1 and 2 respectively.

Table 2. *Excretion of radioactivity in bile and urine of rats after intravenous injection of (+)-[U-¹⁴C]catechin*

The flavonoid was given to rats I and II (two males) as a single dose and the bile and urine were collected for 24 h. Rats III and IV (two males) were also given the flavonoid as a single dose, but only urine was collected and the common bile duct was ligated. For rats V and VI (two males) each received a single dose of the flavonoid and the ureter was cannulated but the common bile duct was left intact.

Time after injection (h)	Distribution of radioactivity							
	Rats I and II				Rats III and IV		Rats V and VI	
	Bile		Urine		Urine		Urine	
	(d.p.m.)	(% of dose)	(d.p.m.)	(% of dose)	(d.p.m.)	(% of dose)	(d.p.m.)	(% of dose)
$\frac{1}{2}$	375287	13.8	369410	13.6	322069	11.8	250718	9.2
1	382744	14.0	143650	5.3	243887	8.9	123472	4.5
$1\frac{1}{2}$	171828	6.0	83072	3.1	161796	5.9	117180	4.3
2	56835	2.1	60040	2.2	151506	5.6	70026	2.6
$2\frac{1}{2}$	34070	1.3	42070	1.5	149187	5.5	56556	2.1
3	26595	1.0	32648	1.2	107893	4.0	46760	1.7
$3\frac{1}{2}$	16487	0.6	20017	0.7	88815	3.3	24450	0.9
4	23457	0.9	25520	0.9	66635	2.4	21390	0.8
$4\frac{1}{2}$	13007	0.5	12690	0.5	59479	2.2	29445	1.1
5	9162	0.3	12852	0.5	54901	2.0	28350	1.0
$5\frac{1}{2}$	8076	0.3	11130	0.4	41915	1.5	19705	0.7
6	6930	0.3	8092	0.3	35806	1.3	15912	0.6
$6\frac{1}{2}$	5860	0.2	11360	0.4	30909	1.1	—	—
7	5126	0.2	7670	0.3	28586	1.0	—	—
$7\frac{1}{2}$	4504	0.2	8960	0.3	44528	1.6	—	—
8	7066	0.3	13000	0.5	23946	0.9	52471	1.9
$8\frac{1}{2}$	3194	0.1	5967	0.2	21848	0.8	—	—
9	2781	0.1	4914	0.2	24179	0.9	—	—
$9\frac{1}{2}$	2760	0.1	3960	0.2	20621	0.8	—	—
10	2564	0.1	4536	0.2	—	—	29210	1.1
$18\frac{1}{2}$	13750	0.3	2856	0.1	—	—	231340	8.5
$19\frac{1}{2}$	—	—	—	—	—	—	22550	0.8
20	—	—	—	—	157800	5.8	17493	0.6
21	—	—	—	—	—	—	13044	0.5
22	—	—	—	—	—	—	14573	0.5
23	—	—	—	—	—	—	14910	0.5
24	—	—	—	—	—	—	16092	0.6
Total	1172085	42.7	884414	32.6	1836306	67.3	1215637	44.5

Fig. 2. Excretion of radioactivity after intravenous injection of (+)-[U-¹⁴C]catechin (25 mg): (a) in bile of rats I and II (two males) with common bile duct and ureter cannulated; (b) in urine of rats I and II (two males) with common bile duct and ureter cannulated; (c) in urine of rats III and IV (two males) with common bile duct ligated; (d) in urine of rats V and VI (two males) with intact common bile duct. Results are expressed as the mean values of two experiments.

Excretion of radioactivity in urine

Cannulation of the common bile duct and ureter.
Each rat was injected intravenously with 25 mg of

(+)-[U-¹⁴C]catechin (sp. radioactivity 17.8 mCi/mol). The radioactivity distributions in the periodic collection of bile and urine are presented in Table 2. The values given represent differences from the 0 h

collection, i.e. before injection of the labelled flavonoid. The total radioactivities present over 24h collection for urine and bile amounted to 32.6 and 42.7% of the administered dose respectively. The radioautographs of the bile collections were very similar to that described above (spots 3-7; Table 1). The chromatograms of the urine collections (obtained both by ether extraction and direct urine application) revealed the presence of metabolites 2, 3 and 6 in large amounts, with traces of metabolites 5 and 1. The metabolites appeared mainly in the $\frac{1}{2}$ h, 1 h and $1\frac{1}{2}$ h samples. The radioactivity during the $\frac{1}{2}$ h, 1 h and $1\frac{1}{2}$ h urine collections also showed maximum values (rats I and II; Table 2). The pattern of excretions of radioactivity in the bile and urine are presented as histograms in Figs. 2(a) and 2(b) respectively.

Ligation of the common bile duct and cannulation of the ureter. Urine samples from two rats were collected for 24h. The total radioactivity in the 24h urine samples amounted to 67.3% of the administered dose (rats III and IV; Table 2). The chromatographic analysis of each sample of urine (obtained both by ether extraction and direct urine application) for rats III and IV showed the presence of metabolites 1, 2, 3, 5, 6 and 7 in large amounts in the $\frac{1}{2}$ h, 1 h and $1\frac{1}{2}$ h urine collections. The radioactivity during the $\frac{1}{2}$ h, 1 h and $1\frac{1}{2}$ h urine collections also showed maximum values (Table 2 and Fig. 2c). This is to be expected, since the common bile duct has been ligated and all the metabolites that were excreted in the bile previously would now appear in the urine. Further, the urine collections had a typical jaundiced appearance.

Cannulation of the ureter only. Each of two rats was injected intravenously with 25mg of (+)-[U- 14 C]catechin. The radioactivity distributions in the periodic collection of urine are shown in Table 2 (rats V and VI) and presented as a histogram in Fig. 2(d). The values given represent differences from the 0h collection, i.e. before injection of the labelled flavonoid. The total radioactivity over 24h urine collection amounted to about 44.5% of the administered dose. The chromatograms showed a pattern for the excretion of metabolites 2, 3, 6, 5 and 1 similar to that observed with the chromatograms of urine from rats I and II. They were detected only from the $\frac{1}{2}$ - $1\frac{1}{2}$ h urine samples.

Chromatography of the ether extract from urine collections. Ether extracts of the unhydrolysed urine of rats I and II, III and IV and V and VI were analysed by two-dimensional chromatography. The patterns of metabolites detected for rats I and II and III and IV were similar. The metabolites detected in the ether extract of the unhydrolysed urine of both groups of rats include in addition to metabolites 3, 5, 6 and 1, unchanged (+)-[U- 14 C]catechin, traces of *p*-hydroxyphenylacetic acid, *m*-

hydroxyphenylpropionic acid and *p*-hydroxyphenyl-lactic acid. The (+)-[U- 14 C]catechin and metabolite 1 were excreted in large amounts in the conjugated form and were liberated by acid hydrolysis.

The ether extract of the unhydrolysed urine from rats V and VI contained in addition to metabolites 3, 5, 6 and 1 unchanged (+)-[U- 14 C]catechin, traces of *p*-hydroxyphenylacetic acid and *p*-hydroxyphenyl-lactic acid. In addition, four metabolites, namely A, B, C and D, were detected in trace amounts. Metabolites A, B, C, D, 1 and (+)-[U- 14 C]catechin were excreted in large amounts in the conjugated form and were liberated by acid hydrolysis. Subsequent isolation, purification and chromatographic analysis of metabolites A, B, C and D revealed that they were identical with δ -(3-hydroxyphenyl)- γ -valerolactone, *m*-hydroxyphenylpropionic acid, δ -(3,4-dihydroxyphenyl)- γ -valerolactone and *p*-hydroxyphenylpropionic acid respectively. These metabolites were reported to be excreted in the urine of the rat and the guinea pig after the oral administration of (+)-catechin (Das & Griffiths, 1968) and during incubation *in vitro* of (+)-catechin with rat intestinal contents (Das, 1969). The metabolites, namely δ -(3-hydroxyphenyl)- γ -valerolactone, *m*-hydroxyphenylpropionic acid, δ -(3,4-dihydroxyphenyl)- γ -valerolactone and *p*-hydroxyphenylpropionic acid, were absent from the ether extracts of the hydrolysed and unhydrolysed urine of rats I and II and III and IV.

DISCUSSION

In normal rats biliary excretion was shown to be of great importance in the elimination of (+)-[U- 14 C]catechin and its metabolites. In contrast, Calvey *et al.* (1970) reported that in normal rats biliary excretion was of little or no importance in the elimination of [14 C]trimophonium iodide (a phenolic quaternary amine) and its metabolites. They reported that only small amounts of radioactivity were detected in bile; about 3% of the dose was recovered in 4h.

Additional metabolites of (+)-[U- 14 C]catechin are excreted in the bile and urine of rat after intravenous injection of the labelled flavonoid. The histogram presented in Fig. 2(d) shows a distinct rise in radioactivity in the 9-10h urine collection that is absent from the histogram in Fig. 2(b). Fig. 2(b) shows the excretion of radioactivity in urine of rats I and II (common bile duct and the ureter cannulated) after the intravenous injection of (+)-[U- 14 C]catechin (25mg). Fig. 2(d) shows the excretion of radioactivity in urine of rats V and VI (ureter cannulated and the common bile duct left intact) after the intravenous injection of (+)-

[U-¹⁴C]catechin (25 mg). In view of the very marked excretion of radioactivity in the bile as early as $\frac{1}{2}$ –1 $\frac{1}{2}$ h after intravenous injection of (+)-[U-¹⁴C]-catechin, it is likely that the second radioactive peak in the 9–10 h urine collection shown in Fig. 2(d) may be attributed to a reabsorption of the radioactivity from the large intestine. This observation was substantiated by the detection of four metabolites, namely *m*-hydroxyphenylpropionic acid, *p*-hydroxyphenylpropionic acid, δ -(3-hydroxyphenyl)- γ -valerolactone and δ -(3,4-dihydroxyphenyl)- γ -valerolactone, in the ether extracts of urine from rats V and VI, whose bile was allowed to flow into the intestinal tract. These additional metabolites are probably formed by the action of the intestinal micro-organisms on the metabolites excreted through the bile duct. It has been reported that the incubation of rat intestinal contents with (+)-catechin *in vitro* resulted in the formation of the phenolic acid and lactone metabolites (Das, 1969). However, these metabolites were not detected in the ether extract from urine of rats I and II and III and IV, in which the bile excretions were not allowed to flow into the intestinal tract of the animals.

It is noteworthy that additional metabolites, namely 3, 4, 5, 6 and 7, were present in the bile (Table 1) after intravenous injection of (+)-[U-¹⁴C]-catechin. Enzymic hydrolysis of the bile collections showed that metabolite 6 was a glucuronide of (+)-catechin (metabolite 2) and that metabolite 5 was a glucuronide of metabolite 1. Metabolites 3, 4 and 7 were resistant to glucuronidase and arylsulphatase action. With the exception of metabolite 2, which was identified as (+)-[U-¹⁴C]catechin, all the other metabolites were not reported to be present in the ether extract of rat urine after the oral administration of (+)-catechin (Griffiths, 1964; Das & Griffiths, 1968, 1969).

The rapid excretion of metabolites in both the urine and bile of rats (as early as $\frac{1}{2}$ h) after intravenous injection of the labelled flavonoid strongly suggests that tissue metabolism (most probably in the liver) or transformation of (+)-catechin could

occur. Therefore, depending on the route of administration of (+)-catechin, it is likely that in the rat there exists an alternative pathway for the metabolism of (+)-catechin (involving biliary excretion) that is different from the pathway proposed in earlier papers (Griffiths, 1964; Das & Griffiths, 1968, 1969).

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