

STUDIES ON FLAVONOID METABOLISM

ABSORPTION AND METABOLISM OF (+)-CATECHIN IN MAN

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Abstract—Oral administration of (+)-catechin to man results in absorption, rapid metabolism and excretion of the compound largely within 24 hr. Eleven (+)-catechin metabolites are detected in the urine. The major phenolic acid metabolite is *m*-hydroxyphenylpropionic acid and the major lactone metabolites include δ -(3,4-dihydroxyphenyl)- γ -valerolactone and δ -(3-hydroxyphenyl)- γ -valerolactone. The phenolic compounds in the urine are excreted in both free and conjugated forms, including their glucuronides and to a lesser degree their ethereal sulphates. Absorption of (+)-catechin occurs in the gastrointestinal tract as early as 6 hr after the oral administration of the compound and appears in the urine together with several unidentified metabolites both in the free and conjugated forms. The amount excreted within 24 hr is about 7.5 per cent of the administered dose. The stomach gastric juice do not degrade (+)-catechin *in vitro* but prolonged contact with the gastric juice causes some polymerisation of the compound. Unchanged (+)-catechin amounts to about 18.6 per cent of the administered dose in the 48 hr faecal collection. In addition, *m*-hydroxyphenylpropionic acid and one unidentified metabolite are detected in the faeces. The rise in phenolic levels in the blood occurs as early as 6 hr after the oral administration of (+)-catechin and parallel to the rise in urinary excretion of phenolic compounds.

SEVERAL reports on the therapeutic effects, absorption and metabolism of flavonoid substances have been published.¹⁻¹¹ The metabolism of (+)-catechin has so far been limited to experimental animals and the results show species differences.¹¹⁻¹⁶ 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 metabolised, the degradation products appearing in the urine in the free and conjugated forms.^{11,15,16} The differences in the metabolism of (+)-catechin in various species of animals¹¹⁻¹⁶ warrant the investigation of the absorption and metabolism of this compound in man. Data obtained in experimental animals cannot be extrapolated to man and this is of particular importance in view of the potentiality of (+)-catechin being used as a therapeutic agent.¹⁷⁻¹⁹ Pharmacological activity follows largely from oral ingestion of flavonoids in the diet and knowledge of their metabolism is essential to any understanding of their action *in vivo*. Although Gero²⁰ recovered about 20 per cent from the urine of patients fed 50 mg of epicatechin and 65 per cent after intraperitoneal injection in guinea pigs, the urines were not examined for breakdown products. The present investigations report an initial work on the fate of a single oral administration of (+)-catechin to man.

EXPERIMENTAL

Subjects. Healthy, male Chinese medical student volunteers were studied. Their ages and body weights ranged from 18 to 30 years and 44–81.6 kg respectively.

Diet. The diet of all the subjects were similar consisting mainly of meat and carbohydrate. The intake of tea, coffee, fruits, vegetables and flavoured beverages was restricted for a total period of at least 10 days. This was necessary in order to minimize interference by phenolic acids of dietary origin.

(+)-Catechin. This was supplied as cream coloured crystals by Zyma S.A., Nyon, Switzerland.

Administration of (+)-catechin. The administration of (+)-catechin to human subjects was in the form of gelatin capsules taken orally with water after breakfast. The amount administered was 83 mg/kg of body weight.

Collection and extraction of urine. Twenty-four hr urine collection was carried out consecutively for 2 days before the oral intake of (+)-catechin capsules. On the third day, the (+)-catechin capsules were administered and 24 hr urine collection was continued for 4 consecutive days. About 15 ml of 2 N-HCl was added to the urine during the collection in order to reduce bacterial growth.

Each 24 hr urine collection was made up to 2 l. with distilled water. Continuous ether extractions were carried out on aliquots of urine (700 ml) which were acidified to pH 1 with conc. HCl. Each ether extract was evaporated to dryness under reduced pressure in a rotary evaporator and the residue taken up with 5 ml of acetone. Aliquots of 0.05 ml of the extract were used to apply on several sheets of Whatman No. 1 chromatographic papers and the standard procedures for two-dimensional chromatography were carried out as described earlier.¹¹

Acid hydrolysis of conjugates in urine. It was noted that direct boiling of the urine (pH 1) resulted in ring cleavage of some of the unchanged conjugated (+)-catechin molecules giving rise to phloroglucinol and protocatechuic acids. Therefore, the use of enzymic hydrolysis was necessary. The conditions for enzymic hydrolysis were as described earlier.¹¹

Collection of whole blood and colorimetric determination of phenolic compounds. Blood (1 ml) was withdrawn from the subject's arm vein 5 min before the oral intake of (+)-catechin and further blood samples were taken at the 6th, 12th, 24th, 30th, 36th, 48th and 96th hr. Each blood sample was collected into a centrifuge glass tube containing a few crystals of heparin (Sigma Chemical Co., U.S.A.). Colorimetric determination of phenolic compounds in the blood was carried out using a modified method of Theis and Benedict.²¹ The heparinised blood (1 ml) was laked with 7 ml of distilled water. The tube was mixed and 10% sodium tungstate (1 ml) (Sigma Chemical Co., U.S.A.) was added. The mixture was shaken and allowed to stand for 3 min before addition of 2/3 NH₂SO₄ (1 ml) (Sigma Chemical Co., U.S.A.). The precipitate formed was allowed to stand for 10 min before centrifuging. To 2 ml of the supernatant were added 8 ml of distilled water. Then Folin-Ciocaltau reagent²² (1 ml) and 20% Na₂CO₃ (2 ml) were added. The tube was shaken and allowed to stand for 2 hr at ambient temperature (27°) for maximal colour development. At the end of this period, the absorption of the purple colour was measured at 660 mμ in the Hilger spectrophotometer. The phenolic levels in the blood were read from a standard curve freshly prepared for (+)-catechin of concentrations ranging from 10 to 200 μg.

Estimation of phenolic compounds in urine. Daily amounts of phenolic compounds

excreted in the urine throughout the experimental period were estimated according to the procedure described by Bray and Thorpe,²² and the optical absorption was measured at 660 m μ in the Hilger spectrophotometer. The values were expressed as mg of (+)-catechin using a (+)-catechin standard curve.

Colorimetric determination of the phenolic metabolites. This was carried out by the method as described by Griffiths.¹⁵ For those metabolites whose standard compounds were not available, their values were expressed as mg of (+)-catechin.

Determination of glucuronide. This was carried out on the undiluted 24 hr urine collections by the method described by Das and Griffiths.¹¹ A standard curve with a range of 0–80 μ g of glucuronide/2 ml was prepared for each set of determination. Measurements of the extinction at 565 m μ were made in a Hilger spectrophotometer.

Determination of ethereal sulphate. This was carried out on the undiluted 24 hr urine collections as described by Das and Griffiths.¹¹ A standard curve of values ranging from 0.05 to 0.8 mg of SO₃/10 ml was prepared for each set of determination. Turbidity was measured in a Hilger spectrophotometer at 605 m μ .

Incubation of (+)-catechin with stomach gastric juice. Stomach gastric juice was collected from each subject before breakfast. Collection of the stomach juice was done by suctioning out from the stomach tube introduced into the stomach through the nostril. Three different sets of tubes containing the gastric juice were prepared. One set contained 15 ml of gastric juice per tube and these served as controls. The second set contained 15 ml of gastric juice plus 30 mg of (+)-catechin per tube. The third set contained 15 ml of boiled gastric juice (the boiling was carried out for 30 min at 100°) plus 30 mg of (+)-catechin per tube. All the tubes were incubated in a shaker bath maintained at 37° and shaken vigorously during the incubation. The incubation period was either 6.5 or 22 hr. At the end of each incubation period the tubes were subjected to continuous 24 hr ether extraction. Each ether extract was then analysed by two-dimensional chromatography.¹¹

RESULTS

Phenolic constituents of urine before administration of (+)-catechin. Restrictions on the intake of various foodstuffs and beverages resulted in a marked decrease in the excretion of phenolic compounds. These included *p*-hydroxyphenylacetic acid, *p*-hydroxybenzoic acid, *p*-hydroxyphenyllactic acid, *m*-hydroxyhippuric and several other phenolic compounds (5 to 6) which were not identified.

Free metabolites of (+)-catechin. Altogether 12 male volunteers were studied as described in the experimental section. The overall pattern of excretion of (+)-catechin metabolites was similar to earlier reports from this laboratory.^{11,16} Several metabolites of (+)-catechin which were absent from or present in much smaller amounts on the control chromatograms were detected. Eleven compounds reacting with Barton's reagent²³ and diazotised *p*-nitroaniline²⁴ were detected. Compounds corresponding to spots 1, 3, 4, 5 and 6 which were present in large amounts were identified using the methods described previously^{11,16} as (+)-catechin, δ -(3,4-dihydroxyphenyl)- γ -valerolactone, δ -(3-hydroxyphenyl)- γ -valerolactone, δ -(4-hydroxy-3-methoxyphenyl)- γ -valerolactone and *m*-hydroxyphenylpropionic acid respectively. Spots 2, 7, 8, 9, 10 and 11 which were excreted in small amounts were not identified. The *R_f* values and colour reactions of these (+)-catechin metabolites are presented in Table 1. Comparison of the chromatograms obtained on successive days showed that the

metabolites of (+)-catechin were detected mainly on the first and second day after the oral intake of the (+)-catechin capsules. On the third day only traces of metabolites were detected. By the fourth day the pattern of phenolic excretions in the urines returned to that of the control day.

Conjugated metabolites of (+)-catechin. Evidence for the presence of (+)-catechin metabolites present as glucuronide and ethereal sulphate conjugates was obtained by the use of enzymes specific for β -glucuronide or aryl sulphate linkages. Two consecutive 24-hr urine collections were obtained from six male subjects. These urine collections served as controls. On the third day, each subject was administered orally with (+)-catechin capsules (83 mg/kg body weight). Twenty-four hr collections were continued for 4 consecutive days. To remove all free phenolic metabolites, aliquots of the acidified urines were submitted to exhaustive ether extraction for 24 hr. The urine samples were then incubated separately with either β -glucuronidase (EC 3.2.1.31) or arylsulphatase (EC 3.1.6.1) enzymes.¹¹ Ether extracts from the enzymic hydrolysed urine were examined by two-dimensional chromatography.¹¹

TABLE 1. R_f VALUES AND COLOUR REACTIONS OF (+)-CATECHIN METABOLITES

Spots	R_f in solvents					Colour with diazotised <i>p</i> -nitroaniline ²⁴
	A	B	C	D	E	
1	streaks	0.01	0.02	0.57	0.46	brown
2	streaks	0.01	0.02	0.62	0.56	light reddish brown
3	streaks	0.19	0.72	0.92	0.76	reddish brown
4	0.97	0.61	0.84	0.70	0.85	red
5	0.98	0.76	0.93	0.85	0.83	red
6	0.60	0.68	0.65	0.79	0.88	purple
7	0.49	0.01	0.09	0.78	0.88	purple
8	0.38	0.01	0.05	0.77	0.86	red
9	0.34	0.16	0.53	0.87	0.86	reddish pink
10	streaks	0.01	0.42	0.02	0.03	greyish brown
11	0.26	0.62	0.88	0.51	0.53	dark purple

Chromatograms were developed until the solvent front was 30 cm from the origin. All compounds gave a blue colour with Barton's reagent.²³ Spot 9 gave a bright blue fluorescent in the presence of NH_3 fumes under long-wave ultra-violet light. Spot 3 absorbed in ultra-violet light. Solvent A = propan-2-ol-aq. NH_3 (sp.gr. 0.88)-water (8:1:1, by vol.); Solvent B = benzene-glacial acetic acid-water (6:7:3, by vol.); Solvent C = chloroform-glacial acetic acid-water (2:1:1, by vol.); Solvent D = dioxan-water (1:10 by vol.); Solvent E = 2% acetic acid (v/v).

The (+)-catechin metabolites which were present in part as glucuronides were δ -(3,4-dihydroxyphenyl)- γ -valerolactone, δ -(3-hydroxyphenyl)- γ -valerolactone, δ -(4-hydroxy-3-methoxyphenyl)- γ -valerolactone, *m*-hydroxyphenylpropionic acid, unchanged (+)-catechin and 4 unidentified metabolites (spots 2, 9, 10, 11) (Table 1). Metabolites of (+)-catechin which were in part present as sulphate conjugates included δ -(3-hydroxyphenyl)- γ -valerolactone, unchanged (+)-catechin, *m*-hydroxyphenylpropionic acid and two other unidentified metabolites (spots 2, 11).

The amounts of glucuronide and sulphate conjugates present in the daily 24 hr urine collection of three subjects collected over the experimental periods are shown in Table 2.

TABLE 2. EXCRETION OF GLUCURONIDE AND ETHEREAL SULPHATE BEFORE AND AFTER THE ADMINISTRATION OF (+)-CATECHIN TO HUMAN SUBJECTS

Conjugates	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Glucuronide	336.7 (190-430)	418.3 (120-615)	935 (825-1100)	635 (360-1020)	446.7 (340-550)	350 (280-430)
Ethereal sulphate	190 (59.1-373.3)	210 (101-326.7)	455 (240-653.3)	583.4 (154-1026.7)	245 (118-373)	164.5 (48.9-282)

The results are expressed as mg of glucuronide or SO₃ in 24 hr and are the means of three subjects with the ranges shown in parentheses. At the end of the first 2 days of the experiment, each subject received a single dose of (+)-catechin (92.3 mg/kg body weight).

Analysis of 6 hourly and 12 hourly periods of urine and blood collection. Six subjects were selected having an average body weight of 45.5 kg and each subject was administered orally with (+)-catechin capsules (92.3 mg/kg body weight). Urines were collected over the following 0-6 hr, 6-12 hr, 12-24 hr, 24-30 hr, 30-36 hr, and 36-48 hr periods. Collections of urine were continued for the 48-72 hr and 72-96 hr periods. Blood withdrawals from the arm vein were carried out as described in the experimental section. The results of the phenolic estimations in the blood and the urine are presented in Tables 3 and 4 respectively. The chromatographic analyses of the urine extracts are shown in Table 5.

TABLE 3. ESTIMATION OF BLOOD PHENOLS OF HUMAN SUBJECTS AFTER THE ADMINISTRATION OF (+)-CATECHIN (92.3 mg/kg BODY WEIGHT)

Time (hr)	Blood phenols (mg/100 ml plasma)	Average increase over controls
0	11.8 (10-13)	
6th	13.1 (11-16.5)	1.3
(after administration of 4200 mg (+)-catechin)		
12th	12.2 (11.5-12.5)	0.4
24th	13.8 (11.0-18.0)	2.0
30th	13.9 (11.5-16.0)	2.1
36th	12.3 (10.5-13.5)	0.5
48th	13.5 (11.0-16.0)	1.7
96th	11.0 (10.5-12.5)	0

The phenols are expressed as mg of (+)-catechin and the values are the means of six experiments with the ranges in parentheses.

TABLE 4. ESTIMATION OF TOTAL ETHER EXTRACTABLE PHENOLIC COMPOUNDS FROM HUMAN URINE AFTER THE ORAL ADMINISTRATION OF (+)-CATECHIN (92.3 mg/kg BODY WEIGHT)

Time (hr)	Free unconjugated phenolic compounds	Average total 24 hr excretion	Conjugated phenolic compounds	Average total 24 hr excretion
24	58.0 (44-61)	58	51 (36-73)	51
0-6 (after oral administration of (+)-catechin)	51.2 (40.8-58.9)		59.4 (44.8-69.3)	
6-12	20.3 (14.0-26.8)		25.8 (17.5-35.1)	
12-24	34.5 (25.2-45.7)	106.0	122.1 (21.8-405.5)	207.3
24-30	24.3 (11.4-44.1)		70.5 (10.9-237.4)	
30-36	18.6 (15.8-23.0)		73.4 (25.5-163.9)	
36-48	33.5 (21.2-53.9)	76.4	180.6 (28.9-387.2)	324.5
48-72	56.5 (32-66)	56.5	68 (44-84)	68
72-96	55.3 (31.3-59)	55.3	50 (36-66)	50

The phenolic compounds are expressed as mg of (+)-catechin and the values are the means of six experiments with the ranges shown in parentheses.

In vitro studies on (+)-catechin with human stomach gastric juice. In order to determine whether (+)-catechin was destroyed or degraded by the low pH and enzymes existing in the stomach, *in vitro* experiments were carried out whereby fresh human stomach gastric juice was incubated with (+)-catechin. Twelve Chinese medical student volunteers were used for the gastric juice collection as described in the experimental section.

All the chromatograms when sprayed with the diazotised *p*-nitroaniline reagent showed only one coloured spot corresponding to (+)-catechin. Larger amounts of unchanged catechin appeared in the 6.5-hr incubate than in the 22-hr incubate suggesting that some of the (+)-catechin had polymerised on prolonged contact with gastric juice. There were no detectable degraded products of (+)-catechin in all the extracts. The chromatogram of the control tubes showed no spots.

Faecal phenolic compounds and levels of unchanged urinary and faecal (+)-catechin of human subjects. Six Chinese subjects who had earlier volunteered for the oral intake of (+)-catechin were selected for a repetition of the oral intake of the compound. An interval of 3 months was allowed between the first oral intake of the drug and the second intake. The dosage administered was 4200 mg per subject. Twenty-four hr faecal excretion of each subject was collected separately 1 day before the oral intake of the drug and for 2 consecutive days after the oral intake of the drug. The weight of each 24 hr faecal sample was noted. Aliquots of fresh faeces were taken and freeze-dried. Each dried sample was homogenized in 3 × 80 ml of methanol, filtered and the filtrate evaporated to dryness. The residue was taken up in 50 ml methanol and

TABLE 5. THE EXCRETION OF PHENOLIC METABOLITES OF (+)-CATECHIN IN HUMAN URINES

Metabolites	0-6		6-12		12-24		24-30		30-36		36-48		48-72	
	F	C	F	C	F	C	F	C	F	C	F	C	F	C
1	+++	+++++	++	+++	+		+	+++		++				
2	++	++++	+	+++										
3					+	+++	+	+++	++	++	++			
4			+	+++	+	+++	+	+++	+	++	++	++		+
5			+	+++	+	+++	+	+++	+	++	++			
6				++	++	++	++	++		++	++		+	
7	+		++		++		++		++		++		++	
8	++		++		++	++	++	++	++	++	++	++	++	
9	+++	++	++	++	++		++	++	++	++	++		+	
10		+++												
11			++	+++	++	++	++	++	++	++	++	++	++	++

Relative amounts of metabolites excreted are indicated by the number of + signs.

F = free ether extractable; C = conjugated with glucuronides or sulphates.

TABLE 6. R_f VALUES AND COLOUR REACTIONS OF (+)-CATECHIN METABOLITES IN HUMAN FAECES

Metabolites	R_f in solvents					Colour with diazotised <i>p</i> -nitro-aniline ²⁴
	A	B	C	D	E	
'X'	0.72	0.00	0.00	0.18	0.03	greyish brown
<i>m</i> -hydroxyphenyl-propionic acid	0.59	0.68	0.64	0.79	0.89	purple
(+)-catechin	streaks	0.01	0.02	0.56	0.46	brown

Chromatograms were developed until the solvent front was 30 cm from the origin. All compounds gave a blue colour with Barton's reagent.²³ Solvent A = propan-2-ol-aq. NH_3 (sp.gr.0.88)-water (8:1:1, by vol.); Solvent B = benzene-glacial acetic acid-water (6:7:3, by vol.); Solvent C = chloroform-glacial acetic acid-water (2:1:1, by vol.); Solvent E = 2% acetic acid (v/v).

aliquots used for qualitative and quantitative chromatographic analyses. The urinary excretion of each subject was collected for the periods of 0-6 hr, 6-12 hr and 12-24 hr. Each urine sample was ether extracted and hydrolysed by glucuronidase and sulphatase enzymes as described in the experimental section. Each ether extract was analysed and the unchanged (+)-catechin isolated chromatographically and estimated.¹¹

Three spots were detected in the methanolic faecal extract with the usual phenolic spray reagents. One spot was identified to be *m*-hydroxyphenylpropionic acid and the other was unchanged (+)-catechin. The third spot was labelled as 'X' and was not identified. Their R_f values in several solvent systems are presented in Table 6. These spots were absent in the faecal extract of the control day, i.e. before the oral administration of (+)-catechin. The faecal excretions of unchanged (+)-catechin over 48 hr are presented in Table 7. The excretions of unchanged (+)-catechin in the urine over the periods of 0-6 hr, 6-12 hr and 12-24 hr are shown in Table 8.

DISCUSSION

In man, the major phenolic acid metabolites of (+)-catechin has been shown to be *m*-hydroxyphenylpropionic acid which is similar to the earlier report in the rat.¹⁵ *m*-Hydroxybenzoic acid, the major (+)-catechin metabolite in the guinea pig,¹¹ together with vanillic acid and protocathechuic acid detected in the urine of rabbit fed

TABLE 7. FAECAL EXCRETION OF UNCHANGED (+)-CATECHIN BY SIX HUMAN SUBJECTS

Time (hr)	Total (+)-catechin	Mean % of dose
0-24	750.2 (378.9-982.8)	17.9
24-48	30.1 (8.4-35.3)	0.7

The results are expressed as mg of (+)-catechin and are the mean of six subjects with the ranges shown in parentheses. Each subject received a single dose of (+)-catechin (4200 mg).

TABLE 8. EXCRETION OF UNCHANGED (+)-CATECHIN AND SPOT 2 IN URINES OF SIX HUMAN SUBJECTS

Time (hr)	Free (+)-catechin	Conjugated (+)-catechin	Free spot 2	Conjugated Spot 2
0-6	17.4 (14.7-24.6)	224.8 (199.6-283.5)	12.9 (8.5-20.5)	69.0 (59.5-87.1)
6-12	4.8 (3.1-6.7)	59.3 (52.5-69.0)	2.5 (1.8-2.8)	38.3 (30.6-46.2)
12-24	—	9.6 (8.4-11.0)	—	11.8 (11.1-12.6)

The values are expressed as mg of (+)-catechin and are the mean of six subjects with the ranges in parentheses. Each subject received orally a single dose of 4200 mg of (+)-catechin.

Mean grand total: 315.9 [(+)-catechin] 134.5 [Spot 2]

Mean % dose: 7.5 [(+)-catechin] 3.2 [Spot 2]

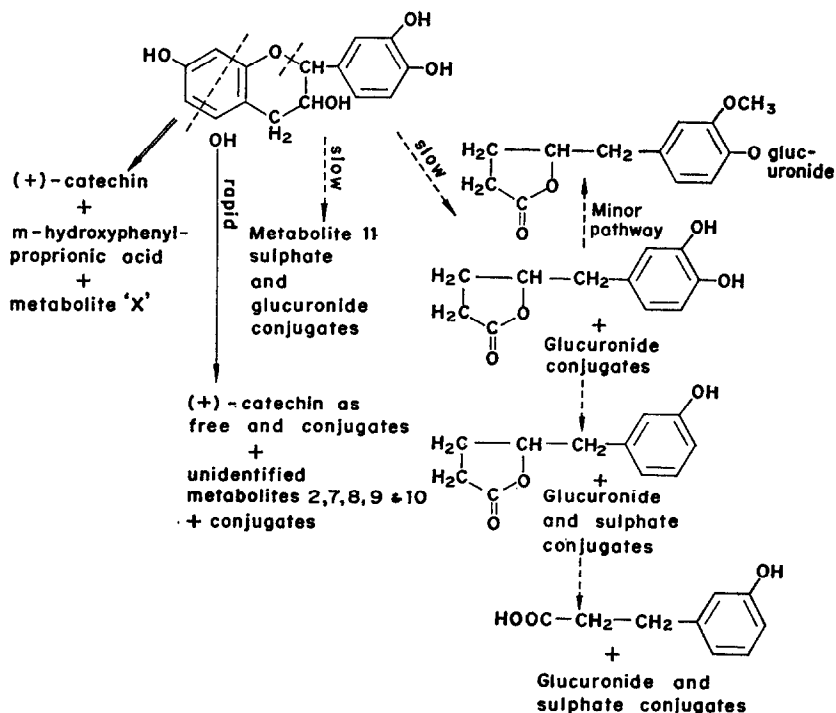
with catechin¹³ were not found in human urine. However, additional metabolites of (+)-catechin namely spots 2, 7, 8, 9, 10 and 11 (Table 1) which were unidentified occurred in human urine and they were absent in the urine of rat and guinea pig. This suggests that in man, the breakdown of (+)-catechin may occur differently from those reported for the guinea pig and rat.^{11,15,16} Several phenyl- γ -valerolactones which were reported for the rat, guinea pig¹¹ and rabbit¹² were also found in human urine after the oral intake of (+)-catechin. Similarly, other reports⁸ described that orally administered hesperidin (hesperetin-7-rutinoside) to man resulted in a different breakdown of the flavonoid and a major metabolite which was identified to be 3-hydroxy-4-methoxyphenylhydracrylic acid was excreted. This compound was absent on similar work using the rabbit. They suggested that in man, the pyran ring of hesperitin is split to yield the hydracrylic acid in contrast to the rabbit where the heterocyclic ring is cleaved. In addition, oral ingestion of naringenin by man⁷ did not decompose the heterocyclic ring to hydroxy acids (which was so in the case of the rat) but instead led to the excretion of naringenin and its glucuronide only.

It is noteworthy that the excretion of unchanged (+)-catechin appeared in the urine as early as 6 hr after administration. In addition, several other metabolites namely spots 2, 7, 8, 9 and 10 also appeared at the same time (Table 5). It is likely that tissue degradation of (+)-catechin must have occurred in order to give rise to the early urinary excretions of the unidentified compounds. According to *in vitro* and *in vivo* tests by Lavollay *et al.*²⁵⁻²⁷ and by Lecoq *et al.*,²⁸⁻³² several flavonoids appear to be involved in metabolic processes in animals, in which they appear to be able to transform the flavonol structure. Rutin was reported³³ to be hydrated at pH 6.8 by the rat liver to red cyanidinrutinoside. Horn *et al.*³⁴ recently reported that in their *in vitro* studies, three compounds of the catechin groups were active on the respiratory activity of cytochrome oxidase in rat liver homogenates and that several coloured unidentified pigments were detected in the incubation mixture at the end of the experiment.

The phenyl- γ -valerolactones and *m*-hydroxyphenylpropionic acid were detected from the 12-24 hr urine collection and persisted up to the 36-48 hr urine collection suggesting that the intestinal bacteria may be involved in their production. This has been reported to occur in the rat.^{11,15,35} Most of the (+)-catechin metabolites were excreted in the urine in the form of conjugates and could be extracted by ether only

after subjection to enzymic hydrolysis. A scheme is presented for the pathway of the metabolism of (+)-catechin in man (Scheme I). Stomach gastric juice did not degrade (+)-catechin *in vitro* but prolonged contact with the gastric juice caused some polymerisation of the compound.

Estimation for blood phenols showed an initial rise as early as 6 hr after the oral administration of (+)-catechin and higher levels occurred at the end of the 24th, 30th and 48th hr. In the 96th hr blood sample, the value returned to about control level (Table 3). The levels for ether extractable phenolic compounds from urine paralleled those observed for blood phenols (Table 4).



SCHEME I. Pathway of the metabolism of (+)-catechin in man. (— — — → metabolites arising from cleavage of the catechin rings and excreted in urine; — — — → metabolites excreted in urine within 0-6 hr after administration of (+)-catechin; ····· → metabolites excreted in faeces).

Large amounts of unchanged (+)-catechin were detected in human faeces in contrast to earlier reports for the rat and guinea pig.^{11,15,16} The amount excreted in the faeces over 48 hr corresponded to about 18.6 per cent of the administered (+)-catechin (Table 7). In addition to the detection of (+)-catechin, two other metabolites were detected in the faeces. One of the two was identified to be *m*-hydroxyphenyl-propionic acid and the other metabolite labelled as 'X' was not identified (Table 6). The latter metabolite was absent in the rat and guinea pig.^{15,16} Furthermore, the present findings showed no phenyl- γ -valerolactones in human faeces after the oral intake of (+)-catechin. A similar report has been given for the guinea pig¹⁶ but in

the rat, phenyl- γ -valerolactones were detected in the faeces after administration of (+)-[U- 14 C]-catechin.¹⁶

Clark and MacKay⁴ reported that in human experiments, the urinary excretion following large oral doses (50 mg/kg body weight) of several flavonoid compounds was negligible and that they were not recoverable from faeces. However, the present investigation shows that appreciable amount of large oral doses of (+)-catechin (83 mg/kg body weight) was absorbed from the gastrointestinal tract and about 7.5 per cent of the administered dose appeared in the 24-hr urine collection as unchanged (+)-catechin. Most of the absorbed catechin were conjugated and excreted in the urine during the 0–6 hr and 6–12 hr periods (Table 8).

No side effects that could be attributed to the large intake of (+)-catechin were observed from any of the human volunteers. A few subjects complained of having diarrhoea about 24 hr after the oral intake of (+)-catechin.

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REFERENCES

1. J. R. GRIFFITH, JR., M. A. LINDAUER and J. F. COUCH, *Med. Soc. State of Pennsylvania, Program*, 93rd Annual Session, Oct. 5–7, p. 20 (1943).
2. J. J. WILLAMAN, *J. Am. Pharm. Assoc.* XLIV 404 (1955).
3. A. M. AMBROSE and F. DEEDS, *J. Nutr.* **38**, 305 (1949).
4. W. G. CLARK and E. M. MACKEY, *J. Am. Med. Assoc.* **143**, 1411 (1950).
5. C. W. MURRAY, A. N. BOOTH, F. DEEDS and F. T. JONES, *J. Am. Pharm. Assoc., Sci. Ed.* XLIII 361 (1954).
6. A. N. BOOTH, C. W. MURRAY, F. T. JONES and F. DEEDS, *J. biol. Chem.* **223**, 251 (1956).
7. A. N. BOOTH, F. T. JONES and F. DEEDS, *J. biol. Chem.* **230**, 661 (1958).
8. A. N. BOOTH, F. T. JONES and F. DEEDS, *J. biol. Chem.* **233**, 280 (1958).
9. A. N. BOOTH and F. DEEDS, *J. Am. Pharm. Assoc., Sci. Ed.* **47**, 183 (1958).
10. N. P. DAS and L. A. GRIFFITHS, *Biochem. J.* **98**, 488 (1966).
11. N. P. DAS and L. A. GRIFFITHS, *Biochem. J.* **110**, 449 (1968).
12. Y. OSHIMA and H. WATANABE, *J. Biochem., Tokyo* **45**, 973 (1958).
13. Y. OSHIMA, W. WATANABE and S. ISAKARI, *J. Biochem., Tokyo* **45**, 861 (1958).
14. H. WATANABE, *Bull. agric. chem. Soc. Japan* **23**, 253 (1959).
15. L. A. GRIFFITHS, *Biochem. J.* **92**, 173 (1964).
16. N. P. DAS and L. A. GRIFFITHS, *Biochem. J.* **115**, 831 (1969).
17. L. TERAS, *Eesti NSV Teaduste Akad.* **11**, 41 (1962).
18. L. TERAS, *Eesti NSV Teaduste Akad.* **11**, 96 (1962).
19. L. TERAS, *Eesti NSV Teaduste Akad.* **14**, 135 (1965).
20. E. GERO, *Arch. int. Physiol.* **54**, 201 (1946).
21. R. C. THEIS and S. R. BENEDICT, *J. biol. Chem.* **61**, 67 (1924).
22. H. G. BRAY and W. V. THORPE, in *Methods of Biochemical Analysis*, Vol. 1 (Ed. D. GLICK), p. 45. Interscience, New York (1954).
23. G. M. BARTON, R. S. EVANS and J. A. F. GARDNER, *Nature, Lond.* **170**, 249 (1952).
24. I. SMITH, *Chromatographic and Electrophoretic Techniques*, p. 297. W. Heinemann, London (1960).
25. J. C. LAVOLLAY, *C.r. Acad. Sci.* **214**, 287 (1942).
26. J. C. LAVOLLAY and J. NEUMAN, *C.r. Acad. Sci.* **212**, 251 (1941).
27. J. C. LAVOLLAY and J. NEUMAN, *C.r. Acad. Sci.* **213**, 193 (1941).
28. R. LECOQ, P. CHAUCHARD and H. MAZOUÉ, *C.r. Soc. Biol.* **141**, 52 (1947).
29. R. LECOQ, P. CHAUCHARD and H. MAZOUÉ, *C.r. Soc. Biol.* **143**, 454 (1949).
30. R. LECOQ, P. CHAUCHARD and H. MAZOUÉ, *Thérapie* **4**, 232 (1949).
31. R. LECOQ, P. CHAUCHARD and H. MAZOUÉ, *C.r. Acad. Sci.* **235**, 585 (1952).
32. R. LECOQ, P. CHAUCHARD and H. MAZOUÉ, *Ann. pharm. franc.* **12**, 179 (1954).
33. J. KUHNAU, *zit. nach Merck's Jber.* 1947/48.
34. R. HORN, M. VONDERMUHLL, M. COMTE and C. GRANDROQUES, *Experientia* **26**, 1081 (1970).
35. N. P. DAS, *Biochim. biophys. Acta* **177**, 668 (1969).