Determination and Significance of L-Tyrosine O-Sulphate and its Deaminated Metabolites in Normal Human and Mouse Urine

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Methods were developed for the determination of the *O*-sulphate esters of L-tyrosine, *p*-hydroxyphenylpyruvate, *p*-hydroxyphenylacetate and *p*-hydroxybenzaldehyde in human urine. The amounts of these esters normally present in human female urine were determined. The quantities and specific radioactivities of L-tyrosine *O*-sulphate and *p*-hydroxyphenylpyruvate *O*-sulphate in mouse urine after labelled tyrosine had been fed were determined and were consistent with the hypothesis that the sole source of *p*-hydroxyphenylpyruvate *O*-sulphate was L-tyrosine *O*-sulphate. It is suggested that the turnover of known polypeptides containing L-tyrosine *O*-sulphate residues can account for only a portion of the quantities of the ester and its metabolite(s) that are present in normal female human urine.

L-Tyrosine *O*-sulphate is present in peptide-bound form in fibrinogen (Bettelheim, 1954; Blombäck, 1960; Blombäck *et al.*, 1965; Jevons, 1963), in the polypeptide hormones gastrin II (Gregory *et al.*, 1964) and cholecystokinin/pancreozymin (Mutt & Jorpes, 1968) and in a number of other physiologically active polypeptides of amphibian origin (see Dodgson & Rose, 1970). The unbound ester is also present in the urine of man and other mammals (Tallan *et al.*, 1955; John *et al.*, 1966) and it may be presumed that this arises in part from the turnover of fibrinogen (see Jones *et al.*, 1963) and from polypeptide hormones containing the ester.

The metabolism of injected L-tyrosine *O*-[³⁵S]sulphate in the rat has been studied by Dodgson *et al.* (1961), who found that the ester was rapidly deaminated to yield *p*-hydroxyphenylpyruvic acid *O*-[³⁵S]sulphate and *p*-hydroxyphenylacetic acid *O*-[³⁵S]sulphate (Powell *et al.*, 1963). This deamination was attributed to L-tyrosine *O*-sulphate-2-oxo-glutarate aminotransferase, an enzyme first described by Rose *et al.* (1966) and which occurs in all mammals so far examined including man (John, 1966). The presence of this enzyme in human tissues suggested that the deaminated metabolites of L-tyrosine *O*-sulphate (*p*-hydroxyphenylpyruvate *O*-sulphate, *p*-hydroxyphenylacetate *O*-sulphate and possibly *p*-hydroxybenzaldehyde *O*-sulphate) might normally be present in appreciable amounts in human urine. The implication of this would be that the amount of L-tyrosine *O*-sulphate that is measured in urine may represent only a portion of that actually being turned over.

The automated method for determination of L-tyrosine *O*-sulphate in urine (John *et al.*, 1966) was based on cation-exchange chromatography. However, the method is not generally suitable for the analysis

of human urine because of the heterogeneity of the L-tyrosine *O*-sulphate peak. A new method was therefore developed which was based on that originally described by Tallan *et al.* (1955) and which employed anion-exchange resin chromatography for fractionation of constituents of urine.

G.l.c. seemed to present the greatest possibility for determining the metabolites of L-tyrosine *O*-sulphate in the light of its successful application to the determination of free phenolic materials in urines in normal and pathological conditions (Horning *et al.*, 1966; Albro & Fishbein, 1971; Coward & Smith, 1969; Blau, 1970). The highly polar nature of the sulphate esters prevented direct analysis by g.l.c. and a system has therefore been developed that involves the prior removal of ester sulphate groups by a sulphatase enzyme, followed by formation of volatile trimethylsilyl derivatives of the liberated free phenols. The possibility that direct replacement of ester sulphate groups by acetyl groups (Paulson & Portney, 1970) might have also been a useful approach was not borne out in practice for these particular esters.

The method developed was not entirely satisfactory for the determination of *p*-hydroxyphenylpyruvate *O*-sulphate and a procedure based on ion-exchange chromatography was therefore developed for this compound. By using these various methods the concentrations of L-tyrosine *O*-sulphate and its metabolites in urine have been measured for normal human subjects.

Experimental and Results

Materials

Chemicals. L-Tyrosine *O*-sulphate was prepared by the method of Dodgson *et al.* (1959), potassium *p*-hydroxyphenylpyruvic acid *O*-sulphate by the

method of Rose & Powell (1963), potassium *p*-hydroxyphenylacetic acid *O*-sulphate by the method of Dodgson *et al.* (1961) and potassium *p*-hydroxybenzaldehyde *O*-sulphate by the method of Flanagan (1963). All other chemicals were of AnalaR grade.

Arylsulphatase. Powder B was prepared from *Alcaligenes metalcaligenes* by the method of Dodgson *et al.* (1954).

2,4-Dinitrophenylhydrazine solution. 2,4-Dinitrophenylhydrazine (396mg, BDH Chemicals Ltd., Poole, Dorset, U.K.) was stirred with 1M-HCl (600ml) for 30min and the whole was then heated to 70°C until solution was complete. After cooling, the solution was diluted to 1 litre with 1M-HCl and stored in a dark bottle until required. This stock solution was then further diluted before use by mixing 500ml with 200ml of methanol and then diluting to 1 litre with water. This solution was also kept in a dark bottle.

Urine collection. The total urine voided by female human subjects was collected over 24h periods. The volume was measured and a portion (100ml) was retained for analysis. Cloudy samples were clarified by centrifuging for 10min at 5°C. Samples that could not be analysed immediately were stored at -10°C until used.

Brij 35 solution. This was prepared by warming 100g of the non-ionic detergent Brij 35 (100g; BDH Chemicals Ltd.), which is a polyoxyethylene dodecyl ether, with 650ml of water until solution was complete.

Analysis of L-tyrosine O-sulphate in urine

Constituents of urine were fractionated on a column (5cm × 0.6cm) of AG1 anion-exchange resin (X8; Cl⁻ form; minus 400 mesh; Bio-Rad Laboratories Ltd., Richmond, Calif., U.S.A.). The resin (50g batches) was prepared by washing it with 2M-HCl (500ml) and water (to neutrality), followed by equilibration with 0.075M-KCl containing 1% (v/v) Brij 35 solution and adjusted to pH 3.0 with 2M-HCl. The resin was stored in the equilibrating buffer and a new column was poured after each fractionation. Samples (2ml) of urine were applied to the column, allowed to drain in under gravity and washed in by a further 2ml of equilibrating buffer. The column was then eluted with the buffer at a flow rate of 26ml/h for 45min, after which time the buffer was automatically changed (Locarte Autochanger, Locarte Instruments Ltd., London W.12, U.K.) to 0.22M-KCl adjusted to pH 2.5 with 2M-HCl and containing 1% (v/v) Brij 35 solution. The column was eluted with this buffer for 2h. The effluent was monitored for ninhydrin-positive material with the Technicon Amino Acid AutoAnalyzer (Technicon Instruments Ltd., New York, N.Y., U.S.A.), the chart speed having been decreased from 30.5 to

3.8cm/h. Nine of the twelve ink pads in the print-out mechanism were removed so that one ink spot/min per colorimeter appeared on the chart. Two samples were used for each determination and to one of the samples was added a known amount of chemically synthesized L-tyrosine *O*-sulphate (0.11 μmol) before analysis. From the increase in the peak area for L-tyrosine *O*-sulphate, the concentration of the ester in the sample could be calculated.

Fig. 1 shows a typical chromatogram for a 2ml sample of normal female human urine analysed for L-tyrosine *O*-sulphate as described. Addition of an authentic sample of the ester to the urine before analysis produced an increase in the size of the appropriate peak in proportion to the amount of ester added. To verify the identity of the peak further experiments were carried out. In the first one samples (2ml) were analysed for L-tyrosine *O*-sulphate as above and for free L-tyrosine by normal autoanalytical procedures (John *et al.*, 1966). A 5ml sample of the same urine was then adjusted to 0.4M with respect to HCl and kept at 100°C for 30min in a tube covered with a bubble condenser. These con-

Fig. 1. Separation of L-tyrosine *O*-sulphate of normal female urine by column chromatography on AG1 anion-exchange resin

For details see the text. The L-tyrosine *O*-sulphate peak is denoted by the arrow. ---- indicates the time at which the eluting buffer was automatically changed.

ditions are sufficient to hydrolyse L-tyrosine O-sulphate completely. The hydrolysate was then neutralized and 2ml portions were analysed for L-tyrosine O-sulphate and free L-tyrosine as before. Under these circumstances the peak appropriate to the former compound had completely disappeared whereas that owing to the latter had increased by an amount corresponding to the quantity of L-tyrosine O-sulphate hydrolysed (cf. John *et al.*, 1966).

In a further experiment, urine (5ml) was adjusted to pH 9.0 with 2M-KOH and incubated for 2h at 37°C with 1 mg of arylsulphatase (powder B). A 2ml portion was then adjusted to pH 3 with 2M-HCl and subjected to chromatography. Again the L-tyrosine O-sulphate peak was no longer present confirming that the material was indeed a sulphate ester.

Analysis of p-hydroxyphenylpyruvate O-sulphate in urine

The chromatographic separation stage used in the preparation of *p*-hydroxyphenylpyruvate O-sulphate (Rose & Powell, 1963) was developed for determining this component in urine. Urine samples (1–5ml) were applied to a column (6cm×0.6cm) of DEAE-cellulose (Whatman DE-11, W. and R. Balston Ltd., Maidstone, Kent, U.K.) prepared in the sulphate form by washing 20g of the material with 4 litres of 0.5M-NaOH, 3 litres of water and 4 litres of 0.25M-H₂SO₄ and equilibrating with 0.01M-K₂SO₄. The column was eluted with 0.01M-K₂SO₄ for 30min at a flow rate of 48ml/h, followed by 0.2M-K₂SO₄ for a further 45min. The effluent was monitored (Technicon AutoAnalyzer) for 2,4-dinitrophenylhydrazine-positive materials by a method based on Technicon AutoAnalyzer Methodology no. N-44. The column effluent was automatically mixed with an equal volume of 2,4-dinitrophenylhydrazine reagent; N₂ at a flow rate of 0.42ml/min was used to segment the stream. After mixing with the reagent 1M-NaOH was introduced into the stream at a flow rate of 1.2ml/min. The extinction at 515nm (15mm flow-cell) of the brown colour produced was automatically recorded. *p*-Hydroxyphenylpyruvate O-sulphate emerged as the first peak after the change in eluent.

Two urine samples were used for each determination, and to one of these a known amount of authentic *p*-hydroxyphenylpyruvate O-sulphate (0.1μmol) was added before analysis. From the increase in the appropriate peak area for this ester the amount present in the original sample could be calculated.

To verify the identity of the peak the acid hydrolysis and arylsulphatase hydrolysis procedures described in the preceding section were applied to urine before chromatographic analysis. The peak ascribed to *p*-hydroxyphenylpyruvate O-sulphate disappeared as a result of hydrolysis, although it was not possible to measure liberated *p*-hydroxyphenylpyruvic acid

owing to the degradation of this compound which occurred under the experimental conditions used.

Analysis of p-hydroxyphenylacetate O-sulphate and p-hydroxybenzaldehyde O-sulphate in urine

A urine sample (10ml) was applied to a column (10cm×2cm) of the cation-exchange resin AG-50W (X8; H⁺ form; 200–400 mesh; Bio-Rad Laboratories Ltd.) and allowed to drain in under gravity. The column was eluted with water and that part of the effluent which was at pH lower than 2.5 was collected in an ice-cold vessel. The effluent was immediately extracted with 3×20ml portions of diethyl ether to remove the free phenols present and the ether extracts were discarded. The aqueous extract was adjusted to approx. pH 7 with 2M-NaOH and 1ml of 0.1M-Tris-HCl buffer, pH 9, was added. The pH of the extract was adjusted to 9.0 if necessary, and the whole was incubated overnight at 37°C with 1 mg of arylsulphatase (powder B). After incubation the pH was adjusted to 1.0 with HCl and the solution was extracted three times with ether (15ml portions). The combined extracts were concentrated to 1ml by directing a stream of dry air on to the surface and were then transferred to a small vial (approx. 1.8ml) before evaporation to dryness. Trimethylsilyl derivatives of the dried extracts were prepared by addition of pyridine-hexamethyl disilazane (1:1, v/v; 0.2ml; Eastman Organic Chemical Co., Kodak Ltd., Kirkby, Liverpool, U.K.). The vial was stoppered, shaken for 2min and left for 1h. Trimethylsilyl derivatives were separated on a glass column (300cm×0.4cm) packed with 10% (w/v) OV-1 silicone gum on Chromosorb W-HP (80–100 mesh; Phase Separations Ltd., Queensferry, Flintshire, U.K.). The column was run in the Pye model 104 gas chromatograph fitted with an identical reference column and dual flame-ionization detectors. Portions (2–5μl) of the derivatives were injected on to the column at an injector head temperature of 170°C, detector temperature of 250°C and an argon carrier-gas flow rate of 60ml/min. The initial temperature of the column was 120°C and a temperature programme of 4°C/min rise up to a maximum of 230°C was initiated immediately after injection of the sample.

Analysis of a sample (10ml) of urine gave the chromatogram shown in Fig. 2(a). Addition of authentic *p*-hydroxyphenylacetate O-sulphate and *p*-hydroxybenzaldehyde O-sulphate to the urine gave increases in the peaks ascribed to the two esters in proportion to the amounts added (Fig. 2b).

To verify the identity of the two peaks in question, chromatography of portions (2.5μl) of the trimethylsilyl derivatives prepared from urine was carried out under different conditions. Stainless-steel columns (150cm×0.6cm) containing 3% (w/v) OV-17 on Chromosorb G (100–200 mesh; non-acid washed;

Fig. 2. Gas-liquid chromatography of trimethylsilyl derivatives of compounds present in treated hydrolysates of female human urine

(a) Normal female urine treated as described in the text. X and Y denote *p*-hydroxyphenylacetate and *p*-hydroxybenzaldehyde peaks respectively. (b) A sample of the same urine to which had been added *p*-hydroxyphenylacetate *O*-sulphate ($0.3\mu\text{mol}$) and *p*-hydroxybenzaldehyde *O*-sulphate ($0.4\mu\text{mol}$) before treatment.

Perkin-Elmer Ltd., Beaconsfield, Bucks., U.K.) were employed in conjunction with the same temperature programme as for the OV-1 column. The chromatogram obtained showed peaks at the retention times of authentic trimethylsilyl derivatives of *p*-hydroxyphenylacetate and *p*-hydroxybenzaldehyde which were quantitatively equivalent to those obtained by chromatography on OV-1.

Excretion patterns in normal female human urine

By using the methods described above, the concentrations of these various esters were determined for a range of normal adult female urines. The results are shown in Table 1.

Relationship between *L*-tyrosine *O*-sulphate and *p*-hydroxyphenylpyruvate *O*-sulphate in mouse urine

Previous work (Dodgson *et al.*, 1961; Powell *et al.*, 1963; Jones *et al.*, 1963; Basford *et al.*, 1966) has established that free or peptide-bound *L*-tyrosine *O*-sulphate injected into experimental animals is finally excreted, in part at least, as *p*-hydroxyphenylpyruvate *O*-sulphate and *p*-hydroxyphenylacetate *O*-sulphate. However, such experiments do not exclude the possibility that a portion of the output of these esters in urine may have arisen from the biological sulphation of *p*-hydroxyphenylpyruvate produced as the first stage in the normal metabolism of *L*-tyrosine. To throw further light on this point the following experiment was performed. Two male albino mice (CSE strain; 20–30g body wt.; Scientific Products Farm, Ash, Canterbury, Kent, U.K.) were each injected with 0.5mCi of *L*-[side-chain-2,3- ^3H]tyrosine (specific radioactivity 13.5Ci/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.). Animals were kept for 4 days in a metabolism cage designed for the separate collection of urine and faeces. During this time the mice were allowed food and water *ad libitum*.

The 4-day urines were pooled and 1 ml was applied to a column (1 cm $\times$ 2 cm) of the cation-exchange resin AG-50W (X8; H^+ form; 200–400 mesh; Bio-Rad Laboratories Ltd.) and allowed to drain in under gravity. This was followed by 1 ml of water and subsequent elution of the column with water. The effluent which emerged at pH values lower than 4.0 was collected and concentrated to 1 ml *in vacuo* at 35°C. Any free *L*-tyrosine present in the original urine sample was retained on the column at this stage. The concentrate was adjusted to 0.4M with respect to HCl and was heated to 100°C for 30 min in a tube fitted with a bubble condenser. Under these conditions any *L*-tyrosine *O*-sulphate would be hydrolysed to yield *L*-tyrosine. The hydrolysate was adjusted to pH 2.0 and subjected to analysis for *L*-tyrosine on the Technicon Amino Acid AutoAnalyzer fitted with a Locarte column and automatic buffer changer. Analysis of a portion (0.2 ml) of the concentrate before hydrolysis showed that *L*-tyrosine was not present in the urine. It followed that any *L*-tyrosine measured after the hydrolysis stage was derived from the *L*-tyrosine *O*-sulphate.

For the determination of the specific radioactivity of the *L*-tyrosine in the hydrolysed urine a fraction collector was connected to the waste outlet of the colorimeter and 1 ml fractions of the emerging solution were collected. Portions (0.5 ml) of each fraction were added to 20 ml of a scintillation mixture consisting of 2,5-diphenyloxazole (7 g) and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (0.6 g) dissolved in toluene-methyl oxitol (3:1, v/v; 1 litre). Radioactivity was determined by counting in the Packard Tri-Carb model 3375 liquid-scintillation spectro-

Table 1. *Urine concentrations of L-tyrosine O-sulphate, p-hydroxyphenylpyruvate O-sulphate, p-hydroxyphenylacetate O-sulphate and p-hydroxybenzaldehyde O-sulphate in normal adult females*

For details see the text. Results are expressed as mg/24h.

Subjects	O-Sulphate of ...	L-Tyrosine	p-Hydroxy- phenylpyruvate	p-Hydroxy- phenylacetate	p-Hydroxy- benzaldehyde	Total (expressed as L-tyrosine O-sulphate equivalents)
1		22	6	4	1	34
2		20	10	6	3	40
3		30	2	9	1	43
4		30	4	5	1	41
5		24	4	3	1	33
6		24	6	2	1	33
7		18	7	7	1	34
8		24	2	3	2	32
9		27	4	9	0.5	42
9		28	4	2	2	37
10		25	1	5	2	34
10		23	6	3	2	35
11		27	7	1	2	38
11		24	7	2	1	34
11		29	3	2	2	37
12		34	2	3	0.5	40
12		26	1	3	1	32
12		28	2	9	1	41
13		29	11	6	2	49
13		27	8	10	1	47
13		29	5	6	2	43
13		27	5	7	2	42
Average		26	5	5	1	38

Table 2. *Analysis of mouse urine for L-tyrosine, L-tyrosine O-sulphate and p-hydroxyphenylpyruvate O-sulphate after injection of 0.5mCi of L-[side-chain-2,3-³H]tyrosine*

For details see the text.

Compound	Amount present (μ mol/ml)	Specific radioactivity (c.p.m./ μ mol)
L-Tyrosine	0.068	30×10^6
L-Tyrosine O-sulphate	0.034	88 500
p-Hydroxyphenylpyruvate O-sulphate	0.140	41 000

meter. The specific radioactivity of the L-tyrosine O-sulphate could then be deduced and is reported in Table 2. In a separate experiment the amount of free L-tyrosine present in the original urine was determined by adjusting a 0.5ml portion of the pooled urine to pH 2.0 with 2M-HCl, clarifying by centrifuging and subjecting to analysis for L-tyrosine on the AutoAnalyzer. The specific radioactivity of the amino acid was determined as described above and is recorded in Table 2.

A further 1ml portion of the 4-day urine was analysed for p-hydroxyphenylpyruvate O-sulphate by the method already described. However, the method could not be applied for the measurement of the specific radioactivity of the ester owing to interference from a 2,4-dinitrophenylhydrazine-negative radioactive peak. To measure specific radioactivity a sample (0.5ml) of urine was adjusted to pH 2.0 with 2M-HCl and extracted with ether (3 $\times$ 1ml portions) to remove contaminating ether-soluble

materials. Portions (25 μ l) of the aqueous residue were then subjected to horizontal paper electrophoresis on Whatman no. 1 paper, together with authentic samples of *p*-hydroxyphenylpyruvate *O*-sulphate. Electrophoresis was for 2 h at a potential gradient of 10 V/cm in the presence of 0.1 M-sodium acetate buffer, pH 4.5. The ester was detected by u.v. light and by reference to the authentic material. The appropriate spot was cut out and added to 10 ml of a scintillation mixture prepared as described above except that toluene alone was used as solvent. Radioactivity was measured as described above and the value obtained was corrected for the difference in quenching between this scintillation mixture and that containing methyl oxitol. The specific radioactivity of the ester is shown in Table 2.

Discussion

The presence in mammalian urine of relatively large amounts of L-tyrosine *O*-sulphate has still not been satisfactorily accounted for. The problem stems from the fact that the biological sulphating system of the cytosol is unable to use free L-tyrosine as an acceptor substrate (Segal & Mologne, 1959; Grimes, 1959; Nose & Lipmann, 1958; Suzuki & Strominger, 1960). Only when the carboxyl group is blocked and the α -amino group is in an undissociated state can biological sulphation occur (Jones & Dodgson, 1965; Jones *et al.*, 1966) and the existence of a sulphotransferase enzyme, which is able to catalyse the process, has been demonstrated in rat liver cytosol (L-tyrosine methyl ester sulphotransferase; Mattock & Jones, 1970; Mattock *et al.*, 1970). In fact, it seems probable that the enzyme is normally responsible for the biological sulphation of tyramine (Barford & Jones, 1972). The question must therefore be posed as to the origins of L-tyrosine *O*-sulphate in the urine. There is indirect evidence suggesting that a portion must be derived from the turnover of fibrinogen, gastrin II and cholecystokinin/pancreozymin. Certainly, in the rabbit, injection of rabbit fibrinopeptide B, the L-tyrosine *O*-sulphate residue of which had been labelled with ^{35}S , was followed by the excretion of free labelled L-tyrosine *O*-sulphate, *p*-hydroxyphenylpyruvate *O*-[^{35}S]sulphate and *p*-hydroxyphenylacetate *O*-[^{35}S]sulphate (Jones *et al.*, 1963). The implications of these various points would be that sulphation of L-tyrosine residues probably occurs after their incorporation into polypeptide chains. Subsequent turnover of the polypeptide would lead to the liberation and excretion of free L-tyrosine *O*-sulphate or its deaminated metabolites. Enzymic liberation of sulphate from L-tyrosine *O*-sulphate cannot occur as the ester cannot serve as a substrate for mammalian arylsulphatases A, B and C (Dodgson *et al.*, 1959). It is of considerable interest that the most generous assessment of the turnover of known L-tyrosine *O*-

sulphate-containing polypeptides could not account for more than a part of the daily output of the ester in urine.

The question now arises as to whether all the *p*-hydroxyphenylpyruvate *O*-sulphate in urine results from the deamination of L-tyrosine *O*-sulphate by L-tyrosine *O*-sulphate-2-oxoglutarate aminotransferase or whether some of the material could be produced by the biological sulphation of *p*-hydroxyphenylpyruvate. This oxo acid is on the main pathway of metabolism of L-tyrosine, but there are two reasons why its biological sulphation is unlikely to occur to any significant extent. First, the acid is normally rapidly metabolized further via homogentisate, and, secondly, it is an extremely poor sulphate acceptor (Hext, 1972). More direct evidence that *p*-hydroxyphenylpyruvate *O*-sulphate in urine is produced entirely from L-tyrosine *O*-sulphate comes from the radioisotope experiments with mice (Table 2). The specific radioactivity of the *p*-hydroxyphenylpyruvate *O*-sulphate isolated from mouse urine after injection of radioactive L-tyrosine (containing ^3H at positions 2 and 3 on the side chain) is approx. 50% of that of the L-tyrosine *O*-sulphate. This is the theoretical value that would be obtained if all the *p*-hydroxyphenylpyruvate *O*-sulphate had been produced from L-tyrosine *O*-sulphate, since 1 ^3H atom is lost from position 2 of the side chain as a result of deamination. It is difficult to envisage how this relationship of specific radioactivities could have been obtained if the sulphation of L-tyrosine had been a process entirely independent of the sulphation of *p*-hydroxyphenylpyruvate.

The origins of the sulphate esters of *p*-hydroxyphenylacetate and *p*-hydroxybenzaldehyde, which are apparently also always present in human urine, cannot be stated with the same degree of certainty, but both are readily produced from *p*-hydroxyphenylpyruvate *O*-sulphate non-enzymically (Powell *et al.*, 1963). *p*-Hydroxybenzaldehyde *O*-sulphate is present in urine in relatively small quantities and *p*-hydroxyphenylacetate *O*-sulphate is the predominant ester of the two (Table 1). *p*-Hydroxyphenylacetate is known to be an important metabolite of tyramine and is excreted at the rate of approx. 30 mg/24 h (Horning *et al.*, 1966). The possibility that a small proportion of the total amount produced might undergo biological sulphation cannot therefore be entirely ruled out at the present. Indeed, Tacker *et al.* (1970) detected traces of *p*-hydroxyphenyl[1- ^{14}C]acetate *O*-sulphate in rat urine after administration of [1- ^{14}C]tyramine. The position is obscured even further by the possibility that the sulphate ester might have been derived from tyramine after the latter had itself been sulphated. Other work in these laboratories (J. G. Jones, personal communication) has shown that *p*-hydroxyphenylacetate *O*-[^{35}S]sulphate can be detected in rat urine after the injection of tyramine

O-[³⁵S]sulphate. Further work is clearly needed to establish the quantitative contribution of tyramine metabolism to the pool of *p*-hydroxyphenylacetate O-sulphate in urine.

On the whole, the collective evidence certainly supports the view that the deamination of L-tyrosine O-sulphate is the principal route by which the three other sulphate esters are formed. It follows that the amount of L-tyrosine O-sulphate being turned over each day may be substantially greater than would appear from the amount of the ester present in urine. The total L-tyrosine O-sulphate equivalents obtained from 22 different samples of normal adult female urine ranged from 32 to 49 mg/24 h, values that certainly cannot be accounted for by the daily turnover of known L-tyrosine O-sulphate-containing polypeptides. Tallan *et al.* (1955) have claimed that the output of the sulphate ester in urine is unaffected by high-protein diets and the possibility exists that other, as yet undiscovered, L-tyrosine O-sulphate-containing polypeptides may make substantial contributions to the concentration of the sulphated amino acid and its metabolites in urine.

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