SUMMARY

1. Invertase and maltase have been detected in a centrifugally isolated particulate fraction of disrupted mucosal cells of rabbit small intestine. The preparation was free of lactase activity.

2. The crude enzyme preparation has been partially purified and a relatively stable and apparently soluble form of the two activities obtained.

3. The kinetic characteristics of the invertase activity have been determined.

4. Oligosaccharide formation by the enzyme preparation incubated with sucrose or with maltose has been demonstrated. In the presence of the latter substrate two oligosaccharides were formed, one of which was possibly maltotriose and the other possibly a tetrasaccharide.

5. The invertase activity has been characterized as an α-glucosidase. On incubation with sucrose the enzyme incorporated [^{14}C]fructose into sucrose and into another apparently non-reducing, benzidine-positive, glucosylfructose. Two benzidine-negative sugars were detected as products of the action of the enzyme on sucrose in the presence of [^{14}C]glucose.

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The Metabolism of Potassium L-Serine *O*[^{35}S]-Sulphate in the Rat

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Dodgson, Lloyd & Tudball (1961*a*) and Dodgson & Tudball (1961) reported the presence in mammalian tissues of an enzyme system capable of bringing about the desulphation of L-serine *O*-sulphate *in vitro*. The enzyme is mainly located in the cell sap of rat liver and therefore coexists with the enzyme system which is responsible for the sulphation of alcohols, phenols and steroids (see Spencer, 1960; Roy, 1956). Dodgson & Tudball (1961) have pointed out that the coexistence of desulphating and sulphating mechanisms could explain why it has not been possible to achieve the

enzymic sulphation of L-serine by rat-liver-supernatant preparations (Spencer, 1960; Dodgson *et al.* 1961*a*). L-Serine and its phosphorylated derivatives occupy important positions in intermediary metabolism and are involved in the active centres of a number of enzyme systems (Schaffer, Harshman & Engle, 1955; Anderson & Jollés, 1957; Hartley, Naughton & Sanger, 1959). The possibility therefore exists that a specific desulphating enzyme system for sulphated L-serine residues might be of importance in maintaining the metabolically reactive hydroxyl group of the amino acid in an unesterified form. It was therefore decided to investigate the metabolic fate of L-serine *O*-sulphate in the intact animal. Some preliminary experiments have been reported by Dodgson, Lloyd & Tudball (1961*b*).

MATERIALS AND METHODS

Potassium L-serine O[^{35}S]-sulphate. This was prepared by a modification of the method described by Dodgson *et al.* (1961*a*). L-Serine (0·5 g.) was added at room temperature to 1 ml. of $H_2{}^{35}SO_4$ (sp.gr. 1·84; specific activity 28 mc/g.) and the mixture stirred until solution was complete. The reaction mixture was kept *in vacuo* for the remainder of 1 hr., then poured into 100 ml. of ice-cold water containing $Ba(OH)_2,8H_2O$ (7 g.). Precipitated $Ba^{35}SO_4$ was removed by centrifuging and the clear supernatant evaporated to small volume (5–10 ml.) *in vacuo* at 38°. The concentrate was then added to a column (1 cm. × 10 cm.) of Dowex 50 ion-exchange resin (20–50 mesh; H^+ form; Dow Chemical Co., Midland, Mich., U.S.A.). The column was washed with 20 ml. of water and the combined eluate and washings were adjusted to pH 7·5 with aq. 5% (w/v) KOH before being evaporated to dryness *in vacuo* at 38°. The residue was dissolved in the minimum amount of water and precipitated by the dropwise addition of ethanol. The white crystalline material was separated at the pump, washed with ethanol and then with ether, and finally dried *in vacuo* over $CaCl_2$ at room temperature. The yield was 547 mg. and the specific activity 10·9 μc/mg. Descending paper chromatography on Whatman no. 1 paper, with butan-1-ol–acetic acid–water (50:12:25, by vol.) or 2-methylpropan-2-ol–formic acid–water (6:3:1, by vol.) as the mobile phase, showed a single component, identical in chromatographic mobility with authentic potassium L-serine *O*-sulphate. Paper electrophoresis on Whatman no. 1 paper in 0·1 M-ammonium acetate showed the material to be homogeneous and free from inorganic [^{35}S]sulphate.

It became apparent that this labelled material slowly liberated inorganic [^{35}S]sulphate on storage in the solid form at 0°, possibly by the process of self-irradiation. Sufficient inorganic [^{35}S]sulphate was formed during 5–6 weeks to warrant periodic removal before use. The material was dissolved in 10 ml. of ice-cold water, acidified with 5 ml. of 0·1 N-H_2SO_4, and a cold saturated solution of $Ba(OH)_2$ was added until the pH was slightly alkaline. Precipitated $Ba^{35}SO_4$ was removed and the ester recovered as described above.

Experimental animals and collection of urine and faeces. Guinea pigs and mice (C.E. strain) were mature animals and M.R.C. hooded rats were 3 months old. Animals were fed and housed and the urine and faeces collected as described by Dodgson & Tudball (1960).

Measurement of radioactivity. The procedures for the precipitation of ^{35}S-activity in the form of $Ba^{35}SO_4$ described by Dodgson & Tudball (1960) were used. Plating of $Ba^{35}SO_4$ samples was carried out according to the method of Lloyd (1961).

Detection of radioactivity on paper chromatograms and paper-electrophoresis strips. The qualitative procedure described by Dodgson & Tudball (1960) and the quantitative method of Dodgson & Tudball (1961) were employed.

EXPERIMENTAL AND RESULTS

Rats were injected intraperitoneally with 5 or 8 μmoles of potassium L-serine *O*[^{35}S]-sulphate in 0·5 ml. of water. Urine and faeces were collected at intervals of 24 and 48 hr. after the injection. Initial experiments showed that negligible amounts of ^{35}S-activity appeared in the excreta at time-intervals greater than 48 hr. after the administration of the dose. After 48 hr. the animals were killed and the residual ^{35}S in the carcass was determined. Table 1 records the results. No significant amounts of ^{35}S appeared in the neutral-sulphur fraction of urine and these results are not included.

With male and female rats receiving doses of 5 μmoles, a high percentage (over 91%) of the ^{35}S-activity in the urine was present in the inorganic sulphate fraction. With female rats receiving doses of 8 μmoles, the corresponding value was 81%.

Micro-organisms present in rat faeces can desulphate some sulphate esters, e.g. chondroitin [^{35}S]sulphate (Dohlman, 1956) and *N*-acetyl-D-glucosamine 6-*O*[^{35}S]-sulphate (Lloyd, 1961). A similar degradation could account, at least in part, for the inorganic [^{35}S]sulphate found in rat urine after the administration of L-serine *O*[^{35}S]-sulphate. Evidence that rat-faeces preparations are able to desulphate L-serine *O*[^{35}S]-sulphate was provided as follows. A 2% suspension of a 24 hr. faeces sample was prepared in 0·5 M-tris–acetic acid and the pH adjusted to that (pH 6·6) of a 24 hr. urine sample from the same animal. The suspension was then centrifuged for 30 min. at 3000*g* and 0°, and to 12 ml. of the supernatant were added 5 μmoles of L-serine *O*[^{35}S]-sulphate in 0·5 ml. of water and the whole kept at room temperature (20°). Duplicate 1 ml. samples were withdrawn at intervals up to 72 hr. and assayed for inorganic [^{35}S]sulphate as described above. Appropriate control determinations were made in which the substrate and faeces preparation were incubated separately and mixed immediately before the precipitation of the inorganic sulphate fraction. After 24 hr., 30% of the available ^{35}S-activity in the incubated faecal

Table 1. *Distribution of* ^{35}S *in the urine and carcass of rats injected intraperitoneally with potassium* L-*serine* O[^{35}S]-*sulphate*

Results are average values with the ranges in parentheses. ^{35}S was precipitated and counted as $Ba^{35}SO_4$ by the procedures described in the text. The radioactivity of the various doses corresponded to $1{\cdot}98 \times 10^4$ counts/min./μmole, measured after hydrolysis as an infinitely thick plate of $Ba^{35}SO_4$.

Sex	Dose (μmoles)	No. of animals	Range of body wt. (g.)	Distribution of ^{35}S in urine (% of ^{35}S recovered in urine)				Residual ^{35}S in carcass after 48 hr. (% of ^{35}S injected)	^{35}S recovered in 48 hr. faeces output (% of ^{35}S injected)	Total % of dose recovered
				Inorganic sulphate fraction		Combined inorganic and ester sulphate fraction				
				24 hr.	48 hr.	24 hr.	48 hr.			
Male	5	3	210	86·0 (83·2–91·8)	5·3 (4·1–6·8)	93·8 (91·8–96·1)	6·3 (4·5–8·1)	4·1 (3·5–4·8)	4·1 (2·6–5·7)	91·3 (87·1–95·6)
Female	5	3	200–220	88·7 (87·6–90·8)	4·6 (3·8–5·2)	95·2 (94·6–96·0)	4·7 (3·9–5·3)	4·8 (2·7–9·5)	7·2 (6·0–8·9)	91·9 (87·3–97·9)
Female	8	2	210–220	76·9 (75·9–77·8)	4·6 (4·2–5·0)	94·7 (94·7–94·8)	5·2 (5·1–5·3)	— —	4·1 (3·5–4·8)	— —
Male*	5	3	270	73·3 (71·5–75·6)	8·8 (7·6–10·3)	89·1 (87·3–90·9)	10·8 (9·1–12·7)	10·9 (10·7–11·1)	11·1 (7·5–14·0)	88·1 (87·2–89·2)

* Animals which have received terramycin and sulphasuxidine.

preparations appeared as inorganic [^{35}S]sulphate (Fig. 1). When the labelled ester was incubated under identical conditions with 24 hr. urine samples, less than 2·5 % of the available ^{35}S appeared as inorganic [^{35}S]sulphate after incubation for 24 hr.

The possibility therefore existed that the inorganic [^{35}S]sulphate present in urine after the administration of L-serine O[^{35}S]-sulphate was a result of the activity of faecal micro-organisms, either by contaminating the urine samples or by acting *in vivo* on L-serine O[^{35}S]-sulphate circulating between intestine and tissue via the bile. To check the first possibility the following experiment was performed. Five samples of L-serine O[^{35}S]-sulphate (each 5 μmoles in 0·5 ml. of water) were placed in individual collection vessels and normal rat urine was collected for 24 hr. in the usual way. Samples of urine were then analysed by the usual quantitative procedure, by quantitative paper electrophoresis and by paper chromatography. Less than 2 % of the available ^{35}S-activity appeared in the inorganic sulphate fraction of the urine and only L-serine O[^{35}S]-sulphate was detectable by paper chromatography and paper electrophoresis. As a check on the second possibility the effect of treating animals with antibiotics before the administration of the ^{35}S-labelled ester was examined. Lloyd (1961) has shown that the treatment of rats with terramycin and sulphasuxidine decreases the ability of faecal preparations from these animals to hydrolyse *N*-acetyl-D-glucosamine 6-O[^{35}S]-sulphate *in vitro* to negligible proportions and also diminishes the degree of hydrolysis of this ester *in vivo*. Rats were treated orally with 300 mg. of terramycin and 1·5 g. of sulphasuxidine over a period of 72 hr. before the injection of the labelled ester. Dodgson, Powell & Tudball (1960) reported

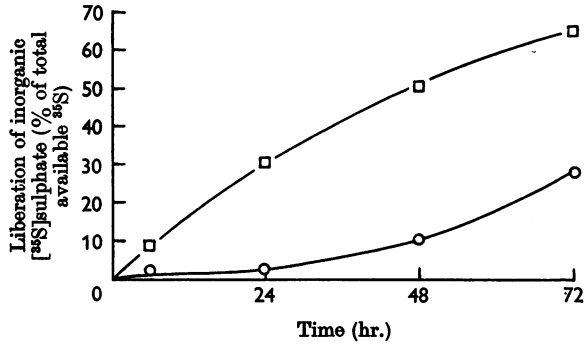

Fig. 1. Effect of time on the liberation of inorganic [^{35}S]-sulphate from potassium L-serine O[^{35}S]-sulphate. Experimental details are given in the text. □, Incubated at 20° with faeces preparation in 0·5 M-tris–acetic acid, pH 6·6: ○, incubated at 20° with urine sample, pH 6·6.

that this treatment decreased the direct microscopic count 100-fold and the number of viable organisms present in the faeces 900-fold. Although the average recovery of ^{35}S in these experiments (88%) was somewhat lower than that obtained with animals not pretreated in this way (91%), over 82% of the ^{35}S-activity recovered in the urine was present as inorganic [^{35}S]sulphate (Table 1).

Paper-chromatographic analysis of urine of experimental animals injected with L-*serine* O[^{35}S]-*sulphate*

Preliminary control experiments were carried out in which 24 hr. normal urine samples from male and female rats and mice were incubated at room temperature for 6, 12, and 24 hr. with either 5 μmoles of L-serine *O*[^{35}S]-sulphate (in 0·5 ml. of water) or with sodium [^{35}S]sulphate [0·1 ml. of a carrier-free solution (100 μc) plus 0·4 ml. of an unlabelled solution (0·15 M)]. Portions (30 μl.) of the incubated preparations were subjected to descending paper chromatography on Whatman no. 1 paper with butan-1-ol–acetic acid–water (50:12:25, by vol.) as solvent. No exchange of ^{35}S occurred between either L-serine *O*[^{35}S]-sulphate or sodium [^{35}S]sulphate and other sulphate esters present in the urine.

Portions (30 μl.) of 12 hr. urine samples voided by animals receiving L-serine *O*[^{35}S]-sulphate (2·5 μmoles/100 g. body wt.) or sodium ^{35}[S]sulphate (50 μc/100 g. body wt.) were subjected to descending paper chromatography on Whatman no. 1 paper with butan-1-ol–acetic acid–water (50:12:25, by vol.), and to two-way ascending chromatography with ethanol–water–urea (160:40:1, v/v/w) as first solvent and propan-1-ol–acetic acid–water (20:3:10, by vol.) as second solvent. Alternatively urine samples were initially dialysed and treated as described by Lloyd (1961).

Table 2 records the chromatographic mobilities of the ^{35}S-labelled compounds in the urines of rat and mouse after the administration of L-serine *O*[^{35}S]-sulphate or sodium [^{35}S]sulphate. In addition to large amounts of inorganic [^{35}S]sulphate, chromatograms of urines of animals dosed with L-serine *O*[^{35}S]-sulphate showed a complex pattern of ^{35}S-labelled compounds. This pattern was identical with that obtained with the urine of animals receiving sodium [^{35}S]sulphate and reflects the utilization of some of the liberated inorganic [^{35}S]sulphate for the synthesis of normal urinary sulphate esters (see also Dodgson & Tudball, 1960). Two-way chromatograms of both urines also showed identical distributions of ^{35}S-labelled materials. In experiments where it was possible to collect 3 and 6 hr. urine samples, the patterns obtained were still complex and similar to a 12 hr. urine pattern, showing the excretion of the ^{35}S-labelled materials to be a rapid process.

Experiments with guinea pigs showed a similar distribution of ^{35}S-activity after the administration of L-serine *O*[^{35}S]-sulphate or sodium [^{35}S]sulphate at dose levels similar to those used for rat and mouse. A urine sample collected 30 min. after the injection of L-serine *O*[^{35}S]-sulphate showed inorganic [^{35}S]sulphate to be the only ^{35}S-labelled material in the urine at this stage. The desulphation of L-serine *O*[^{35}S]-sulphate is thus an extremely rapid process.

Table 2. *Chromatographic mobilities in butan-1-ol–acetic acid–water (50:12:25, by vol.) of radioactive compounds present in the urine of either male or female rats receiving potassium* L-*serine* O[^{35}S]-*sulphate or sodium* [^{35}S]*sulphate*

Experimental details are given in the text. The R_F values quoted are the average values of five experiments.

	Rat		Mouse	
R_F	$Na_2{}^{35}SO_4$	L-serine *O*[^{35}S]-sulphate	$Na_2{}^{35}SO_4$	L-serine *O*[^{35}S]-sulphate
0·06	+	+	+	−
0·11*	+	+	+	+
0·15†	−	−	−	+
0·17	+	−	+	+
0·30	+	+	+	−
0·35	+	+	+	+
0·47	+	−	+	−
0·63	+	+	+	+
0·69	+	+	+	+
0·76	+	+	+	+

* R_F of inorganic [^{35}S]sulphate.
† R_F of L-serine *O*[^{35}S]-sulphate.

DISCUSSION

The administration of L-serine *O*[^{35}S]-sulphate to rats results in more than 75% of the administered dose appearing in the urine as inorganic [^{35}S]-sulphate within 24 hr. Rat-faeces preparations are also capable of desulphating L-serine *O*[^{35}S]-sulphate *in vitro*, but it seems unlikely from the studies with antibacterial agents that appreciable amounts of the inorganic [^{35}S]sulphate that is present in urine could arise from degradation of the ester by faecal organisms. Though the level of ^{35}S-activity associated with faecal sulphur is low when compared with urinary ^{35}S-activity, it is substantially higher than that found in faeces after the administration of other ^{35}S-labelled sulphate esters (cf. Dodgson & Tudball, 1960; Lloyd, 1961; Hawkins & Young, 1954). The relatively high level found in the faeces in the present series of experiments may well indicate a small biliary circulation of L-serine *O*[^{35}S]-sulphate.

Chromatographic examination of urines of rat

and mouse receiving L-serine *O*[^{35}S]-sulphate shows the presence of many ^{35}S-labelled materials (cf. Dodgson & Tudball, 1960). These materials almost certainly arise (via the intermediate formation of 3′-phosphoadenosine 5′-sulphatophosphate) from the inorganic [^{35}S]sulphate liberated *in vivo*.

Although there is a rapid liberation of inorganic [^{35}S]sulphate after the administration of L-serine *O*[^{35}S]-sulphate, it is not certain whether the desulphating enzyme system described by Dodgson & Tudball (1961) is responsible or whether other mechanisms may be involved. Thus deamination of the ester may yield the *O*-sulphate of hydroxypyruvic acid, from which inorganic [^{35}S]sulphate might be liberated spontaneously. Deamination of L-tyrosine *O*-sulphate has been shown to occur in the rat, but without the subsequent liberation of inorganic sulphate from the resulting *O*-sulphate ester of *p*-hydroxyphenylpyruvic acid (G. M. Powell, F. A. Rose & K. S. Dodgson, unpublished work). Ichihara & Greenberg (1957) have demonstrated the conversion of 3-phosphoserine into 3-phosphohydroxypyruvate by a transaminase system. L-Serine itself is involved in many metabolic transformations and it may well be that the liberation of inorganic [^{35}S]sulphate from L-serine *O*[^{35}S]-sulphate *in vivo* is not indicative of a simple desulphation reaction.

Several workers (e.g. Dodgson, Powell, Rose & Tudball, 1961*c*; Dodgson & Tudball, 1960; Hanahan & Everett, 1950; Hawkins & Young, 1954; Lloyd, 1961) have studied the metabolic fate of various ^{35}S-labelled sulphuric acid esters. The results, with the exception of those obtained for oestrone [^{35}S]sulphate (Hanahan & Everett, 1950), showed that only a limited hydrolysis of the ester occurred *in vivo*. In the majority of these studies the results obtained could have been predicted theoretically, since sulphatase enzymes which might have hydrolysed the esters were either absent or were known to have little affinity for the esters. However, an enzyme system is present in mammalian tissues which is extremely efficient in bringing about the hydrolysis of *p*-nitrophenyl sulphate *in vitro* (Dodgson, Rose & Tudball, 1959; Roy, 1960), though only some 25 % hydrolysis occurred after the administration of the ^{35}S-labelled ester to experimental animals (Dodgson & Tudball, 1960). In the light of these investigations the demonstration that 75 % of the dose of L-serine *O*[^{35}S]-sulphate appears in urine as inorganic [^{35}S]-sulphate within 24 hr. seems all the more remarkable. The present findings could indicate a process of some physiological importance, such as a mechanism for the protection of the metabolically reactive hydroxyl group of serine.

SUMMARY

1. After the intraperitoneal administration of potassium L-serine *O*[^{35}S]-sulphate to male and female rats most of the radioactivity appears in the urine within 24 hr.

2. Up to 75 % of the administered dose appears in the urine as inorganic [^{35}S]sulphate. Of the ^{35}S-activity recovered in the urine up to 92 % was in the inorganic sulphate fraction, the remainder being associated with the ester sulphate fraction.

3. Faecal preparations are capable of desulphating L-serine *O*[^{35}S]-sulphate *in vitro*, 30 % of the available ^{35}S appearing as inorganic [^{35}S]-sulphate in 24 hr. at 20°.

4. The administration of antibacterial agents to rats before the intraperitoneal injection of L-serine *O*[^{35}S]-sulphate results in a decrease in the level of ^{35}S-activity associated with the inorganic sulphate fraction.

5. After the administration of the ^{35}S-labelled ester, chromatographic examination of the urines of rat, mouse and guinea pig shows, in each case, a complex pattern of ^{35}S-labelled materials identical with that obtained after the injection of sodium [^{35}S]sulphate.

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