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The Metabolism of Potassium Dodecyl [^{35}S]Sulphate in the Rat

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The metabolic fate of potassium dodecyl [^{35}S]sulphate was studied in rats. Intraperitoneal and oral administration of the ester into free-ranging animals were followed by the excretion of the bulk of the radioactivity in the urine within 12hr., approximately 17% being eliminated as inorganic [^{35}S]sulphate. Similar results were obtained in experiments in which potassium dodecyl [^{35}S]sulphate was injected intravenously into anaesthetized rats with bile-duct and ureter cannulae. Analysis of urinary radioactivity revealed the presence of a new ester sulphate (metabolite A). This metabolite was isolated, purified and subsequently identified as the sulphate ester of 4-hydroxybutyric acid by paper, thin-layer and gas chromatography, by paper electrophoresis and by comparison of its properties with those of authentic butyric acid 4-sulphate. The identity of the metabolite was confirmed by isotope-dilution experiments. When either purified metabolite A or authentic potassium butyric acid 4[^{35}S]-sulphate was administered to free-ranging rats the bulk of the radioactivity was eliminated unchanged in the urine within 12hr., approx. 20% of the dose appearing as inorganic [^{35}S]sulphate. Whole-body radioautography and isolated-liver-perfusion experiments implicated the liver as the major site of metabolism of potassium dodecyl [^{35}S]sulphate. It is suggested that butyric acid 4-sulphate probably arises by ω-oxidation of dodecyl sulphate to a fatty acid-like compound, which is then degraded by β-oxidation.

Dodecyl sulphate is widely used commercially (Allen, 1962) because of its surface-active properties. As a result of its widespread use in industrial and domestic preparations a number of studies (e.g. Epstein, Throndson, Dock & Tainter, 1939; Cascorbi, Rudo & Lu, 1963; Walker, Brown, Ferrigan, Pickering & Williams, 1967) of the toxicity of the ester have been reported. Its biological degradability in sewage and industrial waste by micro-organisms has also received attention (e.g. Bogan & Sawyer, 1955, 1956; Okey, Cohen & Chapman, 1964; de Long, 1964), but no metabolic studies with identification of the products of degradation have been reported. Further, no study of the metabolic fate of dodecyl sulphate appears to have been carried out in mammals.

The present work on the metabolism of potassium dodecyl [^{35}S]sulphate in rats shows that the ester undergoes relatively little desulphation, but is metabolized to yield a major product that has been identified as the sulphate ester of 4-hydroxybutyric acid.

MATERIALS AND METHODS

Potassium dodecyl [^{35}S]*sulphate.* This was prepared by a modification of the method of Kloubeck & Kondelik (1963). Dodecan-1-ol was obtained from British Drug Houses Ltd. (Poole, Dorset) and distilled (b.p. 257–261°) before use. [^{35}S]Sulphuric acid (0·290g., specific radioactivity 6·4mc/m-mole, obtained from The Radiochemical Centre, Amersham, Bucks.) was added dropwise with stirring to a cooled (−15°) solution of dodecan-1-ol (0·55g.) in 10ml. of light petroleum (b.p. 40–60°). After continuous stirring over a period of 4hr. at −15° the whole mixture was allowed to attain room temperature. Thionyl chloride (0·350g.) was added to remove water produced during the reaction and the resultant SO_2 and HCl were removed in a stream of N_2. Light petroleum was removed by evaporation *in vacuo* at room temperature, the residual gum was dissolved in dry methanol (25ml.) and the solution was neutralized with KOH (5%, w/v, in dry methanol) with ethanolic phenolphthalein as external indicator. Precipitated K_2SO_4 was removed by centrifuging and washed with 40ml. of methanol at 50°. The washings and supernatant were combined and, after the addition of 10vol. of ether, the mixture was stored overnight at 4°. Precipitated potassium dodecyl [^{35}S]sulphate was collected by centrifuging, washed with ether (total volume 20ml.) and recrystallized twice from boiling water before being dried *in vacuo* over P_2O_5. The yield was 300mg. and the specific radioactivity 6·4mc/m-mole. Thin-layer chromatography on silica gel G with solvent *A*, butan-1-ol–acetic acid–water (4:1:1, by vol.) (R_F of the product 0·69), and with solvent *B*, benzene–butan-2-one–ethanol–water (1:1:2:1, by vol.) (R_F of the product 0·73), showed the product to be homogeneous and free from inorganic [^{35}S]sulphate. Infrared analysis (see below) showed the product to have an

absorption spectrum identical with that of authentic dodecyl sulphate (Chihara, 1960).

Potassium dodecyl [^{35}S]sulphate was stored as a frozen aqueous 0·1% solution. Under these conditions the ester was found to be stable and no breakdown due to self-irradiation processes was observed.

Potassium butyric acid 4-sulphate. Pyridine–SO_3 reagent (1·3g.), prepared by the method of Baumgarten (1926), was mixed with sodium 4-hydroxybutyrate (1g.; Sigma Chemical Co., St Louis, Mo., U.S.A.) and stirred in dry pyridine (50ml.) for 3hr. at 50°. Pyridine was removed *in vacuo* at 40° and the residue was dissolved in dry methanol (20ml.). The mixture was neutralized with KOH (5%, w/v, in dry methanol), and the precipitate was collected by centrifuging and extracted with methanol at 55° (total volume 80ml.). The combined methanol extracts were concentrated *in vacuo* to a final volume of 50ml., and ethanol at 4° was added until the solution became turbid. After 18hr. the precipitated potassium butyric acid 4-sulphate was separated by centrifuging, washed with ethanol (total vol. 20ml.) and dried *in vacuo* over P_2O_5 at room temperature. The yield was 800mg. (Found: C, 18·7; H, 2·5; K, 28·8; S, 11·8; ester sulphate, 35·4; $C_4H_6K_2O_6S$ requires C, 18·5; H, 2·3; K, 30·0; S, 12·3; ester sulphate, 36·9%).

Further evidence that *O*-sulphation had occurred was provided by infrared spectroscopy. Strong absorption bands were present in the ranges 1210–1250 and 770–810cm.$^{-1}$. There was no absorption band at 3320cm.$^{-1}$ characteristic of the unsubstituted hydroxyl group, showing the preparation to be free from the parent alcohol.

The product was shown to be homogeneous by descending paper chromatography on Whatman no. 1 paper with solvent *A* and with solvent *C*, isobutyric acid–0·5N-NH_3 (5:3, v/v); its R_F in solvent *A* was 0·24 and in solvent *C* 0·36; and by thin-layer chromatography with solvent *A* (R_F 0·45), with solvent *D*, butan-1-ol–acetic acid–water (3:1:1, by vol.) (R_F 0·49), and with solvent *E*, propan-2-ol–water (3:2, v/v) (R_F 0·68).

A ^{35}S-labelled sample of potassium butyric acid 4-sulphate was prepared as described above except that pyridine–$^{35}SO_3$ (0·41g.) was mixed with 0·315g. of sodium 4-hydroxybutyrate and stirred in 25ml. of dry pyridine. The yield was 66mg. and the specific radioactivity 3·3mc/m-mole. Potassium butyric acid 4[^{35}S]-sulphate was stored as a frozen aqueous 0·2% solution. The ester was used as soon as possible after preparation since analysis showed breakdown of the ester (20·2% in 87 days), probably due to self-irradiation processes.

Other materials. γ-Butyrolactone (puriss) was obtained from Koch-Light Laboratories Ltd. (Colnbrook, Bucks.). The potassium salts of propionic acid 3-sulphate and glycollic acid sulphate were kindly provided by Dr N. Tudball.

Chromatography. Paper chromatograms were usually run overnight on Whatman no. 1 paper, and thin-layer chromatograms on silica gel G were run over a distance of 12cm. Sulphate esters were detected on paper chromatograms with rhodizonate reagent by the method of Schneider & Lewbart (1956) and on thin-layer chromatograms with fluorescein (0·2%, w/v, in ethanol).

Radioactive areas on chromatograms were detected either by radioautography on Ilford Industrial B X-ray film for 7–18 days, or by scanning with a Packard Radiochromatogram Scanner (model 7200). The relative amount of radioactivity associated with each spot was measured from the record of the scanner as described by Jones & Dodgson (1965).

Gas-chromatographic analysis was carried out with a Perkin–Elmer F-11 gas chromatograph fitted with dual flame-ionization detectors. The glass columns (6ft.× 0·25in.) were packed with diethylene glycol succinate on hexamethyldisilazane-treated Chromosorb W (80–100 mesh) (3:22, by wt.). All samples were examined isothermally at 130° with the injection-point heaters at 240°. The rate of flow of N_2 carrier gas was 22ml./min.

Paper electrophoresis. Compounds were run horizontally as spots on Whatman no. 1 paper with 0·1M-sodium acetate–acetic acid buffer, pH4·5, for 1·5hr. at a potential gradient of 13v/cm. Radioactive areas were located by radioautography.

Infrared spectroscopy. The spectra of the esters were determined with a Perkin–Elmer 137 Infracord recording spectrophotometer, the esters being examined as mulls in Nujol (liquid paraffin).

Experimental animals. Adult male and female M.R.C. hooded rats were used throughout unless otherwise stated. Intraperitoneal injections of the esters were carried out while the animals were under ether anaesthesia. The animals were placed in metabolism cages that permitted separate collection of urine and faeces, and were allowed water without restriction and food after 24hr. Urine samples were collected after 6, 12, 24 and 48hr., funnel washings being added to the appropriate sample.

In some experiments, the esters were administered by stomach tube to rats under light ether anaesthesia, and urine and faeces were collected as described. Similar experiments were made in which animals had received prior treatment with antibiotics (see Dodgson, Powell, Rose & Tudball, 1961).

In experiments on rats with bile-duct and ureter cannulae, animals were anaesthetized with ether, the jugular vein was cannulated and Nembutal (10–15mg./kg. body wt.) was administered by the cannula. The bile duct and ureters were cannulated and bile and urine samples collected at suitable times after administration of the ester by the jugular-vein cannula. Animals were maintained under Nembutal anaesthesia throughout the experiments.

Whenever possible, urine and bile samples were assayed for radioactivity immediately; otherwise they were stored at 4° until required.

Measurement of radioactivity in biological materials. The determination of inorganic [^{35}S]sulphate (as $Ba^{35}SO_4$) and total [^{35}S]sulphate (as $Ba^{35}SO_4$ after acid hydrolysis) contents of urine and bile samples was as described by Flynn, Dodgson, Powell & Rose (1967). In experiments with potassium dodecyl [^{35}S]sulphate and potassium butyric acid 4[^{35}S]-sulphate, hydrolysis times were 22hr. and 16hr. respectively. Time–hydrolysis curves showed complete hydrolysis of the esters after these times. When inorganic [^{35}S]sulphate had to be precipitated in the presence of dodecyl [^{35}S]sulphate, the $Ba^{35}SO_4$ precipitate was washed with two 25ml. lots of methanol at 50° before being washed with acetone. This was necessary to remove the barium salt of dodecyl [^{35}S]sulphate, which tended to be precipitated with the $Ba^{35}SO_4$. The total ^{35}S contents of faeces and of the whole rat carcass were precipitated and

counted as $Ba^{35}SO_4$ after oxidation with fuming HNO_3 by the method of Young, Edson & McCarter (1949).

Liver perfusion. Isolated rat livers were perfused with blood containing potassium dodecyl [^{35}S]sulphate by the method of Flynn *et al.* (1967).

Whole-body radioautography. Young M.R.C. hooded rats (30–50g. body wt.), under light ether anaesthesia, each received an intraperitoneal injection of potassium dodecyl [^{35}S]sulphate (0·5mg. dissolved in 0·5ml. of water). Rats were killed at 5min., 15min., 30min., 1hr., 2hr. and 4hr., and whole-body radioautograms were prepared by the method of Powell, Curtis & Dodgson (1967).

EXPERIMENTAL AND RESULTS

Free-ranging male and female rats were injected intraperitoneally with potassium dodecyl [^{35}S]-sulphate (1mg. dissolved in 1ml. of water). Urine was collected after 6, 12, 24 and 48hr. and faeces were collected over the 48hr. period. At the end of this period the animals were killed and their carcasses taken for the determination of residual ^{35}S. Only traces of radioactivity were associated with faeces and carcasses, and the bulk of the radioactive dose was recovered in the urine within the first 12hr. (Table 1). The bulk of the urinary radioactivity appeared as ester sulphate in urine from both male and female rats, although some of the radioactivity was present as inorganic [^{35}S]-sulphate. Similar results were obtained in experiments in which male rats received potassium dodecyl [^{35}S]sulphate orally (Table 1).

The relatively small amounts of inorganic [^{35}S]-sulphate found in the urine could result from biliary circulation and subsequent desulphation of the ester by the intestinal flora. Further experiments were therefore carried out in which rats that had been pretreated with antibiotics to eliminate gut flora received the ester orally. The results (Table 1) showed that the intestinal flora do not play a significant role in the metabolism of potassium dodecyl [^{35}S]sulphate, since the distribution of radioactivity in urine, faeces and carcasses was substantially the same as that previously recorded.

Potassium dodecyl [^{35}S]sulphate was also injected intravenously into rats with bile-duct and ureter cannulae. Urine samples were collected at hourly intervals over a period of 12hr. and bile samples were collected at 2hr. intervals. Table 2 records the results but does not include values for the radioactivity content of bile, since the mean recovery of radioactivity in bile over 12hr. was only 1·4% (0·7% as inorganic [^{35}S]sulphate). The greater part of the dose was excreted in the urine within the first 2hr. and virtually the whole of the dose within the first 4hr., 9·4–23·8% of the dose being excreted as inorganic [^{35}S]sulphate.

Table 1. *Distribution of ^{35}S in the urine, faeces and carcass of rats receiving potassium dodecyl [^{35}S]sulphate (1mg. dissolved in 1ml. of water)*

The results are mean values for the numbers of animals stated after 'male' or 'female', with the ranges in parentheses. For experimental details see the text.

Intraperitoneal administration	Time (hr.)	Distribution of ^{35}S in urine (% of ^{35}S injected)								^{35}S recovered in faeces (% of ^{35}S injected)	^{35}S recovered in carcass (% of ^{35}S injected)	Total ^{35}S recovered (% of ^{35}S injected)
		Total sulphate fraction				Inorganic sulphate fraction						
	...	6	12	24	48	6	12	24	48			
Male (6)		49·7	27·3	7·8	1·5	7·2	5·1	1·5	0·6	0·2*	0·4*	86·9
		(41·1–62·2)	(19·6–34·2)	(3·0–11·1)	(0·1–3·6)	(5·1–12·2)	(3·6–6·5)	(1·1–2·5)	(0·05–0·9)	(0·1–0·3)	(0·3–0·5)	(81·7–89·2)
Female (6)		66·6	19·6	5·4	1·6	11·5	4·1	1·8	0·7	0·9*	0·4*	94·4
		(53·1–82·0)	(4·2–39·4)	(2·2–8·1)	(0·8–2·2)	(6·5–17·5)	(2·0–7·8)	(1·4–2·4)	(0·4–1·0)	(0·7–1·2)	(0·4–0·4)	(83·1–98·5)
Oral administration												
Male (3)		55·5	39·9	2·3	1·0	9·9	6·6	0·9	0·5	0·7	0·4	99·8
		(10·4–85·4)	(10·4–81·7)	(2·0–2·7)	(0·9–1·1)	(2·0–14·0)	(1·9–13·4)	(0·7–1·1)	(0·4–0·6)	(0·5–0·9)	(0·3–0·6)	(96·7–102·3)
Male (3)†		61·0	33·1	11·1	1·7	8·5	6·7	2·4	0·4	0·5	0·4	107·8
		(55·9–86·0)	(16·3–60·5)	(3·5–26·2)	(0·9–1·3)	(6·8–11·9)	(4·1–11·4)	(1·0–5·2)	(0·3–0·5)	(0·1–1·0)	(0·3–0·6)	(106·7–108·0)

* Mean of three values.
† Animals pretreated with antibiotics.

Table 2. *Excretion of* ^{35}S *in the urine after intravenous injection of* 1 *mg. of potassium dodecyl* [^{35}S]*sulphate into anaesthetized rats with bile-duct and ureter cannulae*

For experimental details see the text.

	Distribution of ^{35}S in urine (% of ^{35}S injected)							
Time (hr.) ...	1	2	3	4	5	6	7–12	Total
Animal 1 (male)								
Total sulphate	32·9	42·5	12·1	5·5	2·3	1·4	2·7	99·4
Inorganic sulphate	7·9	6·8	2·2	1·3	0·8	0·5	1·2	20·7
Animal 2 (male)								
Total sulphate	27·5	43·6	12·8	2·8	1·3	1·2	2·6	91·8
Inorganic sulphate	6·6	12·8	3·1	1·3	0·6	0·6	1·3	26·3
Animal 3 (female)								
Total sulphate	45·0	32·0	7·0	3·3	2·2	0·9	2·8	93·2
Inorganic sulphate	3·9	3·5	1·3	0·9	0·7	0·3	1·0	11·6
Animal 4 (female)								
Total sulphate	60·3	26·1	6·5	3·4	1·7	0·7	2·8	101·5
Inorganic sulphate	3·5	3·2	1·9	0·8	0·4	0·2	1·1	11·1

Qualitative examination of ^{35}S *in urine of animals receiving potassium dodecyl* [^{35}S]*sulphate*

Free-ranging rats received intraperitoneal injections of potassium dodecyl [^{35}S]sulphate as before and urine was collected after 6 and 12 hr. Urine samples were made up to 100 ml. with water and portions (20 μl.) were applied to Whatman no. 1 paper and subjected to electrophoresis. Portions (20 μl.) were also applied to thin-layer silica-gel plates and subjected to chromatography with solvents *A* and *B*. The following controls were run on the same chromatograms and electrophoretograms as the test urine: normal rat urine to which potassium dodecyl [^{35}S]sulphate had been added, normal rat urine to which inorganic [^{35}S]sulphate had been added, test urine to which potassium dodecyl [^{35}S]sulphate had been added and test urine to which inorganic [^{35}S]sulphate had been added.

The electrophoretograms of test urine showed the presence of one major radioactive component and one relatively minor component (15% of the total radioactivity on the electrophoretogram), the mobility of which was identical with that of inorganic [^{35}S]sulphate. The major component (metabolite A) was not potassium dodecyl [^{35}S]-sulphate, and no trace of the latter could be detected. Similarly, in chromatograms of test urine, two radioactive spots were detected; one of these (the minor component) was chromatographically identical with inorganic [^{35}S]sulphate, and the major component (R_F 0·45 in solvent *A* and 0·31 in solvent *B*) was chromatographically distinct from potassium dodecyl [^{35}S]sulphate (R_F 0·69 in solvent *A* and 0·73 in solvent *B*), which was not detected in test urines. Identical observations were made when urine obtained from animals receiving oral or intravenous injections of potassium dodecyl [^{35}S]-sulphate was subjected to thin-layer chromatography.

Purification of metabolite A

Ten male rats were each injected intraperitoneally with potassium dodecyl [^{35}S]sulphate (1 mg. dissolved in 1 ml. of water); urine was collected over 24 hr. and pooled together with funnel washings (total volume approx. 400 ml.). Thin-layer chromatography (solvent *A*) and subsequent scanning of a sample (20 μl.) of the pooled urine revealed that metabolite A represented approx. 75% of the injected radioactivity. The urine was passed through a column (7·0 cm. × 1·1 cm.) of Dowex 1 ion-exchange resin (X4; 50–100 mesh; Cl^- form) at a flow rate of about 60 ml./hr., and all the radioactivity was retained by the resin. The column was eluted with 0·1 M-potassium chloride (15 ml.) at a flow rate of approx. 30 ml./hr. Successive elution with 15 ml. portions of 0·2 M-, 0·4 M-, 0·6 M-, 0·8 M- and 1·0 M-potassium chloride was carried out. The bulk of the radioactivity (90%) was found in the 0·6 M- and 0·8 M-potassium chloride eluates, and these eluates were combined and concentrated to about 10 ml. *in vacuo* at 37°. This solution was applied to a column (50·0 cm. × 1·2 cm.) of Sephadex G-10, which was subsequently eluted with water at a flow rate of 25 ml./hr. The void volume of the column was discarded and successive fractions (0·5 ml.) were collected and checked for radioactivity and the presence of Cl^- ion. Fractions that contained radioactivity and were essentially free from Cl^- ions (usually fractions 14–28) were combined and concentrated *in vacuo* at 37° to

approx. 1 ml. This solution was applied, as a streak, to a thick-layer plate (thickness 1·25 mm.) of silica gel G, which was developed in solvent *E*. Metabolite A was located by radioautography (4 hr. exposure) and the appropriate area of silica gel was removed and extracted with water (15 ml.). After centrifugation to remove silica gel, the eluate was concentrated to 4 ml. *in vacuo* at 37° and insoluble material was removed by centrifuging. Thin-layer chromatography with solvent *A* showed the solution to contain metabolite A as the single radioactive component. The solution was applied to a column (40·0 cm. × 1·2 cm.) of Sephadex G-25 (fine grade), and metabolite A was eluted with water at a flow rate of approx. 25 ml./hr. The void volume of the column was discarded and successive fractions (0·5 ml.) were collected and assayed for radioactivity. Fractions containing metabolite A were combined (usually fractions 22–36, total vol. 7·5 ml.) and stored at 4°. About 60% of the radioactive ester sulphate fraction of the original urine was present in the final solution of metabolite A. This solution contained a single radioactive component when subjected to thin-layer chromatography in solvents *A*, *B* and *E*.

The possible presence of non-radioactive contaminants in the preparation was investigated by spraying a series of chromatograms with concentrated sulphuric acid, phosphomolybdic acid in ethanol (0·5%, w/v), ninhydrin in acetone (0·2%, w/v) or ethanolic naphtharesorcinol (0·2% solution)–orthophosphoric acid (22:1, v/v) reagent (Randerath, 1963). After treatment with concentrated sulphuric acid, a single spot, coinciding with the radioactive component, was rendered visible on all the chromatograms. No components were revealed with the other sprays, showing that the metabolite was not contaminated with lipid, protein or carbohydrate in detectable amounts.

The solution containing metabolite A is referred to as purified metabolite A.

Identification of metabolite A

Thin-layer and paper chromatography. Purified metabolite A was applied as spots (20 μl.) to silica-gel plates together with the following authentic markers: potassium butyric acid 4-sulphate, potassium propionic acid 3-sulphate and potassium glycollic acid sulphate. After development with solvents *A*, *D* and *E*, radioautograms were prepared and the esters were subsequently rendered visible on the chromatograms with the fluorescein reagent. In all solvent systems the radioactive spot (metabolite A) coincided with the coloration produced by the fluorescein reagent. Further, the mobility of metabolite A was identical with that of potassium butyric acid 4-sulphate and distinct from the mobilities of the other marker ester sulphates (see Table 3).

Further experiments were carried out in which purified metabolite A was applied as spots (20 μl.) to Whatman no. 1 paper together with the authentic markers previously used. Chromatograms were developed with solvents *A* and *C*. Radioautograms were prepared and the esters were rendered visible by the rhodizonate reagent. The radioactive spot (metabolite A) coincided with a rhodizonate-positive spot. The mobility of metabolite A again coincided with that of potassium butyric acid 4-sulphate (see Table 3).

Gas chromatography. Purified metabolite A (1 ml.) was diluted to 15 ml. with water and mixed with 5 ml. of 4 N-hydrochloric acid. The solution was heated at 110° for 16 hr. (a time–hydrolysis curve for metabolite A showed complete hydrolysis after 16 hr.) after which it was extracted with four 20 ml. lots of benzene. The benzene extract was concentrated *in vacuo* at room temperature to about 20 μl. and portions (1 μl.) were applied through the rubber septum directly into the chromatographic column. The following controls (1 μl. portions in each case) were also subjected to the analytical procedure: (*a*) benzene (80 ml.) evaporated to a volume of approx. 20 μl.; (*b*) a benzene extract (four 20 ml. lots, evaporated to approx. 20 μl.) of unhydrolysed purified metabolite A (1 ml. diluted to 15 ml. with water). For purposes of comparison, authentic γ-butyrolactone (1 ml.) was mixed with benzene (9 ml.) and portions (1 μl.) were analysed by gas chromatography.

Three major peaks were obtained with the

Table 3. *Chromatography of metabolite A and authentic standards*

Details of the solvent systems are given in the text.

		R_F (paper chromatography)		R_F (thin-layer chromatography)		
Compound	Solvent ...	*A*	*C*	*A*	*D*	*E*
Metabolite A		0·24	0·36	0·45	0·49	0·68
Butyric acid 4-sulphate		0·24	0·36	0·45	0·49	0·68
Propionic acid 3-sulphate		0·20	0·26	0·40	0·46	0·63
Glycollic acid sulphate		0·14	0·17	0·23	0·30	0·61

benzene extract of hydrolysed metabolite A. Two of these peaks were derived from the benzene, since they were also obtained with the controls (*a*) and (*b*). The remaining peak had a retention time (8·9min.) identical with that of γ-butyrolactone. This peak was reinforced when γ-butyrolactone in benzene was added to the concentrated benzene extract of hydrolysed metabolite A before gas chromatography.

These findings collectively demonstrated that the product of acid treatment of metabolite A was γ-butyrolactone and indicated that metabolite A itself was butyric acid 4[^{35}S]-sulphate. This possibility was explored by gas-chromatographic analysis of potassium butyric acid 4-sulphate. The ester (20mg.) was dissolved in 15ml. of water and mixed with 5ml. of 4N-hydrochloric acid. The mixture was heated at 110° for 16hr. and extracted with four 20ml. lots of benzene, and the extract was concentrated to approx. 1ml. Gas-chromatographic analysis of portions (1μl.) revealed a peak with a retention time identical with that obtained with γ-butyrolactone.

Isotope-dilution experiments. Potassium butyric acid 4-sulphate (100mg.) was dissolved in a solution of purified metabolite A (3ml.). The solution was warmed to 50°, ethanol was added until turbidity was incipient, and the solution was cooled to 0°. The crystals were separated by centrifuging and dried *in vacuo* over phosphorus pentoxide. A sample (1mg.) was retained and the remainder was recrystallized a further six times, a 1mg. sample being retained after each recrystallization. Each sample was dissolved in approx. 15ml. of water, 4N-hydrochloric acid (5ml.) was added and the whole was heated to 110° for 16hr. After cooling, $Ba^{35}SO_4$ was precipitated and washed, and the radioactivity was determined. Within the limits of experimental error, material of constant specific radioactivity was obtained after the first recrystallization.

These experiments established the identity of metabolite A as the ^{35}S-labelled sulphate ester of 4-hydroxybutyric acid.

Injection of butyric acid 4[^{35}S]-sulphate

Potassium dodecyl [^{35}S]sulphate was metabolized in the rat to yield a single ester sulphate that was identified as butyric acid 4[^{35}S]-sulphate. The urine of animals receiving potassium dodecyl [^{35}S]-sulphate also contained some inorganic [^{35}S]sulphate. The origin of this inorganic [^{35}S]sulphate and the establishment of butyric acid 4[^{35}S]-sulphate as the metabolic end product from dodecyl [^{35}S]sulphate were investigated in experiments in which rats received authentic potassium butyric acid 4[^{35}S]-sulphate.

Table 4. *Distribution of ^{35}S in the urine, faeces and carcass of male rats injected intraperitoneally with either butyric acid 4[^{35}S]-sulphate or purified metabolite A*

The doses were: butyric acid 4[^{35}S]-sulphate, 1mg. dissolved in 0·5ml. of water; metabolite A, 0·7ml. of the purified preparation. The results are mean values for three animals with each compound, with the ranges in parentheses. For experimental details see the text.

Compound	Time (hr.)	Distribution of ^{35}S in urine (% of ^{35}S injected)								^{35}S recovered in faeces (% of ^{35}S injected)	^{35}S recovered in carcass (% of ^{35}S injected)	Total ^{35}S recovered (% of ^{35}S injected)
		Total sulphate fraction				Inorganic sulphate fraction						
		6	12	24	48	6	12	24	48			
Butyric acid 4[^{35}S]-sulphate		88·1 (76·9–98·0)	9·2 (3·2–20·6)	1·1 (1·0–1·1)	0·7 (0·7–0·8)	20·6 (17·8–25·4)	2·9 (1·1–5·9)	0·3 (0·2–0·3)	0·2 (0·1–0·2)	0·0	0·1 (0·0–0·2)	99·1 (94·3–103·6)
Metabolite A		99·7 (99·0–100·0)	4·0 (2·6–5·0)	1·0 (0·6–1·9)	0·6 (0·4–0·8)	19·2 (13·0–21·0)	1·5 (1·3–1·6)	0·3 (0·1–0·7)	0·2 (0·1–0·3)	0·2 (0·2–0·2)	0·2 (0·2–0·3)	105·7 (102·7–107·7)

Three male free-ranging rats each received 1 mg. of potassium butyric acid 4[^{35}S]-sulphate dissolved in 0·5 ml. of water. Urine was collected over 6, 12, 24 and 48 hr. and faeces were collected over the 48 hr. period. At the end of this time the animals were killed and the carcasses taken for the determination of residual ^{35}S. Radioactivity could not be detected in faeces and only trace amounts in carcasses. The bulk of the radioactivity (up to 74%) appeared in the urine as ester sulphate within 12 hr. Approx. 23·5% of the injected radioactivity was present as inorganic [^{35}S]sulphate (Table 4). This value falls within the range recorded with rats receiving potassium dodecyl [^{35}S]sulphate, and suggests that the inorganic [^{35}S]sulphate appearing in the urine of rats receiving this compound may have originated from its metabolic end product, butyric acid 4[^{35}S]-sulphate.

Samples of the urine collected in these experiments were subjected to thin-layer chromatography with solvent *A*. The following controls were run on the same chromatograms as the test urine: normal rat urine to which potassium butyric acid 4[^{35}S]-sulphate had been added, normal rat urine to which inorganic [^{35}S]sulphate had been added, test urine to which potassium butyric acid 4[^{35}S]-sulphate had been added and test urine to which inorganic [^{35}S]sulphate had been added. Chromatograms of test urine showed the presence of one major radioactive component that was chromatographically identical with butyric acid 4[^{35}S]-sulphate and a minor component chromatographically identical with inorganic [^{35}S]sulphate.

Identical experiments were also carried out in which three male free-ranging rats each received intraperitoneal injections of purified metabolite A (0·7 ml.). The results obtained (Table 4) were in close agreement with those recorded when animals received potassium butyric acid 4[^{35}S]-sulphate. When the urine was chromatographed, butyric acid 4[^{35}S]-sulphate and inorganic [^{35}S]sulphate only were detected.

Whole-body radioautography

The most striking feature of the whole-body radioautograms was the cellular accumulation of radioactivity in the liver. Thus radioautograms of animals killed 5 min. after injection showed significant amounts of radioactivity within the liver, although a considerable amount of the ^{35}S remained in the intraperitoneal cavity, the site of injection. The concentration of radioactivity in the liver increased up to 30 min. and then gradually declined, and only trace amounts remained after 4 hr. In all the radioautograms only traces of radioactivity were associated with the circulating blood. Apart from the liver, the kidney was the only other organ in which any appreciable cellular accumulation was recorded.

These observations demonstrated that, after the administration of dodecyl [^{35}S]sulphate, liver and kidney were the only organs that became labelled to any significant extent. The appearance of radioactivity in the kidney might reflect the urinary excretion of ^{35}S, and the ability of the cells of the liver to accumulate radioactivity might implicate the liver as the major site of metabolism of dodecyl [^{35}S]sulphate.

Liver-perfusion experiments

The experiments described above indicated that potassium dodecyl [^{35}S]sulphate was metabolized in the liver in the intact animal. To confirm this, isolated rat livers were perfused with blood containing 10 mg. of potassium dodecyl [^{35}S]sulphate. A sample of the perfusate was withdrawn from the system before the insertion of the liver and incubated at 37° for the duration of the experiment. No hydrolysis occurred in this control sample. After insertion of the liver, samples of blood (0·5 ml.) were collected at zero time, after 15 min., 30 min. and 1 hr., and after every following hour, and bile samples (approx. 0·6 ml.) were collected hourly. The blood samples were centrifuged to remove erythrocytes; preliminary experiments showed that no significant amounts of radioactivity sedimented with the erythrocytes. Portions (5 μl.) of the supernatants were subjected to thin-layer chromatography in solvent *A*. The blood sample withdrawn at zero time contained one radioactive component only, which was chromatographically identical with dodecyl [^{35}S]sulphate. After 15 min. small amounts (approx. 8% of the circulating radioactivity) of butyric acid 4[^{35}S]-sulphate were detected; after 1 hr. the bulk (77%) of the radioactivity was associated with butyric acid 4[^{35}S]-sulphate and only small amounts (10%) of the original ester could be detected. After 2 hr. dodecyl [^{35}S]sulphate could not be detected. Inorganic [^{35}S]sulphate was detected on chromatograms of samples withdrawn after 30 min. and represented 10% of the circulating radioactivity. This concentration increased to approx. 20% after 2 hr. These findings collectively demonstrated that the isolated liver was capable of metabolizing potassium dodecyl [^{35}S]sulphate. The rapid disappearance of the ester from the circulating blood was paralleled by the appearance of butyric acid 4[^{35}S]-sulphate. Furthermore, inorganic [^{35}S]-sulphate production was dependent on the prior appearance of butyric acid 4[^{35}S]-sulphate, thus providing further evidence that inorganic [^{35}S]-sulphate is derived from this ester. About 3% of the circulating radioactivity was eliminated in the bile over the 6 hr. period.

DISCUSSION

Quantitative and qualitative experiments show that potassium dodecyl [^{35}S]sulphate is extensively metabolized in the rat to yield a single ester sulphate that has been identified as butyric acid 4[^{35}S]-sulphate. The metabolic fate of potassium dodecyl [^{35}S]sulphate is not affected by the route of administration. When potassium butyric acid 4[^{35}S]-sulphate is injected into rats, it is eliminated largely unchanged in the urine. Some inorganic [^{35}S]sulphate also appears in the urine after the injection of this ester; the amounts are comparable with those found in the urine of animals receiving potassium dodecyl [^{35}S]sulphate. These findings suggest that, when potassium dodecyl [^{35}S]sulphate is administered to rats, it is initially metabolized to butyric acid 4[^{35}S]-sulphate, which may then be desulphated with the liberation of inorganic [^{35}S]sulphate. This metabolic route for the degradation of potassium dodecyl [^{35}S]sulphate is supported by isolated-liver-perfusion experiments in which the production of inorganic [^{35}S]sulphate is preceded by the appearance of butyric acid 4[^{35}S]-sulphate. Further, isolated-liver-perfusion studies, together with whole-body radioautography, strongly implicate the liver as the site of metabolism of potassium dodecyl [^{35}S]sulphate.

Previous work on the degradation of long-chain hydrocarbons and their derivatives by micro-organisms (e.g. Foster, 1962; Treccani, 1963) has shown that these compounds are degraded by a process involving initial ω-oxidation followed by β-oxidation of the fatty acids so produced. Similar degradative pathways have been reported in mammals, and enzyme systems involved in the ω-oxidation of fatty acids and their derivatives have been found in liver (Wakabayashi & Shimazono, 1963; Preiss & Bloch, 1964) and kidney (Robbins, 1968). The mechanism of ω-oxidation apparently involves first the conversion of the monocarboxylic acid into the ω-hydroxy acid by direct hydroxylation of the ω-methyl group. Subsequently the ω-hydroxy acid is converted into the oxo acid and finally into the dicarboxylic acid, which is then degraded by β-oxidation (Den, 1965). It is tentatively suggested that potassium dodecyl [^{35}S]sulphate is metabolized by an analogous series of reactions involving initially the oxidation of the terminal methyl group with the formation of 12-hydroxydodecyl [^{35}S]sulphate. This is then further oxidized to the 12-oxo derivative and finally to the corresponding carboxylic acid. This substituted fatty acid could then undergo oxidation, analogous to normal β-oxidation, with the successive elimination of C_2 fragments. The final product of degradation of potassium dodecyl [^{35}S]sulphate is butyric acid 4[^{35}S]-sulphate, and further β-oxidation of this ester to glycollic acid sulphate does not occur. The reason for this is not apparent.

Since dodecyl sulphate is extensively used in a number of industrial and domestic preparations these studies on the metabolism of the ester have a number of implications. It is shown that the ester is extensively metabolized in rats to yield butyric acid 4-sulphate, and the same degradative pathway may operate in both micro-organisms and in man. Rigorous studies on the toxicity of dodecyl sulphate have been carried out, but there is no available information on the relative toxicity of butyric acid 4-sulphate. Further, the present studies have shown that some degradation of butyric acid 4-sulphate with the liberation of inorganic sulphate occurs in the rat. It may be that desulphation is accompanied by the simultaneous production of 4-hydroxybutyric acid, which has been shown to depress the central nervous system in rats (Giarman & Roth, 1964).

Preliminary experiments on the enzyme systems involved in the metabolism of dodecyl sulphate in rats have shown that dodecyl [^{35}S]sulphate can be degraded by rat liver preparations *in vitro*.

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